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Bearth, Natalie D ; Donati, Brice ; Beckmann, Katrin ; Fürst, Anton E ; Suarez, Jose ; Polster, Sabrina ; Jackson, Michelle Amanda

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CASE REPORT

Femoral B-cell neurolymphomatosis in a horse with multicentric lymphoma

Natalie D. Bearth[1] | Brice Donati[2] | Katrin Beckmann[3] | Anton E. Fürst[1] | Jose Suarez[2] | Sabrina Polster[4] | Michelle A. Jackson[1]

[1]Vetsuisse Faculty, Clinic for Equine Surgery, University of Zurich, Zurich, Switzerland

[2]Vetsuisse Faculty, Clinic of Diagnostic Imaging, University of Zurich, Zurich, Switzerland

[3]Vetsuisse Faculty, Clinic for Small Animal Surgery, University of Zurich, Zurich, Switzerland

[4]Vetsuisse Faculty, Institute of Veterinary Pathology, University of Zurich, Zurich, Switzerland

Correspondence: Michelle A. Jackson
Email: mjackson@vetclinics.uzh.ch

Summary

An 18-year-old Freiberger gelding was presented with a history of intermittent left hindlimb lameness after an acute onset 2 weeks prior to referral. The lameness had not responded to anti-inflammatory therapy prescribed by the referring veterinarian. Physical, orthopaedic and neurological examinations revealed signs compatible with ipsilateral femoral nerve dysfunction. Transrectal sonographic examination showed a marked increase in the diameter of the left femoral nerve. The results of electromyography of the left hind quadriceps muscle were compatible with denervation attributable to neuropathy. Evaluation of cerebral spinal fluid (CSF) revealed pleocytosis consisting of medium-sized lymphocytes, 5%–10% of which were positive for CD3 and CD79a. Neoplasia was suspected and steroid therapy was started. However, exacerbation of the clinical signs occurred and the horse was euthanised. Histopathological evaluation showed neurolymphomatosis of the left femoral nerve as the cause of the lameness. Infiltration of tumour cells was also seen to a lesser extent in the right femoral nerve, spinal cord, various parts of the cardiovascular system, renal capsules, an abdominal lymph node and the subcutis of the left stifle and upper lip. Histochemical evaluation revealed 60%–70% of the neoplastic lymphocytes were positive for CD20 and CD79a and up to 40% were positive for CD3. This report describes the uniqueness of a femoral mononeuropathy as the main clinical finding of B-cell neurolymphomatosis and multicentric lymphoma.

KEYWORDS
horse, electromyography, femoral nerve, lymphoma, ultrasonography

INTRODUCTION

Neurolymphomatosis is a rare form of lymphocytic invasion of the peripheral nervous system, which may involve isolated structures or be associated with diffuse neoplastic diseases such as multicentric lymphoma and acute leukaemia (Grisariu et al., 2010). Cranial nerves, spinal nerve roots and spinal nerves may be affected, and clinical presentation in human medicine is divided into painless or painful neuropathy and mono- or polyneuropathy (Baehring et al., 2003). Lymphomatous infiltration of cranial or spinal nerves is the cause of the nerve dysfunction, which must be differentiated from impairment attributable to compression by a mass, other neuropathies and neurotoxicity (Bamford et al., 2018; Sano et al., 2017). This is particularly important in human medicine because paraneoplastic syndromes and chemotherapy toxicity are other causes of neuropathy (Antoine & Camdessanché, 2007). Cranial or spinal nerve involvement in

lymphoma has been described in dogs, cats and horses, and the term neurolymphomatosis has been increasingly used (Hsueh et al., 2019; Lehmbecker et al., 2014; Mandrioli et al., 2012; Rupp et al., 2014; Sakurai et al., 2016; Schaffer et al., 2012).

In addition to sarcoid, melanoma and squamous cell carcinoma, lymphoma is a relatively common malignancy in horses (Knowles et al., 2016). Smith and George (2009) described lymphoma as the most frequent haematopoietic neoplasm in horses, and in a database survey of equine tumours in the UK, it represented 14% of all neoplasms (Knowles et al., 2016). Lymphoma is divided into multicentric, alimentary, mediastinal, cutaneous and solitary forms depending on its anatomical distribution (Canisso et al., 2013; Miglio et al., 2019). Tumour growth is usually silent, and non-specific signs such as pyrexia, weight loss, anorexia and lethargy may be seen initially. Thus, the disease may not be recognised until an advanced stage. More specific clinical signs can be seen when the tumour infiltrates a particular organ, a solitary tumour impairs the function of an organ or paraneoplastic syndrome occurs (Axiak & Johnson, 2012).

Studies in horses describe lymphomatous invasion of the femoral, saphenous, sciatic and cranial nerves and cauda equina (Lehmbecker et al., 2014; Sano et al., 2017; Torrent et al., 2019). Presenting complaints included cranial nerve deficits, lameness, pelvic limb paresis, tail atony, muscle atrophy and, in some cases of infiltration of central nervous system, ataxia. Post-mortem examination of these cases revealed lymphocytic infiltration of a wide range of organs in addition to the central and peripheral nervous system (Lehmbecker et al., 2014).

To the authors' knowledge, femoral mononeuropathy as the primary clinical finding in horses with neurolymphomatosis has not been reported. This case report therefore describes the results of clinical examination, transrectal peripheral nerve ultrasonography, electromyography, cytological examination of CSF and postmortem assessment in an 18-year-old Freiberger gelding with neurolymphomatosis of the left femoral nerve.

CASE DETAILS

History

An 18-year-old Freiberger gelding was referred to the Equine Hospital, Vetsuisse Faculty, University of Zurich, because of sudden onset of left pelvic limb lameness that had started 2 weeks prior to admission. The owner had not observed a traumatic event before the onset of lameness. The referring veterinarian had done an abaxial sesamoid nerve block and radiographic examination of the stifle, hock and pastern of the left pelvic limb, all of which were unremarkable except for mild osteoarthritis of the left stifle joint (periarticular osteophytes on the proximomedial aspect of the tibia on the caudal 60°lateral–craniomedial oblique view). A non-steroidal anti-inflammatory drug (phenylbutazone, 2.2 mg/kg bwt s.i.d. p.o., Covetrus) was administered for 2 weeks but at the control examination the veterinarian could not detect any improvement.

Clinical examination

At the time of presentation, the gelding was bright, alert, responsive and in good body condition (BCS 3/5). Severe muscle atrophy of the left quadriceps musculature was observed and the left pelvic limb was consistently held in a toe-touching position while standing. The horse had a moderate gait deficit at the walk characterised by inability to extend the stifle joint (Video S1). Other than muscle atrophy of the left quadriceps (especially the vastus lateralis muscle), no abnormalities were detected on palpation of the affected limb. Application of hoof-testers and manipulation of the limb elicited no abnormal reactions. Diagnostic regional anaesthesia including a high-four-point nerve block and intraarticular anaesthesia of the left tarsometatarsal and femorotibial joints did not resolve the lameness. Transrectal palpation revealed subjectively enlarged left medial iliac lymph nodes. Haematological analysis showed mild to moderate leucocytosis (10.4×10^3 cells/μL; reference range $4.7–8.2\times10^3$ cells/μL), hypocalcaemia (2.67 mmol/L, reference range 2.9–3.3 mmol/L) and a mild increase in serum amyloid A concentration (9.7 mg/L, reference range 0.5–1.2 mg/L). Serum thymidine kinase concentration was lower than 1 U/L (reference range <5 U/L).

Radiography

Further evaluation included laterodorsal-lateroventral oblique and cranioventral-caudodorsal radiographic views of the pelvis to rule out fractures and luxation of the coxofemoral joint. All the radiographs were unremarkable.

Ultrasonography

Transrectal ultrasonography showed that the sacroiliac joints and contour of the pelvic bones were normal and no haematomas were identified in the pelvic canal and caudal abdomen. The left and right femoral nerves were seen along the external iliac arteries and disappeared distally into the femoral triangle. The diameter of the left femoral nerve was markedly larger than the right femoral nerve and it was relatively hypoechoic (Figures 1 and 2). The widest part of the nerve was seen proximally in the region of the iliopsoas muscle. The left medial iliac lymph nodes were larger, rounder and diffusely hypoechoic compared with the right nodes.

Electromyography

Electromyography (EMG) was done with the horse sedated using a combination of detomidine hydrochloride (0.01 mg/kg bwt iv) and butorphanol tartrate (0.02 mg/kg bwt iv). Needle EMG of both pelvic limbs showed moderately prolonged insertional activity and moderately abnormal spontaneous activity (fibrillation potentials and positive sharp waves) confined to the left vastus lateralis muscle. This

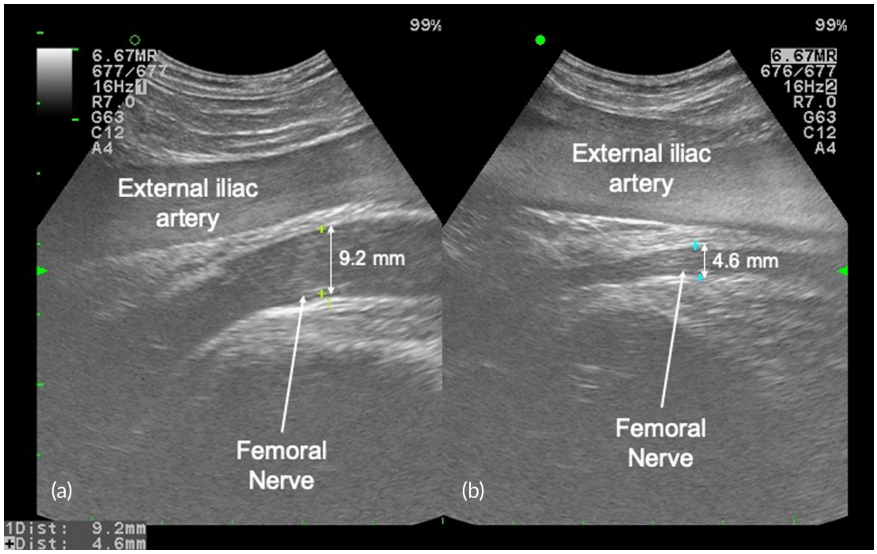

FIGURE 1 Sonograms obtained via transrectal ultrasonography of the left (a) and right (b) femoral nerves located adjacent to the external iliac artery (longitudinal views). The diameter of the left femoral nerve (9.2 mm, green marks) is larger than that of the right (4.6 mm, blue marks).

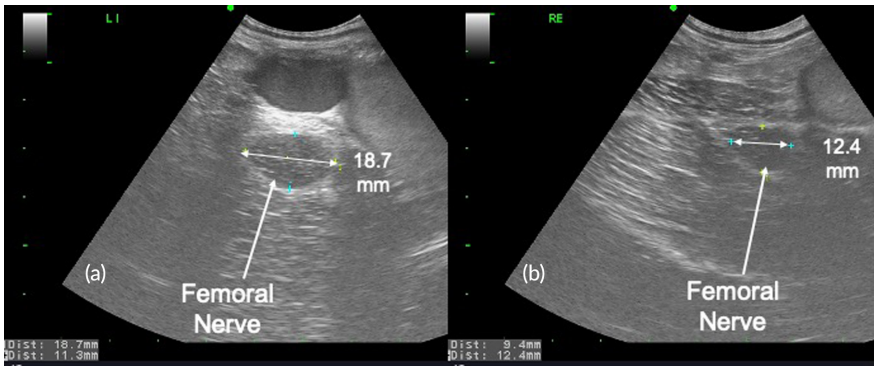

FIGURE 2 Sonograms obtained via transrectal ultrasonography of the left (a) and right (b) femoral nerves located adjacent to the external iliac artery (cross-sectional views). The diameter of the left femoral nerve (18.7 mm) is larger than that of the right (12.4 mm).

suggested denervation secondary to left femoral nerve neuropathy (Figure 3).

Cerebrospinal fluid analysis

Cerebrospinal fluid collected from the lumbosacral space was light red and slightly turbid on gross examination. Laboratory analysis showed a mild increase in protein concentration at 1.4 g/L (reference value <1 g/L), an erythrocyte count of 9 erythrocytes/μL and marked pleocytosis with a leucocyte count of 6144 leucocytes/μL (ref. <10/μL). The latter comprised 98% lymphocytes, 1% neutrophils and 1% monocytes. Microscopic evaluation showed that the vast majority of lymphocytes were medium-sized and had a moderate amount of basophilic cytoplasm, a round lobulated nucleus with a reticular chromatin structure and 1–4 large nucleoli. A few mitotic figures were seen. Based on the cytological findings, lymphoma consisting of medium-sized lymphocytes was diagnosed. Immunohistochemical evaluation of cytospin preparations for B-cell- (CD3) and T-cell marker (CD79a) showed that 5%–10% of the lymphocytes were positive for both markers, but no predominant population could be identified. The suspicion of lymphoma was based on cytology only and could not be supported by immunohistochemistry.

Treatment

The horse was treated with a single-dose of a corticosteroid (dexamethasone 0.04 mg/kg bwt iv, MSD Animal Health GmbH) followed by a non-steroidal anti-inflammatory drug (phenylbutazone 2.2 mg/kg bwt s.i.d. p.o., Covetrus), vitamin B complex (B-neuron ad.us.vet. 3 mg/kg bwt iv, Vetoquinol AG), an anticoagulant (Dalteparin 50 IU/kg bwt s.i.d. s.c., Pfizer), a corticosteroid (prednisolone 1.5 mg/kg s.i.d. p.o., Vetoquinol), and an antiepileptic drug used against neuropathic pain (gabapentin 5 mg/kg bwt b.i.d p.o., Mepha Pharma AG). Despite treatment, the condition of the horse deteriorated over the next 5 days, so that the lameness increased, and euthanasia was elected. Post-mortem examination

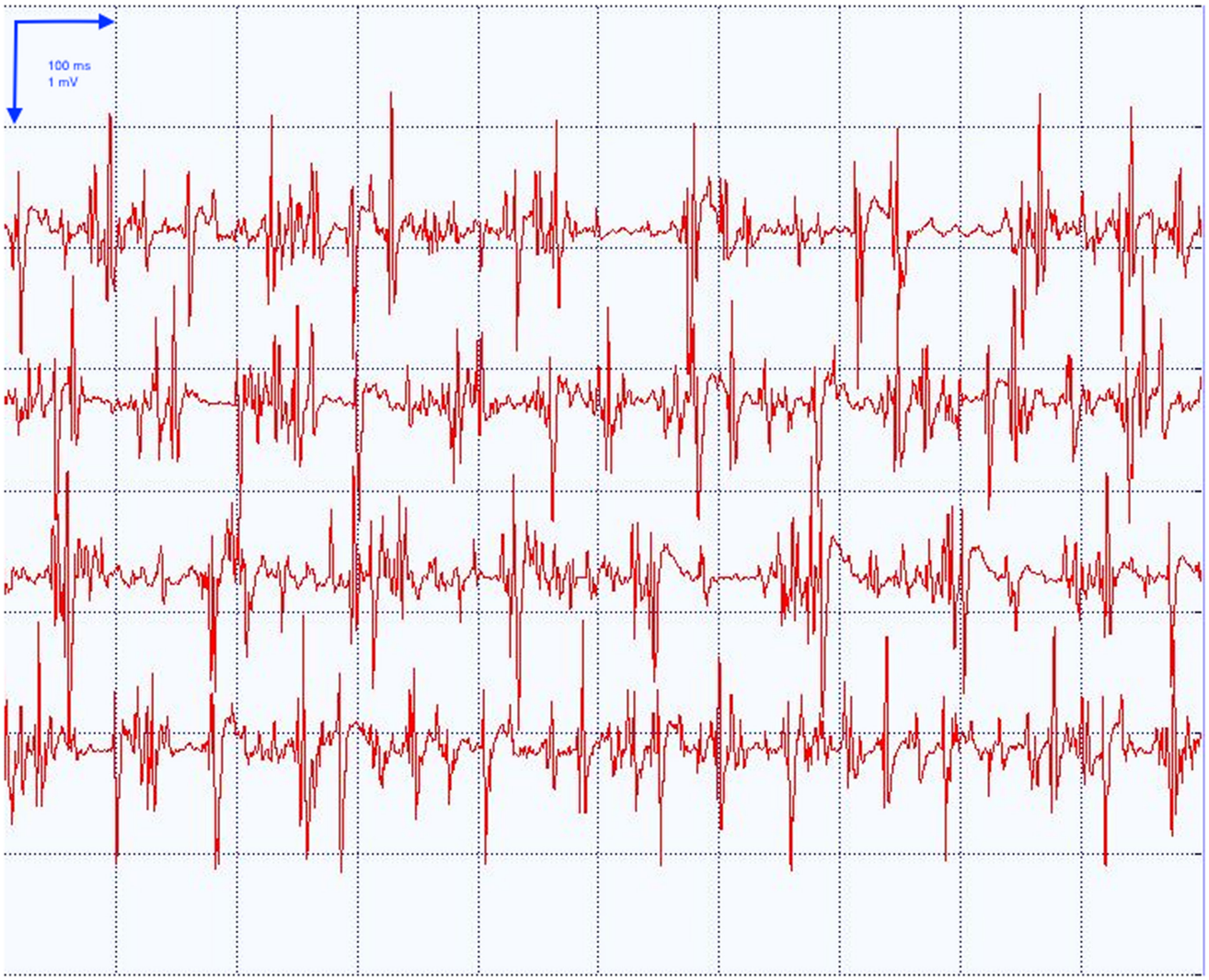

FIGURE 3 Tracing obtained by needle electromyography of the left vastus lateralis muscle showing moderately abnormal spontaneous activity.

including histopathological evaluation was carried out with the owner's consent.

Post-mortem findings

Macroscopic changes were noticed in the nervous and cardiovascular systems, digestive and urinary tracts and lymphatic tissues. Focal thickening of the dura mater over the most cranial aspect of the thoracic segment of the spinal cord and 15 cm caudal to this site was seen. The left femoral nerve was approximately 2.5 cm in diameter compared with the right which was about 2.0 cm in diameter (Figure 4).

Multiple, light brown, firm, 4×4×1 cm masses were seen on the pericardium. Similar light brown, firm nodules approximately 2.5 cm in diameter were observed on all leaflets of the aortic valve. Thickening of the mitral and pulmonary valve leaflets, approximately 20 cm of the proximal aspect of the aortic wall and about 10 cm of the pulmonary trunk wall were observed. The altered vascular walls were up to 1 cm thick and were diffusely infiltrated with firm gelatinous, light brown strands of tissue. Multiple, firm, light brown nodules approximately 2 cm in diameter were present in the capsule of both kidneys. One lymph node in the abdominal cavity of the right flank area was moderately enlarged (approximately 8×4×2 cm).

Histopathological findings

Severe infiltration of medium-sized lymphoblasts was seen in the endoneurium, perineurium and epineurium of all nerve fascicles of the left femoral nerve (Figure 5). Neoplastic cells were large and had indistinct cell borders, a minimal amount of eosinophilic cytoplasm and round to oval nuclei that were approximately 2–2.5 times the diameter of an erythrocyte with finely stippled chromatin and one nucleolus (Figure 6). Moderate anisocytosis and anisokaryosis and infrequent mitotic figures (5/high-power field, indicating a

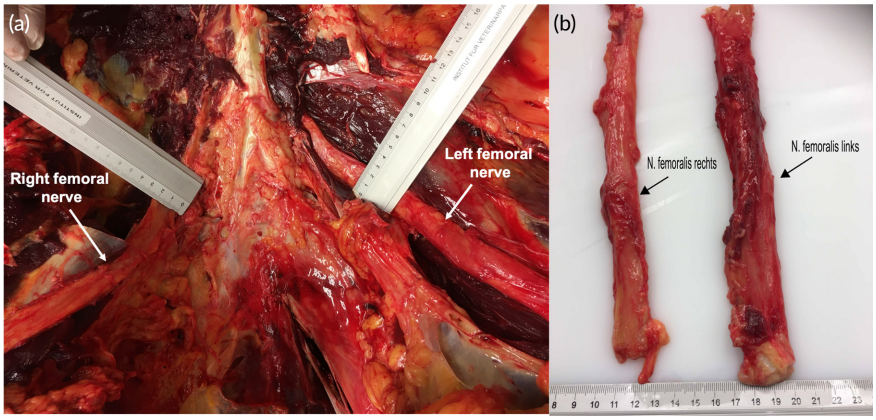

FIGURE 4 Photos of the femoral nerves during preparation (a) and after resection (b). Note that the left femoral nerve (measured thickness about 2.5 cm) is thicker than the right one (measured thickness about 1.5 cm).

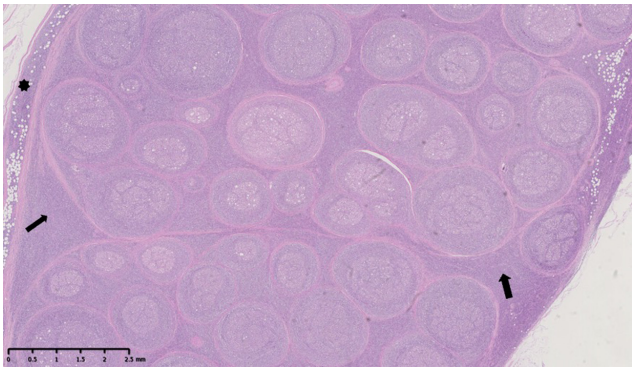

FIGURE 5 Histological section of the left femoral nerve showing that all nerve fascicles (arrows) and the perineural tissue (star) have severe infiltration of medium-sized lymphocytes (Haematoxylin and eosin stain).

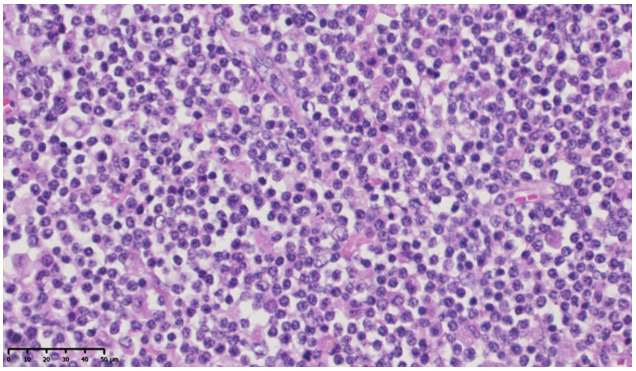

FIGURE 6 Histological section of a nerve fascicle showing infiltration of numerous medium-sized lymphocytes each containing a round nucleus, which is 2–2.5 the diameter of an erythrocyte, and a scant amount of eosinophilic cytoplasm (Haematoxylin and eosin stain).

low-grade neoplasm) were seen. The same neoplastic cells were also seen but to a lesser extent in the following tissues: right femoral nerve; spinal cord, particularly multifocal infiltration of the meninges, perivascular areas of the neuroparenchyma and perineural tissue of the spinal nerves; pericardium; aortic, mitral and pulmonary valves; aortic arch; pulmonary trunk; renal capsules; one abdominal lymph node; and the subcutis of the left stifle and upper lip. Immunohistochemical evaluation of neoplastic cells expressing CD3, CD79a and CD20 in the aorta and aortic valve showed that about 60%–70% were positive for CD20 and CD79a and up to 40% were positive for CD3. Based on all the findings, B-cell lymphoma was tentatively diagnosed (Durham et al., 2012).

DISCUSSION

We used a multimodal diagnostic approach to determine as the cause of unilateral pelvic limb lameness in a horse B-cell neurolymphomatosis and multicentric lymphoma. A lesion in the proximal aspect of the left pelvic limb was suspected and radiography was used to rule out fractures and luxations of the pelvis. Rectal ultrasonography was critical in identifying abnormalities in the shape and size of the left femoral nerve. To the authors' knowledge, ultrasonographic imaging of the equine femoral nerve has not been described. With knowledge of its anatomic course, transrectal ultrasonography can be used to image the femoral nerve from the lumbosacral plexus where the fibres from the third through sixth lumbal spinal nerves meet. Within the iliopsoas muscle, it travels distolaterally to its exit point from the abdominal cavity into the limb at the level of the femoral triangle. Alterations in the size and shape of nerves and nerve dysfunction can be caused by localised compression, infiltration of inflammatory or neoplastic cells, or degeneration. Neoplasia in particular can impair nerve function by compression, in the case of a mass, or by infiltration of neoplastic cells (Lehmbecker et al., 2014; Smith & George, 2009).

Innervation of the affected musculature was assessed using EMG, and the findings were consistent with regional denervation secondary to neuropathy (Wijnberg & Franssen, 2016). Similar electromyographic changes were described in a woman with Hodgkin's lymphoma and paraneoplastic myasthenia gravis (Nanni et al., 2018). The latter seems unlikely in the present case because of the localised

changes in the size of the nerve and the lack of evidence for similar manifestations of paraneoplastic syndrome in horses.

Reports describing femoral nerve dysfunction in horses are rare and focus mainly on postanaesthetic paralysis (Dyson et al., 1988; Mirra et al., 2018). Nonetheless, a differential diagnosis should include all disorders likely to affect the femoral nerve (de Lahunta et al., 2020). The results of ultrasonography, electromyography and clinical examination strongly suggested a neoplastic disease such as lymphoma, which can be multicentric and infiltrative. Finding medium-sized lymphocytic pleocytosis in the CSF sample corroborated a tentative diagnosis of multicentric lymphoma; given its extradural location, femoral nerve involvement alone would not lead to changes in the CSF. Malignancies of equine peripheral nerves other than lymphoid infiltration have been described but appear to be rare (Federici et al., 2015; Nikolaou et al., 2015; Schneider et al., 2010).

Access to the femoral nerve for collection of a biopsy sample was not possible, and thus a definitive ante-mortem diagnosis of the femoral nerve changes could not be made. Surgical resection, which is one of the primary therapeutic approaches to lymphoma in general (Taintor & Schleis, 2011), was not an option either. Other treatment alternatives would have been radiotherapy and chemotherapy. However, inaccessibility of the femoral lesion, treatment-related expenses and the likelihood that the lymphoma was in an advanced stage (due to the enlargement of the left femoral nerve and the cytological findings of the cerebrospinal fluid analysis) culminated in a decision to institute palliative therapy until euthanasia was ethically indicated.

A diagnosis of B-cell lymphoma was confirmed by histological examination of the left femoral nerve, and the term neurolymphomatosis was considered to be an accurate description of the lesion. Most of the case reports of lymphocytic infiltration of neural structures in horses (Lehmbecker et al., 2014) describe central nervous system involvement in the thoracic spinal cord as well, which is in agreement with our findings. Lymphoid infiltration of the central nervous system in equine lymphoma is rare. In a study by Durham et al. (2012), post-mortem examination of 203 horses with lymphoma showed that only 2% had infiltration of neoplastic cells into the central nervous system and none had infiltration of the peripheral nervous system. The clinical signs in the present case included abnormal gait, hindlimb weakness, muscle fasciculations and muscle atrophy, which were consistent with lower motor neuron (LMN) paresis. Signs associated with a more central or more cranial neurological lesion were not seen (Torrent et al., 2019). These may have been overshadowed by the lower motor neuron signs in the left pelvic limb or simply missed because their degree of severity was mild.

A newer diagnostic test used as a tumour marker in equine medicine is serum thymidine kinase (sTK) activity (Larsdotter et al., 2015; Moore et al., 2021). Results of a study that compared clinically normal horses, horses with lymphoma, horses with non-haematopoietic neoplasia and horses with inflammatory disease (Larsdotter et al., 2015) showed the following sTK activities: <2.7 U/L for clinically normal horses; 26.3 ± 91.5 U/L (range 0.8–443 U/L) for horses with lymphoma; 2.3 ± 1.4 U/L (range 0.6–5.7 U/L) for horses with non-haematopoietic neoplasia; and 1.5 ± 0.6 U/L (range 0.6–2.8 U/L) for horses with inflammatory disease. A more recent study suggested that serum thymidine kinase 1 (sTK1) activity could be used as a biomarker for treatment success and detection of early stages of disease and relapse in dogs with lymphoma (Boyé et al., 2019). However, in one recent study, sTK values were not predictive of lymphoma diagnosis in a cohort of horses (Moore et al., 2021). Similarly, in our case the activity of sTK was lower than 1 U/L. Although sTK activity is a useful diagnostic and prognostic biomarker in humans (Ellims et al., 1981) and dogs (Euler et al., 2009) with lymphoma, further studies are needed in horses.

CONCLUSIONS

This unique case report emphasises that, although lymphomatous infiltration of nerves is rare, it should be part of a differential diagnosis for neuropathy in horses. Transrectal ultrasonographic examination and electromyelography were useful for determining femoral nerve dysfunction, and CSF analysis showed neoplastic lymphocytes leading to a tentative diagnosis of multicentric lymphoma.

AUTHOR CONTRIBUTIONS

N. Bearth and M. Jackson contributed to the study design, data analysis and interpretation, and preparation of the manuscript. B. Donati, K. Beckmann, A. Fürst, J. Suarez and S. Polster contributed to data analysis and interpretation. All authors contributed with the final approval of the manuscript.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

FUNDING INFORMATION

No funding received.

ETHICS STATEMENT

The owner was informed about the case report and gave his approval.

ORCID

Katrin Beckmann https://orcid.org/0000-0002-1823-7845
Michelle A. Jackson https://orcid.org/0000-0003-2142-2942

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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