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Polyradiculoneuropathy in dourine-affected horses

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Abstract

Dourine is an equine protozoan disease caused by *Trypanosoma equiperdum*. Dourine-afflicted animals die after developing neurological clinical signs, such as unilateral paresis. The disease has been a problem for many years; however, the pathogenesis regarding the neurological clinical signs of dourine has been unclear. In the present study, we conducted a histopathological examination in order to investigate the mechanisms by which dourine-afflicted horses develop the accompanying neurological clinical signs. Four dourine-afflicted horses in Mongolia were evaluated. An apparently healthy horse exhibited multifocal neuritis without axonal or myelin degeneration. The other horses, which had obvious neurological clinical signs, also exhibited multifocal neuritis. In particular, the nerves that innervated areas associated with neurological clinical signs exhibited neuritis with demyelination in the latter horses. Inflamed, non-demyelinating nerves were infiltrated with B lymphocytes and T lymphocytes; while inflamed, demyelinating nerves were infiltrated with mononuclear phagocytes. Our observations revealed lesion progression in the nerves, such that polyradiculoneuropathy could explain the accompanying neurological clinical signs of dourine. To our knowledge, this is the first report to describe a pathogenic mechanism for the development of the neurological clinical signs found in dourine-afflicted horses.

Keywords: Dourine; Polyradiculoneuropathy; *Trypanosoma equiperdum*.

1. Introduction

Dourine is a contagious equine disease caused by the protozoan organism, *Trypanosoma equiperdum* [1,2]. Depending on the virulence of the *T. equiperdum* strain involved, there are 3 clinical stages associated with the disease [1,2]. The first is the genital stage, characterized by genital swelling [1,2]. The second is the cutaneous stage, characterized by cutaneous plaques; cutaneous plaques constitute pathognomonic lesions in dourine, but may not occur depending on the immunological status of the host and the virulence of the infecting *T. equiperdum* [2,3]. The last stage is the neurological stage [1,2]: initial hyperesthesia is followed by diminished sensitivity or anesthesia, and the afflicted animals develop paresis or paralysis of individual nerves; the most commonly involved nerves are facial nerves and nerves of the pelvic limbs [2]. These clinical stages emerge separately or concurrently [2]. An affected horse gradually deteriorates into a state of emaciation, dehydration, and anemia, concordant with the above-mentioned clinical stages; the infection follows a chronic clinical course, and the horse ultimately dies [1–3].

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Trypanosoma equiperdum belongs to the genus *Trypanosoma*, section Salivaria, subgenus *Trypanozoon* [1]. The subgenus *Trypanozoon* includes several trypanosomes that are of clinical importance in humans and animals. *Trypanosoma brucei gambiense* and *T. b. rhodesiense* cause human African trypanosomosis (HAT) (so-called "sleeping sickness") [4], while *T. b. brucei* and *T. evansi* cause animal African trypanosomosis (so-called "nagana") in cattle [5] and chronic wasting disease (so-called "surra") in a wide range of animal species [6]. The major clinical signs of nagana and surra are fever, progressive anemia and wasting [3,5,6]. The subspecies of *T. brucei* are transmitted solely by the tsetse fly [3–6]. *Trypanosoma evansi* is transmitted by the hematophagous biting fly [3,6,7]. In contrast, *T. equiperdum* is transmitted solely by coitus [1–3]. The taxonomy of *Trypanozoon* trypanosomes has been controversial and indistinguishable by genetic or serological testing because of their close evolutionary relationship [8,9]. A notable difference between *T. evansi* and *T. equiperdum* infection is in the clinical signs of affected hosts [8,9]. However, differential diagnosis is problematic due to the non-constant presence of pathognomonic clinical signs of dourine, which are characteristic of the 3 clinical stages of dourine [8].

Distributions of *T. evansi* and *T. equiperdum* differ in hosts: *T. evansi* localizes to blood, cerebrospinal fluid (CSF), and tissue, while *T. equiperdum* localizes to urogenital mucosa and is rarely found in blood or CSF [1–3,8,9]. Some trypanosomosis involve the development of neurological clinical signs. In HAT, psychiatric disturbances (e.g., the characteristic disturbance of the sleep-wake cycle, motor symptoms, and sensory involvement) can appear in the later central nervous system stage of the disease [4]. Although the detailed mechanisms of these nervous system disorders in HAT remain to be elucidated, encephalitis associated with protozoan invasion is recognized as a causative mechanism [10]. Neurologic abnormalities and encephalitis associated with trypanosomosis have also been recorded in cases of *T. evansi* infection [11]. Histopathologically, the encephalitis that develops in both HAT and *T. evansi* infection is characterized by leptomeningitis and perivascular cuffing by infiltration of mononuclear cells [10,11]. Conversely, dourine-afflicted animals do not develop such encephalitis, despite the dourine-associated clinical signs of peripheral nervous system disease. Early investigators attempted to identify the relevant histopathological alterations in the brain and spinal cord of infected horses; however, they did not detect critical changes within the central nervous system [12,13]. Histopathological alterations, such as nerve inflammation, axonal degeneration, and fibrosis, were found only in the nerves that were distributed within the symptomatic region of sensory disturbances, such as diminished sensitivity or anesthesia [1,2]. In a recent outbreak of dourine in 6 horses in Italy, neurodegenerative lesions and vasculitis of the central nervous system were reported in 1 horse; edematous infiltration in the facial and lingual nerve were reported in the same horse; and neuritis was reported in another horse [14]. The reason that only peripheral nerves in dourine-afflicted animals were affected remains unclear. In the present study, dourine-afflicted horses identified in Mongolia were histopathologically studied to clarify some aspects of the pathogenesis of neurological clinical signs associated with dourine. In brief, inflammatory lesions were not limited to the symptomatic region; rather, they were distributed in nearly all nerves. Herein, we report polyradiculoneuropathy in dourine-afflicted horses in Mongolia.

2. Case reports and methods

Four horses were investigated in the present study, which was conducted in Mongolia from 2014 to 2017. The horses were euthanized and subjected to necropsy. All procedures described in the present report were approved by the Committee on the Ethics of Animal Experiments of Obihiro University of Agriculture and Veterinary Medicine (Permit Numbers 26-37, 27-15, 28-122, and 29-153). All applicable international, national, and institutional guidelines for the care of animals were followed. In the necropsies, liver, spleen, kidney, heart, lung, brain, spinal cord, and nerves were collected and fixed in neutral-buffered 15% formalin. Depending on the situation at the time of each necropsy, nerves were not uniformly collected. Systematic sample collections were performed in cases 2 and 3, while sample collections were incomplete in cases 1 and 4 due to limited equipment and/or operators.

2.1. Case 1

A 9-year-old thoroughbred stallion exhibited genital swelling and paresis of the lip and pelvic limbs. Under the suspicion of dourine, urogenital swab and blood samples were collected; these were then subjected to microscopic observation and polymerase chain reaction (PCR) using the KIN primer [15] and ITS1 primer [16] sets. Anti-*Trypanosoma* antibody was detected in the serum samples by enzyme-linked immunosorbent assay (ELISA) [17] and immunochromatographic test (ICT) [18,19], for animal trypanosomosis. Flagellated protozoa with an undulating membrane and kinetoplast, corresponding to the morphology of *Trypanozoon*, were detected both in genital swabs and blood smears. The PCR-direct sequencing test revealed the identity of the protozoa to be *Trypanozoon*. Based on the clinical features observed and the protozoa detected in the genital swab, the stallion was diagnosed with dourine. The stallion ultimately developed astasia and cachexia (Supplemental Fig. 1); it was euthanised due to poor prognosis, and a necropsy performed. Necropsy revealed severe systemic gelatinous atrophy of adipose tissue, which represented a highly emaciated body condition. There were no other significant lesions found on gross necropsy.

2.2. Case 2

A 7-year-old Mongolian domestic stallion exhibited genital swelling. The horse was seropositive for animal

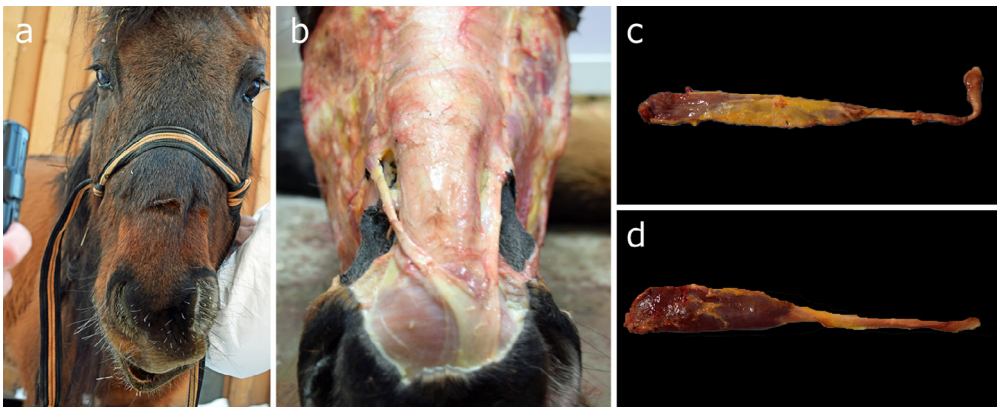

Fig. 1. Deviation of the muzzle in case 3. (a) The muzzle was deviated to the left. Ptosis was also noted on the right eyelid. (b) The levator muscle of the upper lip was constricted, and the right levator muscle was relaxed. (c) The right levator muscle was atrophied and discolored. (d) The left levator muscle appeared normal.

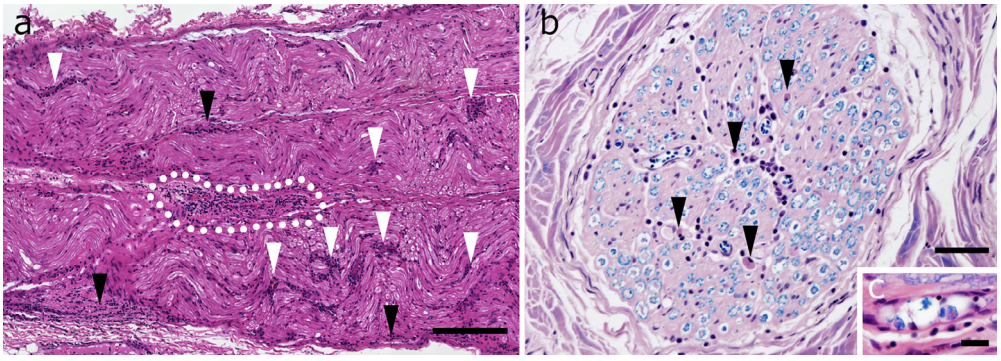

Fig. 2. Photomicrograph of the sciatic nerve. (a) Longitudinal section. Micro-focal aggregates of mononuclear cells were observed around small blood vessels in the epineurium (white dot-lined circle), sub-perineurium (black arrowhead) and interstitially with regard to nerve bundles (white arrowhead). Degenerative nerve changes were not evident. Hematoxylin and eosin stain (HE), bar = 200 μm. (b) Transverse section. Mononuclear cells infiltrated the thin connective tissue that radiated from the perineurium and surrounded small bundles of nerves. In particular, the cells were micro-focally aggregated around capillaries. Activated plasma cells that contained cytoplasmic granules were also observed (black arrowhead). Most of the myelin was normal. Luxol fast blue (LFB) and HE double stain, bar, 50 μm. (c) Limited myelin-laden macrophages and myelin ovoids in vacuolated nerve. Mononuclear cell infiltrates were also observed in affected nerves. LFB-HE stain, bar = 20 μm.

trypanosomosis, as determined by ELISA and ICT. *Trypanozoon* protozoa was detected in the genital swab, blood, and CSF samples via microscopic observation and PCR-direct sequencing test; the protozoa were successfully isolated. Based on the clinical features observed and the protozoa detected in the genital swab, the stallion was diagnosed with dourine. The detailed nature of the isolate has been previously described by Suganuma et al. [20]. The stallion was isolated and kept in the Institute of Veterinary Medicine, Mongolian University of Life Science for 1 year to observe the clinical course. Although trypanosomes were continuously observed in urogenital swabs, the infected horse did not develop any obvious clinical signs. The stallion was subjected to euthanasia and necropsy after 1 year of observation. No significant lesions were detected at necropsy.

2.3. Case 3

A 9-year-old Mongolian domestic mare exhibited facial asymmetry. Ptosis of the right eyelid (Fig. 1(a)) and right ear droop were noted. The right cutaneous muscle of the face and the right masseter muscle were mildly more swollen than the corresponding left muscles. Paresis of the lower lip was noted: some regions of the lower lip did not move in a coordinated manner during feeding. In addition, the muzzle was deviated to the left (Fig. 1(a)). The horse was seropositive for animal trypanosomosis by ELISA and ICT. *Trypanozoon* was detected in a genital swab and CSF samples via microscopic observation and by PCR-direct sequencing. Based on the clinical features observed and the protozoa detected in the genital swab, the mare was diagnosed with dourine and subjected to euthanasia and necropsy. In gross observation, the right levator muscle of the upper lip appeared atonic, while the left muscle was flexed (Fig. 1(b)). The removed right levator muscle exhibited atrophy and was discolored (Fig. 1(c)), while the left levator muscle appeared normal (Fig. 2(d)). There were no gross lesions in other organs or tissues.

2.4. Case 4

A 4-year-old Mongolian domestic mare with suspected dourine was identified in a field. The mare exhibited ear drooping and lip paresis, and was seropositive for animal trypanosomosis by ELISA and ICT. The horse was diagnosed with dourine, based on clinical signs and antibody positivity. The horse was subjected to euthanasia and necropsy in the field for the sake of epidemic prevention and research. No gross changes were detected at necropsy.

2.5. Histopathological examination

The collected samples were embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (HE). Selected specimens were subjected to Luxol fast blue (LFB), Bodian-LFB, and LFB-HE staining, and the periodic acid-Schiff (PAS) reaction. The severity of inflammatory and degenerative changes were assessed in cases 2 and 3, on the basis of the following criteria (Supplemental Fig. 2): ND, not determined due to inadequate sample collection; +, limited (only one focus within the low-power field 100x at the most severely affected region); ++, slight (2 to 5 foci); +++, mild (over 6 foci).

2.6. Immunohistochemistry

Selected samples were also assessed via immunohistochemistry using anti-CD3 rabbit polyclonal antibody (Ready-to-use, IS503, Dako Corp., Glostrup, Denmark), anti-CD20 rabbit polyclonal antibody (1:400, #RB-9013–P1, Thermo Fisher Scientific, Waltham, MA, USA), anti-Iba1 rabbit polyclonal antibody (1:500, 019-19741, Wako, Osaka, Japan) and anti-*T. equiperdum* rabbit antisera (1:400, K. Suganuma). Deparaffinized sections were immersed in 0.3% H_2O_2 to block endogenous peroxidase for 5 min, and then subjected to antigen retrieval with microwave treatment in citrate buffer (15 min, 97 °C). After pre-treatment, the sections were incubated overnight with the primary antibodies at 4 °C. The simple stain MAX-PO polymer reagent (Nichirei Bioscience, Tokyo, Japan) was then applied, and the peroxidase was developed using 3,3′-diaminobenzidine.

3. Results

3.1. Histopathology

No significant lesions were observed within the brains or spinal cords in any of the cases. While there were differences in severity in each case, polyneuritis and perineuritis (inflammation of the perineurium), were observed in all cases.

3.1.1. Case 1

In case 1, muscle fibers within the lips were atrophied with slight endomysial fibrosis and slight infiltration of lymphocytes (Supplemental Fig. 3(a)). In some muscle fascicles within the lip, fibrosis was not evident; however, in these fascicles, nearly all muscle fibers were small angular fibers, indicative of grouped atrophy (Supplemental Fig. 4(a)). Nerves in these tissues exhibited interstitial edema within the endoneurium (Supplemental Fig. 3(b)). A few bundles of nerves were mildly infiltrated with lymphocytes (Supplemental Fig. 3(b)). Although they were slight, similar edematous and inflammatory changes were also present in sciatic nerves, spinal nerves, and unidentified nerves contained in other specimens, such as the parotid gland and unidentified muscles of the pelvic limb. In visceral organs, as has been reported in previous dourine cases [1], splenic hemosiderosis was noted. Focal ascarid-associated eosinophilic granuloma was incidentally observed in the liver, but no other obvious changes were noted in visceral organs.

3.1.2. Case 2

In case 2, mild neuritis and perineuritis were observed systemically; both radicular and distal nerve sites were affected (Supplemental Table 1). The infiltrating cells were primarily identified as lymphocytes and plasma cells. A low level of neutrophil infiltration was observed in the left facial nerve. These infiltrating cells were distributed in the sub-perineurium and associated thin connective tissue, which radiated from the perineurium and enclosed subdivided neuronal bundles within the perineurium (Fig. 2(a) and (b)). Inflammatory cells also infiltrated in the epineurium, especially within the small artery perivascular area. The infiltrating cells sometimes formed foci of small aggregates, which were loosely scattered within the nerves. Blood vessels themselves were unaffected. Although every examined nerve was infiltrated with mononuclear cells, degenerative changes, such as axonal swelling or demyelination, were not evident. Detailed observation revealed few foci, showing the emergence of myelin-laden macrophages (Fig. 2(c)). Neither onion bulb nor onion bulb-like lesions were observed. Muscle was not collected and was not present in other sampled tissue. The brain and spinal cord did not exhibit histopathological alterations. As observed in other cases in the current series, except for hemosiderosis, there was no apparent change in visceral organs.

3.1.3. Case 3

In case 3, neuritis and perineuritis, characterized by infiltration of mononuclear cells, were observed systemically at variable degrees in each nerve (Supplemental Table 2). The specific types of inflammatory cells and infiltrated sites were similar to those observed in case 2. In case 3, however, some inflammatory cells infiltrated the endoneurium. In addition, some of the nerves exhibited demyelination—vacuolation with infiltration by myelin-laden macrophages and myelin ovoid. In such demyelinating nerves, the infiltrating inflammatory cells had a rich cytoplasm and appeared to be monocytes or macrophages. Few axonal swellings were noted within the demyelinating foci. Edematous changes within the endoneurium were also noted in some nerves. Such

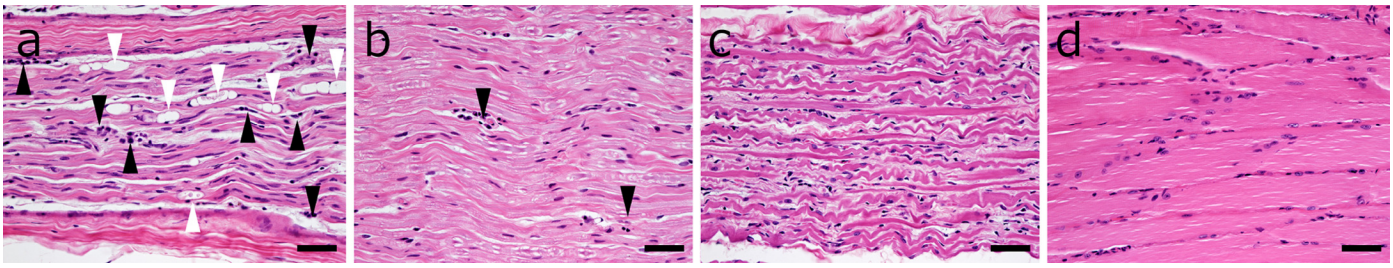

Fig. 3. Photomicrography of the right facial nerve (a), the left facial nerve (b), the right levator muscle of the upper lip (c), and the left levator muscle (d) in case 3. Hematoxylin eosin stain, bars = 50 μm. The inflammatory changes (black arrowhead) were more frequently observed in the right nerve (a) than in the left nerve (b). Vacuolations with myelin ovoid (white arrowhead) were noted in the right nerve (a), while degenerative change was not evident in the left nerve. The muscle fibers of the right muscle (c) were atrophied and thinner than those of the left muscle (d). Some fibers exhibited a wavy appearance. In contrast, the fibers were not atrophied in the left muscle (d). Between each set of atrophied muscle fibers, endomysial fibrosis was also noted in the right muscle (c).

degenerative changes were scattered among nerves; however, the degrees also varied within the same nerve. The facial nerves, which innervate the levator muscle of the upper lip, were affected by neuritis and perineuritis. The inflammatory changes were comparably severe in the right nerve, and were accompanied by multiple vacuolation with myelin ovoid (Fig. 3(a)). In contrast, inflammation within the left nerve was slight and there were no accompanying degenerative changes (Fig. 3(b)). In particular, severe muscular atrophy was noted within the left levator muscle of the upper lip (Fig. 3(c)), while the right had no evidence of muscle atrophy (Fig. 3(d)). In the cross-section of the left levator muscle, large groups of atrophic angular fibers were observed (Supplemental Fig. 4(b)). Muscle atrophy was sporadically observed within lip tissue: there was distinct variation in fiber size, and presence of small angular fibers. The brain and spinal cord were intact. As in other cases in the current series, despite splenic hemosiderosis, there were no apparent changes in visceral organs.

3.1.4. Case 4

In case 4, although the collected samples were limited, neuritis with associated vacuolation and demyelination was observed in the dorsal buccolabial branch of the facial nerve, as well as other nerves within lip tissue and in the ventral muscles of the ear. Lesion severity varied in each bundle of nerves enclosed by perineurium. Muscle atrophy was sporadically observed within lip tissue. As observed in other cases in the current series, despite splenic hemosiderosis, there were no apparent lesions in the visceral organs.

3.1.5. Histological detection of protozoa

Though every specimen was examined repeatedly by several pathologists, etiological organisms were not detected within inflammatory foci of nerves. The PAS reaction was also applied, but infecting protozoa could not be detected. In addition, protozoa was not detected in the other organs or tissues.

3.2. Immunohistochemistry

Immunohistochemistry revealed that infiltrating lymphocytes within nerves consisted of both B and T cells. In case 2, most of the infiltrating cells in the perineurium were B cells, and most of the cells infiltrating interstitially between neuronal bundles, especially those around blood vessels, were T cells (Supplemental Fig. 5(a) and (b)). In the other cases, mixed infiltration of both B cells and T cells was observed within the foci on the nerves. In particular, Iba1-positive cells, which were considered monocytes or macrophages, infiltrated the demyelinating nerves (Supplemental Fig. 5(c)). B cells and T cells did not infiltrate such demyelinating nerves, although both cell types infiltrated the interstitial space between the nerve bundles. Immunohistochemistry using anti-*T. equiperdum* antibody successfully labeled the pathogen in control mouse tissue from mice experimentally infected with the protozoa (data not shown); however, no positively-labeled organisms were detected on the nerves in any of the four cases in the current series.

4. Discussion

The peripheral nervous system signs of dourine are partially explained by the histopathology identified in nerves within affected areas, such as inflammation, edema, and fibrosis [1,2]. Notably, however, no detailed histopathological studies have been reported regarding the peripheral nervous system in dourine-afflicted horses. Previously reported descriptions of neuritis have been brief and without photomicrography [12–14,21]. The mechanisms of the lesion development of the nerves that contribute to the neurological clinical signs remain unclear [2]. Every nerve that was examined in the present study, regardless of its presence at a site related to clinical signs, was inflamed and/or contained lesions, such as edema and fibrosis. In addition, both radicular and distal nerve sites were affected. Demyelination sometimes developed. In the context of these histological findings, the dourine-associated lesions that developed in the nerves constituted polyradiculoneuropathy. To our knowledge,

this is the first report of detailed histopathology describing neuropathy in dourine-afflicted horses.

The presence of neuritis may have been associated with *T. equiperdum* infection. However, there was no evidence that the neuritis was caused by direct injury from the protozoan infection. No *T. equiperdum*-like organisms were detected in the nerves, even in specimens immunostained with anti-*T. equiperdum* antibody. Generally, polyneuritis is caused by immune-mediated mechanisms, nutritional mechanisms, toxic mechanisms, or infection [22]. The present neuritis was similar to immune-mediated neuritis, such as that associated with Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP), in that inflammation occurred multifocally. However, major differences exist among these disorders as follows: In GBS and CIDP, neuritis occurs symmetrically [22]; while neuritis in the current series occurred in a non-symmetrical manner; all nerves observed in the present cases were affected, but the severity of neuritis differed among examined nerves. The severity also differed at locations within the same nerve. In addition, the infiltrating cells observed in GBS and CIDP-affected nerves are primarily macrophages and T cells [23], while those early in the disease course in the present study were B cells and T cells. Onion bulb-like lesions, which constitute pathognomonic lesions of CIDP [23,24], were not observed in the present cases. Because the results of histopathological evaluation of the present cases were not similar to findings of human autoimmunity-associated neuritis, such as GBS and CIDP, dourine-associated neuritis might not involve autoimmunity. In particular, admixed infiltration of T and B cells might exhibit a negative relationship with autoimmunity in the present neuritis. Neuropathies caused by toxic or nutritional mechanisms are characterized by vasculitis or axonal degeneration within nerves [24]. Neither lesion indicating prior vasculitis nor axonal degenerations were observed in the present cases. Therefore, the neuritis present in the current series may not have been associated with nutritional or toxic mechanisms of neuritis; it may be a unique neuritis associated with protozoan infection that has not been reported.

In humans, there are several conditions associated with infectious neuritis, such as leprosy, Lyme disease, HIV-associated neuritis, diphtheria and varicella zoster virus infection [24]. In horses, polyneuritis has been reported in polyneuritis equi and endemic acquired polyneuropathy [25,26]. Polyneuritis equi is characterized by necrotizing pyogranulomatous neuritis [25], and endemic acquired polyneuropathy is characterized by inclusion body schwannopathy [26]. These previously reported neuritis conditions differed from the neuritis present in the current series. The latter may be a unique form of neuritis associated with *T. equiperdum* infection, which has not been previously reported. Immunohistochemical analysis demonstrated that non-demyelinating nerves were infiltrated with B cells and T cells, while demyelinating nerves were infiltrated with mononuclear phagocytes, suggesting a potential relationship between demyelination and mononuclear phagocytes. However, it remains uncertain whether infiltration by mononuclear phagocytes was the cause or result of demyelination.

Considering that obvious lesions were not observed in the central nervous system in the present study, or in previous reports [1,11,12,20], dourine-associated neurological clinical signs appear not to be caused by disorders of the central nervous systems. Case 2 appeared healthy and had not proceeded to the neurological stage. Nevertheless, mild inflammatory changes within nerves were noted in case 2, although axonal degeneration and related changes were minimal. Thus, polyneuritis might not be sufficient to cause dourine-associated neurological signs. Given the readily observable neurological clinical signs, cases 3 and 4 were in the neurological stage of dourine. In particular, deviation of the upper lip due to unilateral atrophy of the levator muscle of the lip was observed in case 3. Considering that the large grouped atrophy was observed in the macroscopically atrophied muscle, and that the neuritis and demyelination were observed in the right facial nerve that innervates the muscle, myofiber atrophy was diagnosed as neurogenic muscle atrophy induced by neuritis and associated demyelination. Paresis was also noted on the lower lips in case 3; however, the buccal branches of the facial nerve that innervate the lip were not histopathologically examined. Although there was no evidence regarding the pathogenesis of paresis on the lower lip, it might have been caused by neuritis and demyelination of the nerve; notably nerves distributed in the face, such as facial nerves and mandibular nerves, exhibited neuritis and demyelination. In case 4, there was limited evidence of muscle atrophy despite sporadically observed muscle atrophy within the lip tissue. However, neuritis and demyelination were observed in the collected nerve, as well as nerves within other tissues. The lesions in the nerves, particularly those involving demyelination might have been linked with dourine-associated neurological clinical signs. Case 1 exhibited the terminal stage that occurs after the neurological stage. Although the collected samples were limited, muscular atrophy, edema, fibrosis, and focal neuritis were observed. These lesions within the nerves were similar to previously reported alterations observed in nerves [1,2,12–14,21]. Lesion transition of the nerve initially began with lymphocytic infiltrates before the neurological stage, then involved demyelination during the neurological stage, and ended with edema and fibrosis in the terminal stage. Thus, neuritis and demyelination appear to be responsible for muscle atrophy and weakness in dourine. Previously reported histological lesions in nerves, such as fibrosis and edema [1,2,12–14,21], result from chronic inflammation and demyelination.

In conclusion, polyradiculoneuropathy was identified via histopathological evaluation of 4 dourine-afflicted horses in the current series. The progression of disease and histopathological lesions suggest that neuropathy is responsible for the clinical signs associated with dourine. The mechanisms by which *T. equiperdum* infection induces neuropathy remain unclear. Further investigations to elucidate

the relationships between *T. equiperdum* infection and the unique inflammatory neuropathy of dourine may facilitate a better understanding of trypanosomosis, a severe disease in humans and animals, via comparison of pathogenesis.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.03.005.

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