PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 45:e07660, 2025
DOI: 10.1590/1678-5150-PVB-7660

Original Article
Livestock Diseases
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An outbreak of Potomac horse fever[1]

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ABSTRACT.- Cerri FM, Basso RM, Pedroso NB, Pessoa MA, Pereira WAB, Oliveira-Filho JP, Amorim RM, Baird JD, Arroyo LG, Borges AS. **An outbreak of Potomac horse fever.** *Pesquisa Veterinária Brasileira 45:e07660, 2025*. Departamento de Clínica Veterinária, Faculdade de Medicina e Zootecnia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Rua Prof. Doutor Walter Maurício Corrêa s/n, Cx. Postal 560, Botucatu, SP 18618-681, Brazil E-mail: alexandre.s.borges@unesp.br

Potomac horse fever (PHF) is caused by *Neorickettsia risticii* and the recently identified *Neorickettsia findlayensis* and is characterized by clinical signs such as diarrhea, hyporexia, and lethargy. PHF outbreaks are uncommon. The aim of this report is to describe the epidemiological, clinical, clinicopathological, and molecular aspects of a PHF outbreak in Brazil. An outbreak of gastrointestinal disease was investigated, and clinical and laboratory evaluations were performed in affected animals. A total of 37 out of 216 horses (17%) were affected by diarrhea, lethargy, and hyporexia during the outbreak. Only one horse developed laminitis, and five horses died. Blood and fecal samples from 17 of 37 affected mares were tested for *N. risticii* by qPCR. *N. risticii* was detected in 16/17 fecal samples and 11/16 blood samples. Oxytetracycline (BID) was an effective treatment for affected horses. The genetic analysis of *N. risticii* revealed similarity with strains described in North America. PHF must be included in the differential diagnosis of adult horses presenting diarrhea in Brazil.

INDEX TERMS: *Neorickettsia risticii*, neorickettsiosis, diarrhea.

RESUMO.- [Um surto de Potomac Horse Fever em equinos.] A Potomac Horse Fever (PHF) é causada por *Neorickettsia risticii* e a recentemente identificada *Neorickettsia findlayensis*, sendo caracterizada por sinais clínicos como diarreia, hiporexia e letargia. Surtos de PHF são incomuns. O objetivo deste relato é descrever os aspectos epidemiológicos, clínicos, clínico-patológicos e moleculares de um surto de PHF no Brasil. Um surto de doença gastrointestinal foi investigado, e avaliações clínicas e laboratoriais foram realizadas nos animais afetados. Um total de 37 dos 216 cavalos (17%) apresentaram diarreia, letargia e hiporexia durante o surto. O principal sinal clínico observado foi a diarreia; apenas um equino desenvolveu laminite, e cinco equinos morreram. Amostras de sangue e fezes de 17 das 37 foram testadas para *N. risticii* por qPCR. *N. risticii* foi detectada em 16 das 17 amostras fecais e em 11 das 16 amostras de sangue. A oxitetraciclina (BID) foi tratamento eficaz para todos os equinos. A análise genética da *N. risticci* demonstrou similaridade com as cepas descritas na América do Norte. A PHF deve ser incluída no diagnóstico diferencial de equinos adultos apresentando diarreia.

TERMOS DE INDEXAÇÃO: *Neorickettsia risticii*, neorickettisiose, diarreia.

INTRODUCTION

Potomac horse fever (PHF), previously known as equine monocytic ehrlichiosis and more recently referred to as neorickettsiosis, is a disease caused by *Neorickettsia risticii* (formerly *Ehrlichia risticii)* (Baird & Arroyo 2013, Taylor 2023). Recently, a novel species, *Neorickettsia findlayensis*, was identified as a cause of PHF in Canada (Teymournejad et al. 2020). *Neorickettsia* species are obligatory intracellular

[1] Received on May 18, 2025.
Accepted for publication on July 15, 2025.
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parasites, with part of their life cycle occurring in trematodes (Gibson et al. 2005, Greiman et al. 2016). As a result, PHF has been observed predominantly near sources of freshwater such as lakes, rivers and irrigation fields, with a high incidence during the summer (Baird & Arroyo 2013, Arroyo et al. 2021, Taylor 2023). However, enzootic transmission cycles of PHF occur in many ecosystems, even fully terrestrial ecosystems (Vaughan et al. 2012).

PHF was formally described in 1983 (Knowles et al. 1983). Identifying the causative agent as *Ehrlichia* was reported shortly thereafter (Rikihisa & Perry 1985), and its isolation, combined with successful experimental reproduction of the disease (Dutta et al. 1988). The clinical signs observed in horses with PHF include acute onset diarrhea, hyporexia or anorexia, lethargy, fever, limb edema and colic (Jones 1990, Bertin et al. 2013, Arroyo et al. 2021). Laminitis is frequently reported as a complication of PHF and is observed a few days after the onset of clinical signs (Arroyo et al. 2021).

The diagnosis of *N. risticii* or *N. findlayensis* can be made by detecting DNA in blood and fecal samples using polymerase chain reaction (PCR) or quantitative polymerase chain reaction qPCR (Durán & Marqués 2016, Paulino et al. 2020, Budachetri et al. 2022, Thirumalapura et al. 2023). The isolation of *Neorickettsia* spp. is challenging as it is time-consuming and requires specialized equipment, cell culture capabilities and highly trained personnel (Mott et al. 1997, Teymournejad et al. 2020). The recommended treatment consists of a short course of intravenous oxytetracycline (6.6 mg/kg of body weight – BW, BID for five days) (Baird & Arroyo 2013, Arroyo et al. 2021, Taylor 2023).

There are well-documented descriptions of PHF cases in North America (Heller et al. 2004, Pusterla et al. 2007, Bertin et al. 2013, Durán & Marqués 2016, Arroyo et al. 2021) and few descriptions in Europe (van der Kolk et al. 1991, Seyyal et al. 1998) and Asia (Pusterla & Madigan 2014). However, they are infrequently reported in South America. In Brazil, the first report of PHF was published by Dutra et al. (2001), involving 12 affected horses in Rio Grande do Sul (this report also includes cases in Uruguay). Subsequent cases were reported in Rio Grande do Sul and Paraná (Coimbra et al. 2006, Marcolongo-Pereira et al. 2014, Marutani et al. 2019). Epidemiological studies later confirmed the circulation of *N. risticii* in the states of Rio de Janeiro (Ferrão et al. 2007, Moreira et al. 2013, Vieira et al. 2013, Roier et al. 2016), Santa Catarina, and São Paulo (Moreira et al. 2013), including its detection by PCR in blood samples from asymptomatic horses in the state of Rio de Janeiro (Paulino et al. 2020).

Reports of PHF outbreaks are scarce (Coimbra et al. 2006). This report aims to describe the epidemiological, clinical, clinicopathological, and molecular aspects of a PHF outbreak in Brazil.

MATERIALS AND METHODS

Ethical approval. All procedures performed at the Veterinary Teaching Hospital were conducted with the prior consent of both the horse's owner and the referring veterinarian. Additionally, the owner was informed and consented to the publication of data related to the animal's admission to the institution. For animals from which biological samples were collected on the properties, all ethical guidelines and animal welfare regulations were strictly followed. The owners authorized sample collection, and the horses were continuously monitored by veterinarians from both the institution and the farms. The study was approved by the Animal Experimentation Ethics Committee (Protocol 282/2024) of the "Faculdade de Medicina Veterinária e Zootecnia", "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (FMVZ-Unesp).

Description of the outbreak. The disease occurred between July and August 2024 (winter in the southern hemisphere) at two Quarter Horse (QH) breeding farms (Farms A and B, located in the state of São Paulo and approximately 8 km apart). Clinical management practices were similar on both farms, and animals were exchanged between farms throughout the year. These facilities are dedicated to breeding QH horses and providing embryo transfer services, housing stallions and embryo donor mares, and recipient mares of various breeds.

At night, the mares were subjected to light therapy via artificial lighting in paddocks (for recipient mares) or individual illuminated stalls (for donor mares) to induce the early onset of estrus through photoperiod control artificially. The mares were exposed to a total of 15 hours of light daily (nine hours of natural light followed by six hours of artificial light). Feeding and water stations were accessible in these illuminated areas (both paddocks and stalls).

The water was supplied via various water troughs located across the paddocks, all sourced from an artesian well. Horses had no access to natural water sources or flooded areas, but small rivers and ponds were located less than 1 km away. According to the information collected and reviewed from annual medical records, sporadic cases of diarrhea had occurred on both farms since 2022. During this period, the artificial lighting program was in use, and cases of diarrhea occurred only in horses exposed to artificial lighting.

In 2023, cases of diarrhea were observed, but no deaths were reported. In 2024, the outbreak started after an embryo donor mare developed diarrhea, lethargy, hyporexia, and limb edema with worsening clinical conditions and was therefore referred to the Large Animal Internal Medicine Service of the Veterinary Teaching Hospital (FMVZ-Unesp, Botucatu). Subsequently, 37 horses developed diarrhea; five did not survive, and two underwent necropsy.

Thirty-seven horses developed diarrhea and were positive for PHF; some did not survive (n = 5), and two were necropsied.

Ancillary test. Blood and fecal samples were collected from untreated animals with clinical signs for qPCR testing to detect *Neorickettsia risticii*, as well as for complete blood counts (CBCs) and serum biochemical evaluation (urea, creatinine, aspartate aminotransferase – AST, alkaline phosphatase – ALP, gamma-glutamyl transferase – GGT, total protein, globulin, albumin, creatine kinase – CK, total bilirubin, direct bilirubin, and indirect bilirubin) using an automated bench-top chemistry analyzer (Cobas Mira Plus, USA). Microscopic screening for *N. risticii* morulae in lymphocytes and monocytes was conducted on blood smear slides. In addition, adult insects, aquatic-stage immature larvae, and water samples from the water troughs were collected. Snails were searched for in the affected paddocks.

qPCR analysis of blood and feces. Blood (n = 16) and fecal (n = 17) samples were collected from diarrheic mares and immediately subjected to testing. According to the manufacturer's instructions, DNA was extracted from feces and blood via the Maxwell® RSC Blood DNA Kit (Promega, MA, USA), with protocol modifications for each sample type. DNA samples were used to detect the *N. risticii*-specific *16S rDNA* gene. The primers and probes used and the thermocycling conditions were performed as previously described (Paulino et al. 2020). As a positive control for the PCR assay, DNA samples from *N. risticii* were kindly provided by Professor Luis Arroyo (University of

Guelph). In addition, a subset of fecal samples (n = 4) was submitted for a qPCR panel to diagnose diarrhea in adult horses: detection of *Salmonella* spp., *Clostridioides difficile, Clostridium perfringens, Rhodococcus equi, Lawsonia intracellularis*, and *N. risticii* was performed via probe qPCR following previously described protocols (Pusterla et al. 2007, Gurjar et al. 2008, Pusterla et al. 2008, Mutters et al. 2009, Pusterla et al. 2010, Paulino et al. 2020). *Enterococcus durans*, *Giardia duodenalis*, and *Cryptosporidium parvum* were detected using qPCR via the SYBR Green technique (Verweij et al. 2003, Stroup et al. 2006, Kim et al. 2022). Probe qPCRs for the *C. difficile* A (*tcdA* gene) and B (*tcdB* gene) toxins (Kilic et al. 2015), and *C. perfringens* alpha (*cpa* gene) (Magdesian et al. 2022), were performed as previously described. For the detection of rotavirus and coronavirus, amplification of the non-structural protein 5 (*NSP5)* fragment and the *N* gene of coronavirus was performed (Soltan et al. 2016).

***16S rDNA* sequencing and phylogenetic analysis.** Three *N. risticii*-positive qPCR DNA samples (from Horses 4, 5 and 28) were subjected to PCR for amplification of a fragment of the *16S rDNA* region (Paulino et al. 2020). DNA samples from Horses 4, 5, 6, 28, 34, and 35 were used for amplification of the *P51* gene via PCR (Gibson et al. 2011). The amplified products were purified and subjected to Sanger sequencing. The sequences obtained from the *16S rDNA* were analyzed for DNA quality. These sequences were submitted to the Basic Local Alignment Search Tool (BLAST) algorithm to verify their similarity with nucleotide sequences available in GenBank™. A phylogenetic tree was constructed based on sequences with the highest similarity. Sequences from the *16S rDNA* region of *Neorickettsia* obtained from horses in Brazil (Paulino et al. 2020), liver samples from bats in Argentina (Cicuttin et al. 2013), insects collected in the USA belonging to genotype B of *N. risticii* (Chae et al. 2003), horses with PHF in the USA belonging to genotype C (Mott et al. 2002), *Neorickettsia sennetsu* obtained from human samples in Japan (Anderson et al. 1991), *N. findlayensis* from horses in Canada (Teymournejad et al. 2020) and *Neorickettsia helminthoeca* from salmon samples in the USA (Greiman et al. 2016) were used for comparison. The sequences were aligned to construct a maximum likelihood phylogenetic tree for medium-length sequences, using both the mentioned sequences and those obtained in the present study, with the assistance of the BV-BRC 3.39.3 platform (Price et al. 2009). The tree was generated via the iTOL online tool and midpoint rooting (Letunic & Bork 2021).

The *P51* gene sequences were obtained and aligned via the Clustal Omega algorithm for multiple sequence alignment (MSA)[5] to evaluate the degree of sequence similarity. The aligned sequences were subsequently queried against a genetic database to identify homologous sequences using the mentioned Basic Local Alignment Search Tool (BLAST)[6].

Pathology. *Post mortem* examinations were performed on two horses (Horse 4, Farm B and Horse 6, Farm A). All intestinal segments and organs exhibiting macroscopic changes were sampled, fixed in 10% buffered formalin, trimmed and stained with hematoxylin and eosin (HE) for microscopic examination.

Testing for *N. risticii* in water and insects. During all farm visits and a fifth visit conducted at night (August 3, 2024), insect larvae, water, and sediment from the bottom of the water troughs were collected. Light traps were set up to capture flying insects (Arandela-15, Ultralight, Brazil). All these samples were subjected to qPCR to detect *N. risticii 16S rDNA* (Paulino et al. 2020).

RESULTS

Outbreak description

During the two-month period of the outbreak investigation, 37/216 horses (17%) developed diarrhea, lethargy, and hyporexia. Among the affected horses, 17/37 (46%) were from Farm A, and 20/37 (54%) were from Farm B. Five of 36 donor mares and 32/180 recipient mares were affected. Figure 1 illustrates the timeline of PHF case occurrence on both farms. There were five stallions on the farms, but none of them developed any clinical signs. All affected animals were observed only in the groups of mares exposed to artificial light therapy. None of the affected mares was pregnant. Five recipient mares did not survive; these were not initially treated or treated with only one dose of oxytetracycline daily. The overall case fatality rate of the outbreak, considering Farms A and B combined was 13% (5/37), with 12% (2/17) on Farm A and 15% (3/20) on Farm B. Seventeen of the 37 affected

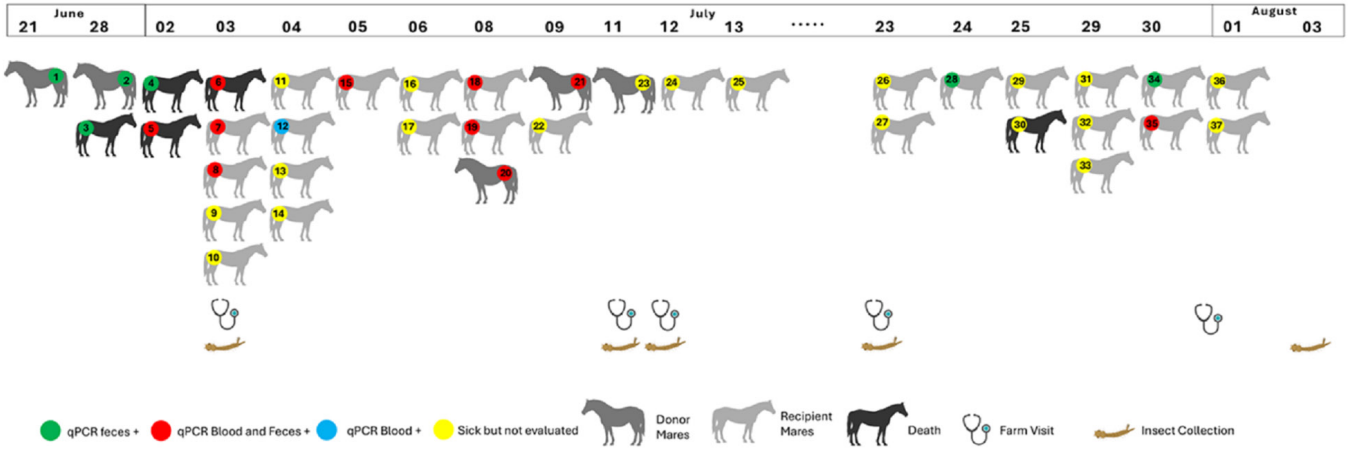

Fig. 1. Timeline of Potomac horse fever outbreak.

[5] Accessed on Nov 17, 2024. https://www.ebi.ac.uk/jdispatcher/msa/clustalo

[6] Accessed on Dec 10, 2024. https://blast.ncbi.nlm.nih.gov/Blast.cgi

horses were tested for *Neorickettsia risticii* via qPCR (16 in both blood and fecal samples and one in only a fecal sample) and qPCR was positive in 16/17 fecal samples and 11/16 blood samples (Table 1).

The first documented case of 2024 (Horse 1: 19-year-old QH mare embryo donor, Farm A) developed clinical signs of anorexia, lethargy, edema of the front limbs, and icteric mucous membranes. Since the mare presented jaundice, a presumptive diagnosis of *Babesia caballi* and/or *Theileria equi* infection was suggested, and the mare was treated at the farm with a single intramuscular administration of sodium dipyrone (25 mg/kg BW) and imidocarb dipropionate (2.2 mg/kg BW). The mare did not improve, developed profuse diarrhea, and was referred to the Veterinary Hospital at Unesp in Botucatu, Brazil, for further diagnosis and treatment. Upon admission, the mare was lethargic with mild tachycardia (48 beats per minute), tachypnea (40 breaths per minute), capillary refill time (CRT) of 2 s, a rectal temperature of 37.5 °C, and a body condition score (BCS) of 3/5. The mucous membranes were pink, and dehydration was estimated at 5%. The intestinal sounds were hypermotile, and the mare exhibited hyporexia and liquid diarrhea (greenish and foul-smelling, Fig. 2-5). Additionally, frequent weight shifting, increased digital pulse, and elevated hoof wall temperature were observed in all limbs, consistent with laminitis.

The CBC revealed severe leukopenia (3,300/µL) due to neutropenia (1,947/µL). The serum biochemistry profile revealed hypoproteinemia (5.2 g/dL), hypoalbuminemia (2.1 g/dL), elevated direct bilirubin (7.3 mg/dL), increased levels of urea (62.5 mg/dL) and creatinine (2.0 g/dL), as well as elevated AST (372 IU/L) and CK activity (820 IU/L). Venous blood gas analysis revealed decreased bicarbonate (HCO_3^-), hyponatremia (Na^+), hypocalcemia (Ca^{2+}), and hypokalemia (K^+) (Table 2).

Table 1. qPCR detection of *Neorickettsia risticii* in blood (n = 16) and feces (n = 17) from horses in Brazil, 2024

Horse	Farm	qPCR			
		Blood	Ct	Feces	Ct
1	A	Negative	-	Positive	31
2	A	Negative	-	Positive	32
3*	B	Not performed		Positive	16
4*	B	Negative	-	Positive	16
5*	B	Positive	36	Positive	22
6*	A	Positive	34	Positive	18
7	A	Positive	35	Positive	23
8	B	Positive	35	Positive	24
12	B	Positive	34	Negative	-
15	B	Positive	31	Positive	32
18	B	Positive	31	Positive	28
19	B	Positive	34	Positive	32
20	B	Positive	31	Positive	31
21	A	Positive	34	Positive	32
28	A	Negative	-	Positive	25
34	A	Negative	-	Positive	21
35	A	Positive	32	Positive	23
TOTAL		11/16 (69%)			16/17 (94%)

qPCR = quantitative polymerase chain reaction, Ct = threshold cycle values; * Nonsurvivors; (-) not applicable.

Fecal samples were submitted to screening for equine enteropathogens via a qPCR panel to investigate the causes of diarrhea in adult horses. DNA of *N. risticii* and *Clostridium perfringens* was detected in the qPCR fecal samples. In contrast, the qPCR results for the blood samples were negative for *N. risticii, B. caballi* and *T. equi*. Based on medical history, clinical signs, and laboratory test results, a definitive diagnosis of PHF was made.

Medical management included fluid therapy using a balanced polyionic solution (lactated Ringer's) and correction of electrolyte imbalances with calcium gluconate (1 mL/kg of BW) and potassium chloride (0.3 × BW × deficit). Sodium bicarbonate (8.4%) was administered to correct metabolic acidosis, and omeprazole (4 mg/kg of BW) was given for gastric protection.

This PHF case (*N. risticii*) was treated with intravenous oxytetracycline (6.6 mg/kg of BW, BID/7 days). Laminitis was managed with cryotherapy on all four limbs for 72 hours, combined with acepromazine (0.02 mg/kg of BW, IM, TID), flunixin meglumine (0.25 mg/kg of BW, IV, QID), and sodium heparin (80 IU/kg of BW, SC, SID).

The fecal consistency and general condition of the horse gradually improved (two days) after starting antibiotic therapy; however, the signs of laminitis persisted. After 10 days of hospitalization, the horse was discharged, showing clinical resolution of diarrhea and laminitis.

Since several horses were affected on both farms and because of the newly available data from PCR testing, farm visits were conducted. Over the course of the outbreak, five visits were made to the farms to assess the horses and sample collection (Fig. 2-5). The most common clinical signs observed included diarrhea, hyporexia, and lethargy, which were reported in all affected horses (100%, 37/37). Notably, laminitis was not observed in any subsequently affected horses during the farm visits.

Farms visits

Farm visit 1 (July 3, 2024). During these visits to the two farms, two recipient mares were found dead and submitted to necropsy (Horse 4 and 6). Neither mare received any treatment. Four other horses were examined during the visit: a donor (Horse 2) and a recipient mare (Horse 7) on Farm A, as well as two recipient mares (Horse 5, which had developed diarrhea three days prior, and Horse 8) on Farm B. Horse 2 improved with medication but still exhibited diarrhea. Horses 5, 7, and 8 also had profuse diarrhea.

Fecal samples were collected and submitted to investigate possible diarrhea-associated infectious agents (*Clostridioides difficile-16s RNA* and *tcdB-*, *C. perfringens-cpa-*, *Cryptosporidium* spp., *Giardia* spp., *Lawsonia intracellularis*, *N. risticii*, *Rhodococcus equi*, rotavirus, and *Salmonella* spp.) and blood for qPCR detection of *N. risticii* in Horses 5, 6, 7, and 8. *N. risticii* was tested in the feces (Table 3). Blood samples were collected for a complete blood count and serum biochemistry (Table 4 and 5).

Since *N. risticii* was detected in the feces of all four Horses tested, the initial oxytetracycline treatment protocol was changed from once daily to twice daily. The day after this visit (July 4), six new horses developed clinical signs (Horses 9–14). Additionally, Horse 5 died, but no necropsy was performed.

Farm visits 2 and 3 (July 10 and 11, 2024). During this visit to Farm B, seven Horses (15, 18–23) presented clinical

signs consistent with PHF (diarrhea, hyporexia, and lethargy). Blood and fecal samples were collected for complete blood counts and qPCR *for N. risticii*. The following day (July 11, 2024), two new cases developed clinical signs (Horses 16 and 17).

Farm visit 4 (July 23, 2024). Twelve horses presented diarrhea (Horses 26–37). After the 4th visit (July 31, 2024), fecal and blood samples from Horses 34 and 35 were collected for qPCR.

Laboratory results

For 37 affected mares, blood and fecal samples were collected for PCR analysis from 17 animals (Horses 1, 2, 3, 4-8, 12, 15, 18-21, 28, 34, and 35), and a blood sample was collected from only one mare (Horse 3). Fecal samples from 16/17 (94%) and blood from 11/16 (69%) patients tested positive (Table 1).

The CBC results for *N. risticii*-positive mares via qPCR are summarized in Table 4. Monocytosis was observed in 6/12 affected mares. During the examination of leukocytes, the

Table 2. Serial complete blood count, serum biochemistry profile, and venous blood gas analysis results from a Potomac horse fever affected mare (Horse 1) admitted to the Veterinary Teaching Hospital, São Paulo, Brazil

Parameters	Admission	Ad+6 h	D1	D1+7 h	D2	D4	D6	D9	D11	Reference[a,b]
RBC (x10^6/µL)	6.93		5.24			4.39	5.17		6.33	6.0-13.0
Hemoglobin (g/dL)	10.9		8.5			6.8	8.1		9.7	10.6-18.9
PCV (%)	32		25			21	25	32	29	34-49ab
MCV (fL)	46.2		47.7			47.8	48.4		45.8	37-58.5
MCHC (%)	33.8		34			32.4	32.4		33.4	31-38.6
Plasma Protein (g/dL)	5.6		4.8			4.6	5.4	6.0	6.2	5.8-8.7
RDW (%)	16.7		17.7				16.8		16.6	
Platelets (cells/µL)	189.375		93.425				119000		144000	
Fibrinogen (mg/dL)	200		400				400		600	100-400
Leukocytes (µL)	300		5300			9800	13500		11600	5200–13800
Neutrophils (cells/µL) (%)	1974 (59)		2544 (48)			6566 (67)	9585 (71)		9048 (78)	2270-8500
Lymphocytes (cells/µL) (%)	1155 (35)		2385 (45)			2842 (29)	2970 (22)		2204 (19)	1100–6800
Eosinophils /µL (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	135 (01)		116 (01)	0-1000
Basophils /µL (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	135 (01)		0 (0)	0-290
Monocytes (cells/µL)	198 (06)		371 (07)			392 (04)	675 (05)		232 (02)	0.0–1400
Urea (mg/dL)	62.5		39		35	30	43	21		21-51
Creatinine (mg/dL)	2.0		1.2		1.2	0.93	1.32	1.09		1.2-1.9
AST (IU/L)	372				339.8	521	550			226-366
ALP (IU/L)	174.5				128	170				143-395
GGT (IU/L)	8.5				12.2	15.9	20.2			4.3-13.4
Total protein (g/dL)	5.2				3.7	4.2	4.9	5.4	5.6	5.2-6.9
Globulin (g/dL)	3.1				2.2	2.5	2.9	3.10	3.2	2.6-3.7
Albumin (g/dL)	2.1				1.5	1.7	2.0	2.30	2.4	2.62-4.04
CK (IU/L)						820	620			2.4-340
Total bilirubin (mg/dL)					7.3	1.7				0-2
Direct bilirubin (mg/dL)					7.3	0.6				0-0.4
Indirect bilirubin (mg/dL)					0.0	1.1				0.2-2
pH	7.39	7.33	7.32	7.37	7.38	7.36				7.32-7.44
pCO_2 (mmHg)	38.2	38.2	37.2	41.4	39.2	43.7				38-46
pO_2 (mmHg)	61	59	49	114	115	30				
HCO_3 (mmol/L)	22.9	20	19.3	24.2	23.3	24.5				20-28
BE (mmol/L)	-2	-5,4	-6,2	-0,9	-1,7	-0,8				±6
Anion Gap (mmol/L)	9.4	8.9	8.3	10.3	7.4	7.4				10
Cl^- (mmol/L)	99	106	107	102	104	105				99-109
K^+ (mmol/L)	2.55	3.04	2.93	3.14	2.97	3.06				2.4-4.7
Na^+ (mmol/L)	131	134	135	137	135	137				132-146
SID_3 (mmol/L) *	34.55	31.04	30.93	38.14	33.97	35.06				38-44
Ca^{2+} (mmol/L)	1.34	1.55	1.51	1.42	1.45	1.59				1-1.3
Lactate (mmol/L)	0.9	0.5	0.6	0.5	<0,3	<0,3				1.11-1.78

RBC = red blood cells, PCV = packed cell volume, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gamma-glutamyl transferase, CK = creatine kinase, pH = potential hydrogen, pCO_2 = partial pressure of carbon dioxide, pO_2 = partial pressure of oxygen, HCO_3 = bicarbonate ion, BE = base excess, Cl^- = chloride, K^+ = potassium, Na^+ = sodium, SID_3 = strong ion difference = $(Na^+ + K^+) - Cl^-$, Ca^{2+} = calcium ion; [a] Grondin et al. (2010), [b] Kaneko et al. (2008)

presence of *N. risticii* was not detected. Serum biochemistry was performed on four horses (Horses 2, 5, 7, and 10). An increase in urea, creatinine, and bilirubin (total, direct, and indirect) was observed in 3/4 of the sampled horses.

Treatment

Initially, before the first farm visit, the protocol for oxytetracycline administration on both farms consisted of daily administration of this drug (single dose daily). All untreated affected horses (n = 3) or those treated with a single dose of oxytetracycline daily (n = 2) died. Once oxytetracycline treatment (6.6 mg/kg IV BID) for seven days was initiated, the feces of these mares had a normal consistency (from fecal balls) by the second day of treatment.

Blood and fecal samples were collected from three horses (Horses 16, 17, and 22) on the last day of treatment for *N. risticii* qPCR testing. The feces of these mares were normal in consistency, and both samples tested negative for all mares.

Pathology

Gross examination of Horses 4 and 6 revealed necrotizing colitis characterized by distension and liquid intestinal contents, coalescent areas of hyperemia, hemorrhage, and edema of the mucosa, as well as prominent Peyer's patches in multiple sections of the small intestine (duodenum, jejunum, and ileum) and in the cecum and colon.

Microscopic examination showed lymphocytes and plasma cells in the lamina propria and, to a lesser extent, in the submucosa of both the large and small intestines (lymphocytic enteritis) (Fig.

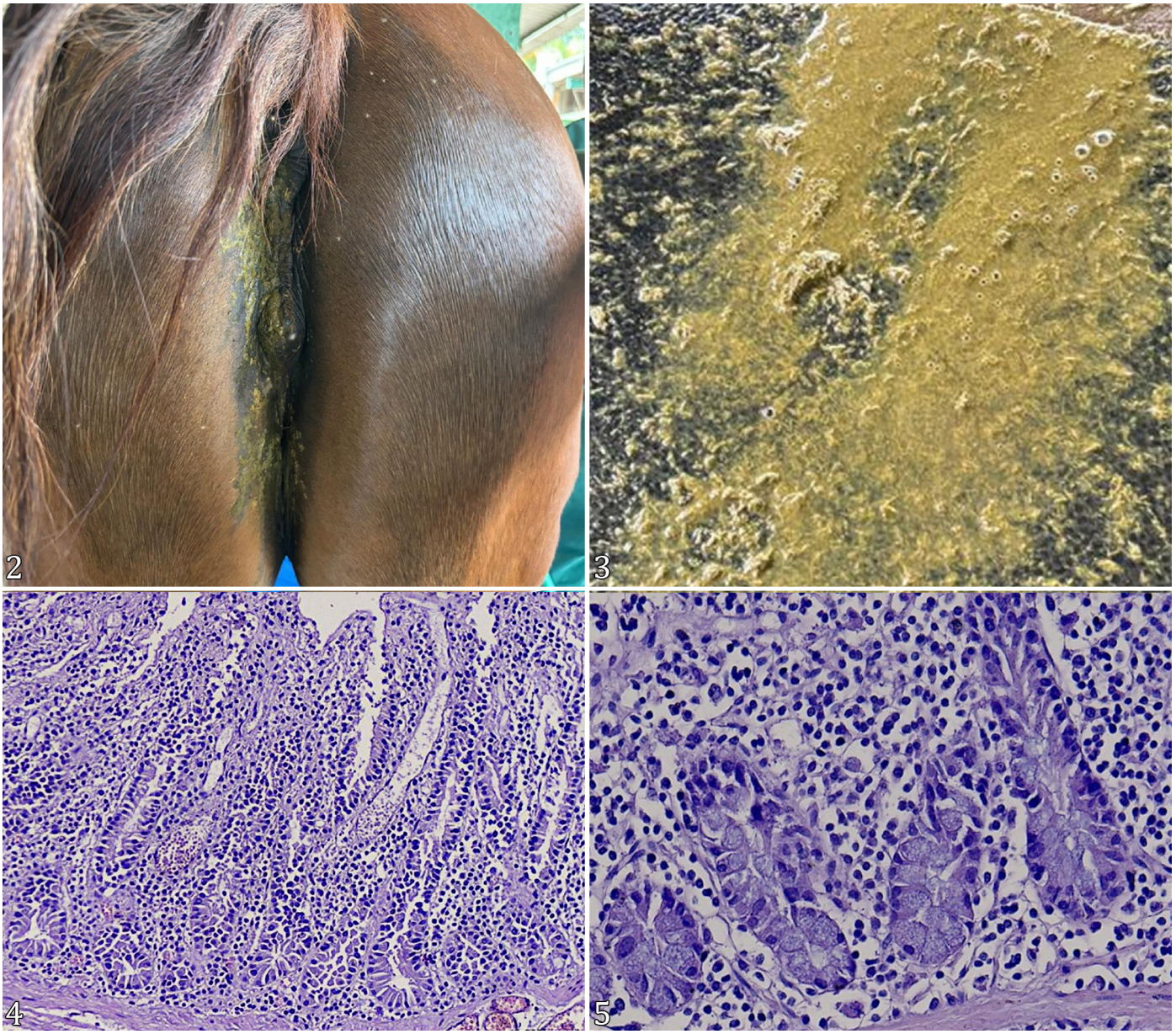

Fig. 2-5. Horse 1. (**2**) Neorickettsia-associated diarrhea in a horse. (**3**) Perineal region of a horse stained with diarrheic fecal material. Horse 5. (**4**) Small intestine: Loss of superficial epithelium, congestion, and mononuclear infiltrate in the lamina propria. HE, obj. 200x. (**5**) Large intestine: mild to moderate mononuclear inflammatory infiltrate within the lamina propria. HE, obj. 400x.

4 and 5). The large colon and caecum also exhibited congestion and hemorrhage. Notably, Horse 4 had foci of mild macro- and microvesicular degeneration of centrilobular hepatocytes, accompanied by mild lymphocytic infiltration in the portal space.

Phylogenetic analysis

The phylogenetic analysis revealed high genetic similarity among the *16S rDNA* sequences obtained from the tested samples (PV067268, PV067269, PV067270), as well as between these sequences and *N. risticii* sequences belonging to Genotype C (Fig. 6), including Brazilian *N. risticii* sequences previously obtained from equine isolates The *P51* sequences identified in the horses studied (PV845751, PV845752, PV845753, PV845754, PV845755, PV845756) presented 99.98% genetic similarity among themselves. Compared with other sequences, these sequences presented 96.75% similarity with sequences obtained from bats in Argentina (KX986614.1), 93.83% similarity with sequences derived from snails in the USA (AF036675), and 90.3% similarity with sequences from naturally infected horses in the USA (AF380265.1).

qPCR for *N. risticii* in environmental samples and insects

No snails were found at the pasture during visits to the two farms. However, many insects in various developmental stages (Fig. 7-10) and organic matter were found in the troughs in illuminated areas. During the first visit, four pooled samples were collected, comprising water samples and larvae present in the water troughs from both farms. On the second visit, eight pooled insect samples and two water samples were collected. The following day, three water samples and three insect samples were collected. At the fourth visit, six samples were collected: three insect samples and three water samples from the troughs. Insects were grouped into pools according to species and origin. The insects identified included damselfly nymphs, stonefly larvae, midge larvae, dragonfly larvae and mayfly larvae (Baptista et al. 2001, Heckman 2011, Duarte & Lecci 2023). A fifth visit was conducted at night on August 3, 2024, and adult insects were collected near the water troughs with light traps. The water and sediment samples from the troughs were analyzed individually. Adult insects and larvae were grouped into pools of five specimens per analysis, with approximately two to three pools of each type tested via qPCR. All the insect pools (n = 20) tested negative for *N. risticii* by qPCR. However, *N. risticii* was detected in one of the water and sediment samples (1/11) collected from a trough on Farm A.

Table 3. qPCR enteropathogen diagnostic panel results for detection in fecal samples (n = 5) from horses with Potomac horse fever, SãoPaulo, Brazil, 2024

Horse		C. dif	C. perf	ECov	Rota	Cryp	Gia	Neor	Salm	Law	R. equi
1	A	N	P	N	N	N	N	P	N	N	N
5*	B	N	P	N	N	N	N	P	N	N	N
6*	A	N	P	N	N	N	N	P	N	N	N
7	A	N	P	N	N	N	N	P	N	N	N
8	B	N	P	N	N	N	N	P	N	N	N

C. dif = *Clostridioides difficile* (*16S rRNA* and *tcdB*), C. perf = *Clostridium perfringens* (*cpa*), ECov = equine coronavirus, Rota = Rotavirus A, Cryp = *Cryptosporidium* spp., Gia = *Giardia* spp., Neor = *Neorickettsia risticii*, Salm = *Salmonella* spp., Law = *Lawsonia intracellularis*, ***R. equi*** = *Rhodococcus* equi, N = negative, P = positive; * Death.

Table 4. Complete blood cell counts results in horses with Potomac horse fever (n = 12). State of São Paulo, Brazil, 2024

Horse	1	2	5*	7	8	11	12	15	18	19	20	21	Median	Min-Max	Referrence[a,b]
RBC (x10^6/µL)	10.1	7.99	12.1	8.87	9.93	10.1	8.27	8.35	10.1	7.22	7.3	8.93	8.87	7.22-12.1	6.0-13.0
Hemoglobin (g/dL)	15.6	12.1	17.9	14	16.3	15.1	12.5	12.1	16	12.1	11.6	15.3	14	11.6-17.9	10.6-18.9
PCV (%)	47	37	54	43	50	44	38	36	47	36	36	46	43	36-54	34-49
MCV (fL)	46.6	43.3	44.6	48.5	50.4	43.6	45.9	43.1	46.5	49.9	49.1	51.5	46.5	43.1-51.5	37-58.5
MCHC (%)	33.2	32.7	33.1	32.6	32.6	34.3	32.9	33.6	34	33.6	32.2	33.3	33.1	32.2-34.3	31-38.6
Total protein (g/dL)	7.0	6.4	7.2	8	9.6	6.8	6.6	7	6.6	6.2	5.8	8.2	6.8	5.8-9.6	5.8-8.7
Fibrinogen (mg/dL)	800	400	NP	NP	NP	600	NP	1000	NP	NP	400	600	500	400-1000	100-400
SAA (µg/mL)	NP	<20	>3000	283	<20	<20	<20	2136	<20	2020	<20	>3000	<20	<20->3000	0-20
Leukocytes/µL	13,400	7,400	8,300	13,500	8,700	22,700	11,800	25,400	19,400	10,100	8,800	12,900	11,800	7,400-25,400	5200-13800
Neutrophils/µL (%)	670 (5)	4,810 (65)	2,490 (30)	3,370 (62)	5,655 (65)	15,663 (69)	5,074 (43)	14,904 (76)	16,490 (85)	3,131 (30)	4,400 (50)	7,869 (61)	4,810 (62)	2,540 (50) -7,400 (85)	2,270-8,500
Lymphocytes/µL (%)	1,742 (13)	2,072 (28)	4,399 (53)	3,780 (28)	1,914 (22)	5,675 (25)	3,894 (33)	4,318 (17)	1,940 (10)	4,141 (41)	2,904 (33)	3,096 (24)	3,780 (28)	1,742 (10)-5,940 (53)	1100-6800
Eosinophils/µL (%)	0 (0)	370 (5)	0 (0)	0 (0)	0 (0)	227 (1)	236 (2)	508 (2)	0 (0)	606 (6)	0 (0)	0 (0)	0 (0)	0 (0)-660 (6)	0-1000
Basophils/µL (%)	0 (0)	0 (0)	0 (0)	22 (0)	0 (0)	227 (1)	118 (1)	0 (0)	0 (0)	303 (3)	0 (0)	0 (0)	0 (0)	0 (0)-370 (0)	0-290
Monocytes/µL (%)	10,988 (82)	148 (2)	1,411 (17)	1,350 (10)	1,131 (13)	454 (2)	2,478 (21)	1,270 (5)	970 (5)	1,919 (19)	1,496 (17)	1,935 (15)	1,350 (13)	148 (1)-10,988 (82)	0.0-1400

RBC = red blood cells, PCV = packed cell volume, MCV = mean corpuscular volume, MCHC = mean corpuscular hemoglobin concentration, SAA = serum amyloid A, NP = not performed; * Death; [a] Grondin et al. (2010), [b] Kaneko et al. (2008).

DISCUSSION

The first PHF cases in Brazil were documented in 2001 in the southern bordering region between Brazil and Uruguay (Dutra et al. 2001). Horses developed clinical signs over five years, with the agent confirmed by PCR. In the subsequent years, additional cases were reported within a four-month period, mostly during the summer, in areas near lakes and rivers, environments conducive to the proliferation of aquatic insects (Coimbra et al. 2006). Some years later, cases were reported in horses admitted to veterinary hospitals in Brazil (Marcolongo-Pereira et al. 2014, Marutani et al. 2019). Additionally, epidemiological studies have identified the circulation of the agent in horses by serology (Ferrão et al. 2007, Moreira et al. 2013, Vieira et al. 2013, Roier et al. 2016) and molecular detection in blood samples from horses without clinical signs (Paulino et al. 2020) and snails

Table 5. Serum biochemistry results (n = 4) of horses with Potomac horse fever. São Paulo, Brazil, 2024

Variable	2	5*	7	8	Median	Min-Max	Reference[b]
Urea (mg/dL)	36	166	102	56	79	36-166	21-51
Creatinine (mg/dL)	1.69	6.73	2.17	1.98	2.08	1.69-6.73	1.2-1.9
AST (UI/L)	277	287	314	406	305.5	277-406	226-366
ALP (UI/L)	231	323	425	384	353.5	231-425	143-395
GGT (UI/L)	11.5	7.3	9.1	14.4	10.3	7.3-14.4	4.3-13.4
Total Protein (g/dL)	6	6.5	6.7	8.5	6.6	6-8.5	5.2-6.9
Albumin (g/dL)	2.8	2.1	2.8	3.1	2.8	2.1-3.1	2.6-3.7
Globulin (g/dL)	3.2	4.4	3.9	5.4	4.15	3.2-4.5	2.62-4.04
Total bilirubin (mg/dL)	4.2	1.5	6.0	3.3	3.75	1.5-6.0	0-2
Direct bilirubin (mg/dL)	0.5	1.5	0.8	0.8	0.8	0.5-1.5	0-0.4
Indirect bilirubin (mg/dL)	3.7	0.8	5.2	2.5	3.1	0.8-5.2	0.2-2
CK (UI/L)	789	1551	655	535	722	535-1551	2.4-340

* Death; AST = aspartate aminotransferase, ALP = alkaline phosphatase, GGT = gammaglutamyl transferase, CK = creatine kinase; [b] Kaneko et al. (2008).

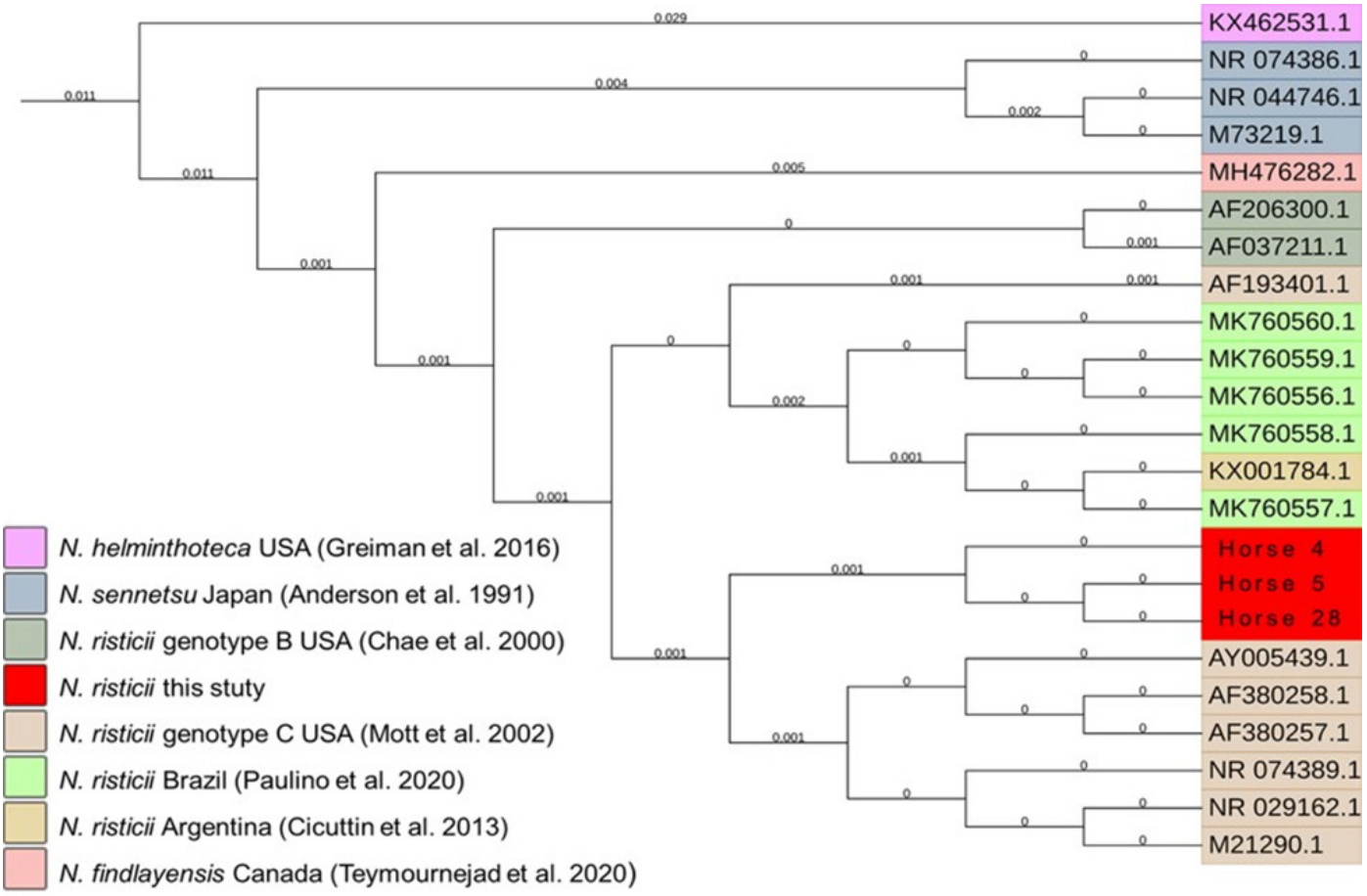

Fig. 6. Phylogenetic analysis of *Neorickettsia risticii* and other species of *Neorickettsia* compared on the *16S rDNA* sequences identified, their respective GenBank accession numbers and sequences from the present study.

(Coimbra et al. 2005, Costa et al. 2016). *Neorickettsia risticii* was also detected in wild animals in Brazil, including bats (*Desmodus rotundus* and *Diaemus youngi*) (Mello et al. 2023, 2024), Iowland tapirus (*Tapirus terrestris*) (Mongruel et al. 2024) and wild ring-tailed coatis (*Nasua nasua*) (Perles et al. 2023). These studies provide evidence of the presence and circulation of *N. risticii* in horses and other mammals in Brazil. Geographically, PHF cases are commonly reported near lakes (Dutta et al. 1988, Baird & Arroyo 2013, Arroyo et al. 2021) or artificial irrigation fields (Pusterla et al. 2013). However, in the present study, this outbreak occurred on farms where the animals had no access to natural or artificial waterways, reservoirs, or flooded areas, although there were small rivers and ponds less than a kilometer away, which may have contributed to the agent's transmission cycle. This outbreak occurred during the winter months (minimum: 13.8 to 14.9 °C and maximum: 24.4 to 27 °C) (Climate Data 2025), a season characterized by lower precipitation levels in São Paulo state, unlike most reports that link PHF cases or the detection of *N. risticii* in horses, with periods of higher rainfall or summer months (Perry et al. 1986, Baird & Arroyo 2013, Kopper et al. 2021, Willette et al. 2022, Taylor 2023).

The clinical signs observed were similar to those described in other PHF reports (Dutta et al. 1988, McLaughlin & Gough 1996, Madigan et al. 1997, Dutra et al. 2001, Baird & Arroyo 2013, Bertin et al. 2013, Arroyo et al. 2021, Taylor 2023). Therefore, *N. risticii* should be included among the infectious agents commonly associated with enterocolitis in horses in this region (*Clostridioides difficile, Clostridium perfringens*, equine coronavirus, *Cryptosporidium* spp., *Lawsonia intracellularis,* rotavirus, and *Salmonella* spp.) (Shaw & Stämpfli 2018, Gomez et al. 2022, Uzal et al. 2022, Gomez et al. 2024). PHF has previously been associated with the development of laminitis (Bertin et al. 2013, Luethy et al. 2021), which occurred in a horse during this outbreak.

Hematological findings in PHF associated with enterocolitis may include either neutrophilia or neutropenia, depending on the stage of the disease and the extent of enteric involvement (Cordes et al. 1986, Fortin-Trahan et al. 2023). Leukocytosis, a response frequently associated with enterocolitis, as well as neutrophilia, was identified in 26% of the evaluated horses, suggesting an underlying inflammatory process. Variations in hematological results may be related to the stage of the disease, the extent of intestinal lesions, and individual differences among the animals (Ziemer et al. 1987).

Serum biochemistry alterations observed in this study were consistent with excessive fluid and electrolyte loss through feces (hyponatremia, hyperchloremia, acidosis and azotemia), leading to electrolyte abnormalities, dehydration and hypovolemia (Rikihisa et al. 1992, Bertin et al. 2013). Blood gas analysis at the time of admission revealed hyponatremia and metabolic acidosis due to strong ions (reduction in SID_3), which persisted until day 2. Acid-base and electrolyte balance alterations are described in cases of PHF (Heller et al. 2004,

Fig. 7-10. Insects collected during the second farm visit (July 10 and 11, 2024). (**7**) Damselflies nymphs, (**8**) stonefly nymphs, (**9**) midge larvae, and (**10**) dragonfly nymphs.

Durán & Marqués 2016, Fortin-Trahan et al. 2023) and bacterial enterocolitis (Gomez et al. 2013, 2020). In horses with PHF, the absorption rate of sodium and chloride decreases due to alterations in the intestinal mucosa, particularly in the large colon (Rikihisa et al. 1992).

The observed fatality rate was lower than that reported in cases of PHF treated in veterinary hospitals in North America (Bertin et al. 2013, Arroyo et al. 2021), which may be related to the disease severity of the animals referred to equine hospitals. The affected farms in this study presented daily animal assessment for reproductive purposes and consequently may have played a role in the early diagnosis and timely treatment of affected horses. Most deaths (3/5) occurred before the first farm visit and before the changes to the antimicrobial treatment protocol, with a subsequent reduction in the duration of clinical signs observed thereafter. The gross changes and microscopic lesions of different intestinal segments in the two horses that underwent necropsy examination were similar to those previously reported (Cordes et al. 1986, Dutra et al. 2001, Coimbra et al. 2006, Uzal et al. 2022).

The qPCR assay was useful for detecting *N. risticii* in fecal and blood samples, as previously described (Bertin et al. 2013, Pusterla et al. 2000a, 2000b, Arroyo et al. 2021, Budachetri et al. 2022). *Neorickettsia risticii* was found in greater proportions in the fecal samples (94%) than in the blood samples (69%), which agrees with previous reports. The threshold cycle (Ct) values observed in the qPCR analysis were lower in the fecal samples, except for Horses 15 and 20, which presented approximately equal Ct values in both and Horse 12, which tested positive only in the blood sample. These findings align with the results of Arroyo et al. (2021), who reported a higher detection rate in fecal samples than in blood samples. While Ct values were not mentioned in their study, the lower Ct values observed in the present study's fecal samples may partly explain these findings. As a result, testing fecal and blood samples for bacteria is the recommended practice when investigating PHF cases. Direct observation of the agent in blood samples from the current outbreak was unsuccessful and yielded no diagnostic benefit.

Both genetic analyses of the *16S rDNA* and *P51* gene sequences revealed high genetic similarity, indicating that the same strain circulated on both farms. Phylogenetic analysis of the obtained sequences, based on *16S rDNA*, revealed clustering among the *N. risticii* sequences of genotype C in the USA (Mott et al., 2002). The identification of *N. risticii* in South America and its genetic similarity to North American strains suggest a common ancestor or perhaps the exchange of bacteria between these regions of the continent (Dutra et al. 2001, Budachetri et al. 2022). A possible route of bacterial exchange between these landmasses is via trematode permanent host migration. Although *N. risticii* has already been detected in bats in South America, this hypothesis remains to be proven (Cicuttin et al. 2013).

The detection of *N. risticii* in aquatic insects has been previously reported (Mott et al. 2002, Park et al. 2003, Wilson et al. 2006, Madigan et al. 2000, Greiman et al. 2014). Multiple cases on a farm are atypical manifestations of PHF, which is generally sporadic, with isolated or scattered cases occurring over extended intervals (Dutta et al. 1988, McLaughlin & Gough 1996, Dutra et al. 2001, Baird & Arroyo 2013, Bertin et al. 2013, Arroyo et al. 2021, Taylor 2023). It was hypothesized that the artificial lighting system may have contributed to the outbreak by attracting insects to the area where the mares were housed (Farren 2007). Targeted studies are therefore necessary to improve the understanding of aquatic insect and bat populations and their correlation with the developmental cycle of metacercariae, and consequently, *N. risticii*. These knowledge gaps likely result from the inherent complexity of the transmission cycle involved in the epidemiology of PHF.

The detection of *N. risticii* in the pooled water sample from the troughs may result from contamination. Another possible explanation is the presence of metacercariae infected with *N. risticii* in the water. To test this hypothesis, amplification and sequencing of *18S rRNA* gene segments from samples would be necessary to identify the presence of metacercariae larval stages, as Park et al. (2003) suggested. This represents one of the limitations of the present study. Additional limitations include the absence of quantitative qPCR to compare DNA loads between blood and fecal samples, the lack of serological testing, and the inability to perform laboratory evaluations on all clinically affected animals. Although partial sequencing was performed, whole-genome sequencing (WGS) was not conducted. This molecular approach would have allowed for a more detailed characterization of the detected strain, including its relationship with other *N. risticii* strains and the identification of potential virulence-associated genes.

CONCLUSION

Neorickettsia risticii is associated with an outbreak in an area of São Paulo state where neorickettiosis has not been previously reported. This study, therefore, supports the current practice of polymerase chain reaction (PCR) testing for the presence of the agent in fecal and blood samples to diagnose suspected Potomac horse fever (PHF).

Acknowledgments.- This study was supported by the Veterinary Hospital of the "Faculdade de Medicina e Zootecnia", "Universidade Estadual Paulista 'Júlio de Mesquita Filho'" (FMVZ-Unesp), Botucatu/SP. The authors are thankful for the support provided by the Brazilian government agencies, "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES), Brazil, for funding this, as well as to OBJETIVAVET - Bauru/SP (Diagnóstico Veterinário) for sharing data and images of post-mortem examinations.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

Credit author statement.- FMC: conceptualization, data curation, formal analysis, investigation, methodology and validation, writing – original draft. RMB: conceptualization, investigation, methodology, validation and writing – original draft. NBP: investigation, methodology, validation, writing – review & editing. MP: investigation, validation and writing – original draft. JPOF: investigation, methodology, formal analysis, supervision, validation and writing – review & editing. WABP: investigation, methodology, formal analysis, validation and writing – review & editing. RMA: investigation, formal analysis, methodology, validation and writing – review & editing. JDB: investigation, formal analysis, validation and writing – review & editing. LGA: investigation, formal analysis, validation and writing – review & editing. ASB: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, supervision, visualization and validation, writing – original draft.

Data availability statement.- The essential data for interpreting the results have already been made available in this paper.

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