

***Histophilus somni*-induced thrombotic meningoencephalitis in cattle from northern Paraná, Brazil¹**

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ABSTRACT.- Headley S.A., Bracarense A.P.F.R.L., Oliveira V.H.S., Queiroz G.R., Okano W., Alfieri A.F., Flaiban K.K.M.C., Lisbôa J.A.N. & Alfieri A.A. 2015. ***Histophilus somni*-induced thrombotic meningoencephalitis in cattle from northern Paraná, Brazil.** *Pesquisa Veterinária Brasileira* 35(4):329-336. Laboratório de Patologia Animal, Departamento de Medicina Veterinária Preventiva, Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, PR-445 Km 380, Cx. Postal 10.011, Londrina, PR 860571-970, Brazil. E-mail: selwyn.headley@uel.br

Thrombotic meningoencephalitis (TME) is a fatal neurological disease of cattle, predominantly from North America, that is caused by *Histophilus somni* with sporadic descriptions from other countries. This manuscript describes the occurrence of spontaneous TME in cattle from northern Paraná, Brazil. Most cattle had acute neurological manifestations characteristic of brain dysfunction. Hematological and cerebrospinal fluid analyses were not suggestive of bacterial infections of the brain. Histopathology revealed meningoencephalitis with vasculitis and thrombosis of small vessels that contained discrete neutrophilic and/or lymphocytic infiltrates admixed with fibrin at the brainstem, cerebral cortex, and trigeminal nerve ganglion of all animals. All tissues from the central nervous system used during this study were previously characterized as negative for rabies virus by the direct immunofluorescence assay. PCR and RT-PCR assays investigated the participation of infectious agents associated with bovine neurological disease by targeting specific genes of *H. somni*, *Listeria monocytogenes*, bovine herpesvirus -1 and -5, bovine viral diarrhoea virus, and ovine herpesvirus-2. PCR and subsequent sequencing resulted in partial fragments of the 16S rRNA gene of *H. somni* from brain sections of all animals with histopathological diagnosis of TME; all other PCR/RT-PCR assays were negative. These findings confirmed the participation of *H. somni* in the neuropathological disease observed in these animals, extend the geographical distribution of this disease, and support previous findings of *H. somni* from Brazil.

INDEX TERMS: *Histophilus somni*, thrombotic meningoencephalitis, bovine disease, neuropathology, central nervous system, molecular diagnostics, histophilosis.

RESUMO.- [Meningoencefalite trombótica-induzida por *Histophilus somni* em bovinos da região norte do Paraná.] Meningoencefalite trombótica (*Thrombotic meningo-*

encephalitis- TME) é uma doença neurológica fatal de bovinos ocasionada por *Histophilus somni*. A infecção tem sido descrita predominantemente na América do Norte e de forma esporádica em outros países. O objetivo deste estudo é relatar a ocorrência de TME em bovinos da região norte do estado do Paraná, Brasil. A maioria dos animais apresentaram sinais clínicos neurológicos característicos de disfunção cerebral aguda. Análises hematológicas e do fluido cerebrospinal não foram sugestivas de infecção bacteriana do cérebro. A histopatologia revelou meningoencefalite com vasculite e trombose de pequenos vasos com discreto infiltrado neutrofílico e/ou linfocítico mesclada com fibrina no tronco e córtex cerebral e no gânglio do nervo trigêmeo de todos os animais. As amostras de sistema nervoso central incluídas nesse estudo foram previamente caracterizadas

¹ Received on October 3, 2014.

Accepted for publication on February 10, 2015.

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como negativas para raiva por meio de técnica de imunofluorescência direta. A participação de agentes infecciosos associados à doença neurológica em bovinos foi avaliada por técnicas moleculares como PCR e RT-PCR para amplificação parcial de genes de *H. somni*, *Listeria monocytogenes*, herpesvírus bovino 1 e 5, vírus da diarreia viral bovina e herpesvírus ovino 2. As seções do cérebro de todos os animais com diagnóstico histopatológico de TME foram positivas em PCR para a detecção do gene 16S rRNA de *H. somni*. O sequenciamento dos produtos amplificados confirmou a presença de DNA de *H. somni* nos fragmentos de cérebro avaliados. As reações de PCR/RT-PCR para todos os outros micro-organismos avaliados resultaram negativas. Os resultados desse estudo confirmaram a participação do *H. somni* nos episódios de doença neurológica observada nos animais avaliados, amplia a distribuição geográfica da TME e ratifica estudos prévios realizados no Brasil que demonstraram a presença de *H. somni* em outras formas de manifestação clínica das infecções por essa bactéria.

TERMOS DE INDEXAÇÃO: Meningoencefalite trombótica, *Histophilus somni*, doença de bovinos, neuropatologia, sistema nervoso central, diagnóstico molecular, histofilose.

INTRODUCTION

Histophilosis is a multisystemic disease entity of ruminants caused by the Gram-negative bacterium *Histophilus somni* (formerly known as *Haemophilus somnus*) which produces several clinical syndromes including thrombotic meningoencephalitis (TME), bronchopneumonia, pleuritis, polysynovitis, septicemia, myocarditis, otitis media, infertility, abortion, and mastitis (Inzana & Corbeil 2004, Pérez et al. 2010). Histophilosis has been a major problem of cattle in North America (Van Dreumel et al. 1970, MacDonald et al. 1973, George 2009) and Australia (Lancaster et al. 1984, Hick et al. 2012) for decades, resulting in severe economic loss to the affected livestock industry (Saunders et al. 1980). Additionally, there are sporadic reports of histophilosis from countries such as the UK (Wessels & Wessels 2005), Argentina (Descarga et al. 2002), and Japan (Tanaka et al. 2005). Recently, we have described histophilosis in cattle from different geographical regions of Brazil due to the characterization of systemic disease in five animals (Headley et al. 2013b), and reproductive manifestations have been confirmed in bovine fetuses (Headley et al. 2015). Further, we believe that histophilosis might be a threat to the beef cattle feedlot industry of Brazil and South America (Headley et al. 2014a), while *H. somni* is considered as an emerging disease pathogen of beef cattle feedlots in Argentina (Descarga et al. 2002).

TME is a fulminating neurological disease of cattle that occurs after septicemia induced by *H. somni* (George 2009), which produces fatality rates in affected herds and affects predominantly feedlot cattle relative to dairy or pastured animals (Fecteau & George 2004). Affected cattle can demonstrate lateral recumbency, profound depression, a sleep appearance (MacDonald et al. 1973; Fecteau & George 2004), head tilt, nystagmus, strabismus, blindness, coma, and convulsions (MacDonald et al. 1973, George 2009).

Gross neurological lesions are characteristic and visible in most untreated cases, and consist of 1 – 30 mm diameter hemorrhagic areas randomly distributed throughout the brain (Van Dreumel et al. 1970, Maxie & Youssef 2007, Zachary 2012). Histologically, TME is characterized by vasculitis and thrombosis, which predominantly affects small arteries, resulting in tissue necrosis with a marked neutrophilic response (Inzana & Corbeil 2004, Maxie & Youssef 2007, George 2009); thrombi frequently contains intraleisional bacterial colonies (Maxie & Youssef 2007, Zachary 2012). This paper presents the clinical, hematological, cerebrospinal fluid, and neuropathological findings observed in cases of *H. somni*-induced thrombotic meningoencephalitis from northern Paraná.

MATERIALS AND METHODS

Animals, clinical history, and necropsy. All affected cattle originated from several breeds, and between 1.5-6 years of age; the signalment and principal clinical manifestations of the affected cattle are given in Table 1. Further, these animals were part of an ongoing study that is investigating the cause of neurological disease in cattle from the state of Paraná. Most properties ($n=4$) were small subsistence farms, while herd n° 5 raised cattle for fattening. Cattle at all herds are routinely vaccinated against Foot and Mouth Disease and clostridiosis, maintained predominantly on green pastures, and supplemented with commercially prepared mineral salts; water was given *ad libitum*.

The first two cases occurred in late July 2013, with the other three cases occurring during August, September, and December 2013. All animals were depressed, but most ($n=4$) were in permanent recumbency, a few were blind ($n=2$), and with convulsions ($n=2$); only one animal was febrile (n° 1). The course of clinical manifestations until spontaneous death or euthanasia was acute in most cases ($n=4$), with variation of 2 to 8 days, but animal n° 2 was reportedly ill during 22 days. This animal was hospitalized due to a history of progressive emaciation and muscular weakness, and was maintained on supportive therapy. Additionally, most animals ($n=4$) were euthanized *in extremis*, with owners consent, due to the severity of clinical manifestations; animal n° 4 died spontaneously. Blood samples and cerebrospinal fluid (CSF) of all animals were collected before death for laboratory analyses.

Routine necropsies were performed soon after death; selected tissue fragments (brain, liver, lungs, kidneys, spleen, intestine, myocardium, and lymph nodes) from each animal necropsied were fixed by immersion in 10% buffered formalin solution and routinely processed for histopathological evaluation. Neurological tissue fragments of all animals were obtained from five pre-defined anatomical locations of the brain: brainstem, cerebrum, cerebellum, thalamus, and the trigeminal nerve ganglion and the carotid rete mirabile (TNG-CRM) complex. Duplicate sections of the organs mentioned above were collected freshly during necropsy, and maintained at -80°C until processed for molecular diagnostics. Additionally, to avoid cross contamination, necropsy equipment used for sample collection were cleaned and then immersed in a mixture of antiseptic and disinfectant solution between each collected sample.

This study adhered to the guidelines for the usage of animals/samples in studies as required by CONCEA (approved by CEUA/UEL protocol # 32340.2012.04).

Characterization of rabies virus. The direct immunofluorescence assay was performed for the identification of Lyssavirus from brain samples of all animals submitted to the Official Dia-

Table 1. Signalment, principal clinical manifestations, and outcome of cattle with thrombotic meningoencephalitis from the northern Paraná

Animal nº	Signalment, herd size, and geographical location	Principal clinical manifestations	Course (days)	Clinical outcome
1	Nellore, male, 1.5 years 50 cattle, Curiúva county	Blindness, depression, fever, flaccid anal sphincter, flaccid tail, loss of facial sensibility, loss of flexor reflex (all limbs), nystagmus, permanent lateral recumbency, tetraparesis, reduced tongue tonus	6	Euthanasia
2	Girolando, male, 1.5 years 30 cattle, Londrina	Depression, motor incoordination, muscular weakness, permanent lateral recumbency, progressive emaciation, respiratory difficulty, sleepy appearance	22	Euthanasia
3	Gir, male, 6 years 20 cattle, Londrina	Depression, flaccid tail, loss of flexor reflex (all limbs), permanent sternal recumbency	6	Euthanasia
4	Crossbreed, female, 4 years 3 cows, Londrina	Convulsions, depression left and right cervical movement, muscular weakness, permanent lateral recumbency, no menace reflex	8	Spontaneous death
5	Nellore, female, 2.5 years 250 cattle, Alto Paraná	Absence of cutaneous and palpebral reflexes, bilateral blindness, bruxism, convulsions, depression, flaccid tongue, opisthotonos	2	Euthanasia

gnostic Laboratory (Centro de Diagnóstico Marcos Enrietti; Curitiba, Paraná, Brazil).

PCR/RT-PCR characterization of infectious agents associated with neurological disease in cattle. Nucleic acids were extracted from frozen brain fragments (cerebrum, cerebellum, brainstem, TNG, and thalamus) of all animals as described (Boom et al. 1990), in association with Proteinase K (Ambion, Grand Island, NY, USA), and a modified method (Alfieri et al. 2006), and then used in PCR/RT-PCR assays designed to identify infectious agents associated with neurological disease of cattle from Brazil. These protocols targeted specific amplicons of the glycoprotein C gene of bovine herpesvirus type 1 (BoHV-1) and -5 (Claus et al. 2005), and the 5'-UTR region of the bovine viral diarrhea virus (Vilček et al. 1994), the listeriolysin gene of *Listeria monocytogenes* (Wesley et al. 2002), the 16s rRNA gene of *Histophilus somni* (Angen et al. 1998). The tegument protein gene of ovine herpesvirus type 2 (OvHV-2) was also targeted to detect DNA of sheep-associated malignant catarrhal fever, SA-MCF (Baxter et al. 1993).

Positive controls consisted of extracted nucleic acid from prototype strains of BoHV-1 (Los Angeles) and -5 (AA-Par) amplified in Madin-Darby bovine kidney cells (Claus et al. 2005); and field strains of previous cases of OvHV-2 (Headley et al. 2013c), *L. monocytogenes* (Headley et al. 2013a; Headley et al. 2014b), *H. somni* (Headley et al. 2013b), and OvHV-2 (Headley et al. 2013c). Nuclease-free water (Invitrogen Corp., Carlsbad, CA, USA) was used as negative controls in all PCR/RT-PCR assays. All RT-PCR/PCR products were separated by electrophoresis in 2% agarose gels, stained with ethidium bromide, and examined under ultraviolet light.

Sequencing and phylogenetic analyses. The amplified PCR products were then purified (illustra GFX PCR DNA and Gel Band Purification Kit, GE Healthcare, Little Chalfont, Buckinghamshire, UK) and submitted for direct sequencing using the forward and reverse primers. The obtained sequences were examined for quality analysis of chromatogram readings by using the PHRED software (<http://asparagin.cenargen.embrapa.br/phph>); sequences were only accepted if base quality was equal to or greater than 20. Consensus sequences were then generated by the CAP3 program (<http://asparagin.cenargen.embrapa.br/cgi-bin/phph/cap3.pl>), after which the partial nucleotide sequences obtained were compared by the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>) with similar sequences deposited in GenBank to confirm sequence identity and validate the isolates identified. The partial

nucleotide sequences of *H. somni* obtained during this investigation were deposited at GenBank.

Phylogenetic trees and sequence alignments were created by using MEGA 6.01 software (Tamura et al. 2011), constructed by the Neighbor-Joining method, based on 1,000 bootstrapped data sets of the nucleotide sequences of selected strains of the 16S rRNA gene of *Pasteurellaceae* family; distances values were calculated by using the Kimura 2 parameter model.

RESULTS

Hematological and biochemical analyses of blood and cerebrospinal fluid

Overall CSF analysis from all animals did not indicate definite bacterial infections, but there were variations to the complete blood cell count (CBC) of these animals. Hematological analyses revealed (Table 2) neutrophilia in all animals; hyperfibrinogenemia (animals nº 3 and 4), and leucocytosis (nº 2) were observed. Significant biochemical abnormalities included elevated urea concentration (nº 1 and 5), with increased enzymatic activity of creatine kinase (nº 1, 3-5), aspartate aminotransferase (nº 1-4), and alkaline phosphatase (nº 1, 2 and 5). CSF alterations were observed only in animal nº 5 and consisted of hyperproteinorraquia and mononuclear pleocytosis (Table 3).

Pathological findings

The most constant gross neurological alterations observed in these animals were moderate congestion of meningeal vessels ($n=3$), shallow sulci with distended gyri ($n=2$), and foci of hemorrhage at the cerebral cortex of animal nº 4 (Fig. 1A-B); significant pathological findings were not observed in animal nº 5 (Table 4).

Histologically, thrombi occurred predominantly within small vessels of the meninges and in all regions of the brain (Fig. 1C-E) of all animals investigated; the distribution of TME within the brain sections of these animals is given in Table 4. Briefly, TME was observed at the cerebral cortex ($n=5$), cerebellar white matter ($n=2$), grey and white matter of the thalamus ($n=3$) and brainstem ($n=5$), and at the TNG-

Table 2. Hematological and biochemical findings of cattle with thrombotic meningoencephalitis

Laboratory Parameters	Reference values	Animals n ^o				
		1	2	3	4	5
Hemogram						
Packed Cell Volume (%)	27 – 48	42.8	36.8	44.1	42.1	38.0
Red Blood cells (x10 ⁶ /μL)	6 – 11.6	8.51	8.20	7.88	7.6	7.97
Hemoglobin (g/dL)	8.50 – 16.5	11.2	11.1	11.1	10.7	12.6
Total plasma protein (g/dL)	6.6 – 7.8	7.8	7.0	8.0	9.8	7.4
Fibrinogen (mg/dL)	300 – 700	400	400	1,000	800	600
Leucocytes (x10 ³ /μL)	6,200 – 12,200	10,100	14,200	8,800	7,600	7,000
Lymphocytes (x10 ³ /μL)	3,300 – 8,000	4,141	5,112	1,496	1,672	2,030
Eosinophils (x10 ³ /μL)	0 – 2,400	0	0	0	0	0
Monocytes (x10 ³ /μL)	200 – 700	0	284	0	0	0
Bastonetes (x10 ³ /μL)	0 – 200	0	0	88	304	0
Segmented neutrophils (x10 ³ /μL)	1,300 – 3,400	5,959	8,804	7,216	5,624	4,970
MCV (fL)	30.5 – 55.5	50.3	44.9	56.0	55.5	47.6
MCHC (%)	28 – 38	26.1	30.1	35.1	25.4	34.0
MCH (pg)	9.7 – 19	13.1	13.5	14.0	14.0	15.8
Serum biochemistry						
Albumin (g/dL)	2.7 – 4.3	2.3	1.5	2.6	2.3	2.6
Creatinine (mg/dL)	1 – 2.7	2.7	0.60	1.5	1.2	2.3
Urea (mg/dL)	5.8 – 35.9	111	15	35	39	70
Creatine kinase, CK (UI/L)	35 – 280	952	136	4,117	4,164	5,676
Aspartate aminotransferase, AST (UI/L)	32 – 71	207	88	962	399	ND
Gamma-glutamyl transferase GGT (UI/L)	3.7 – 31.3	34	45	23	41	30
Alkaline phosphatase, ALP (UI/L)	33 – 100	256	160	ND	ND	198
Total bilirubin (mg/dL)	0.32 – 0.74	0.47	0.55	0.54	0.22	0.47
Conjugated bilirubin (mg/dL)	0.01 – 0.34	0.35	0.35	0.12	0.05	0.06
Unconjugated bilirubin (mg/dL)	0.16 – 0.67	0.12	0.20	0.42	0.17	0.41

ND = not determined.

Table 3. Cerebrospinal fluid analyses of cattle with thrombotic meningoencephalitis

Parameters	Reference values	Animals				
		1	2	3	4	5
Color	Uncolored	Uncolored	Uncolored	Uncolored	Uncolored	Uncolored
Aspect		Clear	Clear	Clear	Clear	Clear
Protein (mg/dL)	40	39.3	21.6	19.9	17.7	133.7
Density	>1,010	1,004	1,008	1,006	1,006	1,004
Total nucleated cell count (mm ³)	10	2	0	0	2	14
Segmented neutrophils (%)		0	0	0	0	28
Band neutrophils (%)		0	0	0	0	0
Mononuclear cells (%)		0	0	0	0	72

-CRM complex ($n=5$). Thrombi consisted of discrete intravascular accumulations of neutrophils and/or lymphocytes admixed with fibrin and moderate to marked proliferation of the affected vascular endothelium; intralesional bacteria were not frequently observed and there was no associated perivascular inflammatory exudate. Additionally, varying degrees of cortical edema, gliosis, and hemorrhage were observed in the brain sections evaluated. Other significant non-neurological pathological alterations included thrombi within small vessels of the lung (Fig.1F), myocardium (n^o 2 and 5), liver and intestine (n^o 5).

PCR, sequencing, and phylogenetic analysis

The *H. somni* PCR successfully amplified bacterial DNA from the brain of all animals with histopathological dia-

gnosis of TME (Table 4). Bacterial DNA was amplified from multiple sections of the brain from all animals, except n^o 2, who had *H. somni* DNA identified only from the trigeminal nerve ganglion. In summary, *H. somni* DNA was amplified from the TNG-CRM complex ($n=5$), thalamus ($n=4$), the brainstem and cerebral cortex ($n=3$), and cerebellum ($n=2$) of affected animals. All other PCR/RT-PCR assays provided negative results and all animals were negative for rabies virus by the immunofluorescence assay.

Direct sequencing revealed the desired 408 base pairs of the partial nucleotide sequence of the *H. somni* 16 rRNA gene from the TNG-CRM complex (animals n^o 1, 2, and 4), cerebral cortex (n^o 3), and the brainstem (n^o 5). Initial BLAST analyses revealed that all nucleotide sequences (GenBank Accession n^o KM374589, animal n^o 1; KM374590, n^o 2; KM374591, n^o 3; KM374592, n^o 4; and KM374593, n^o 5) derived from this study demonstrated 99-100% identity with similar sequences deposited in GenBank. Further, the generated phylogenetic tree revealed that all isolates of *H. somni* formed a large cluster, which was distinct from other members of the *Pasteurellaceae* family (Fig. 2).

DISCUSSION

The histopathological findings observed within several brain sections of these animals are consistent with those described in *H. somni*-induced TME of ruminants (Fecteau & George 2004; Maxie & Youssef 2007; George 2009), and similar to the brain lesions previously described in cases of systemic (Headley et al. 2013b) and reproductive (Headley et al. 2015) histophilosis from Brazil. Additionally, the amplification and consequent sequencing of *H. somni* from these

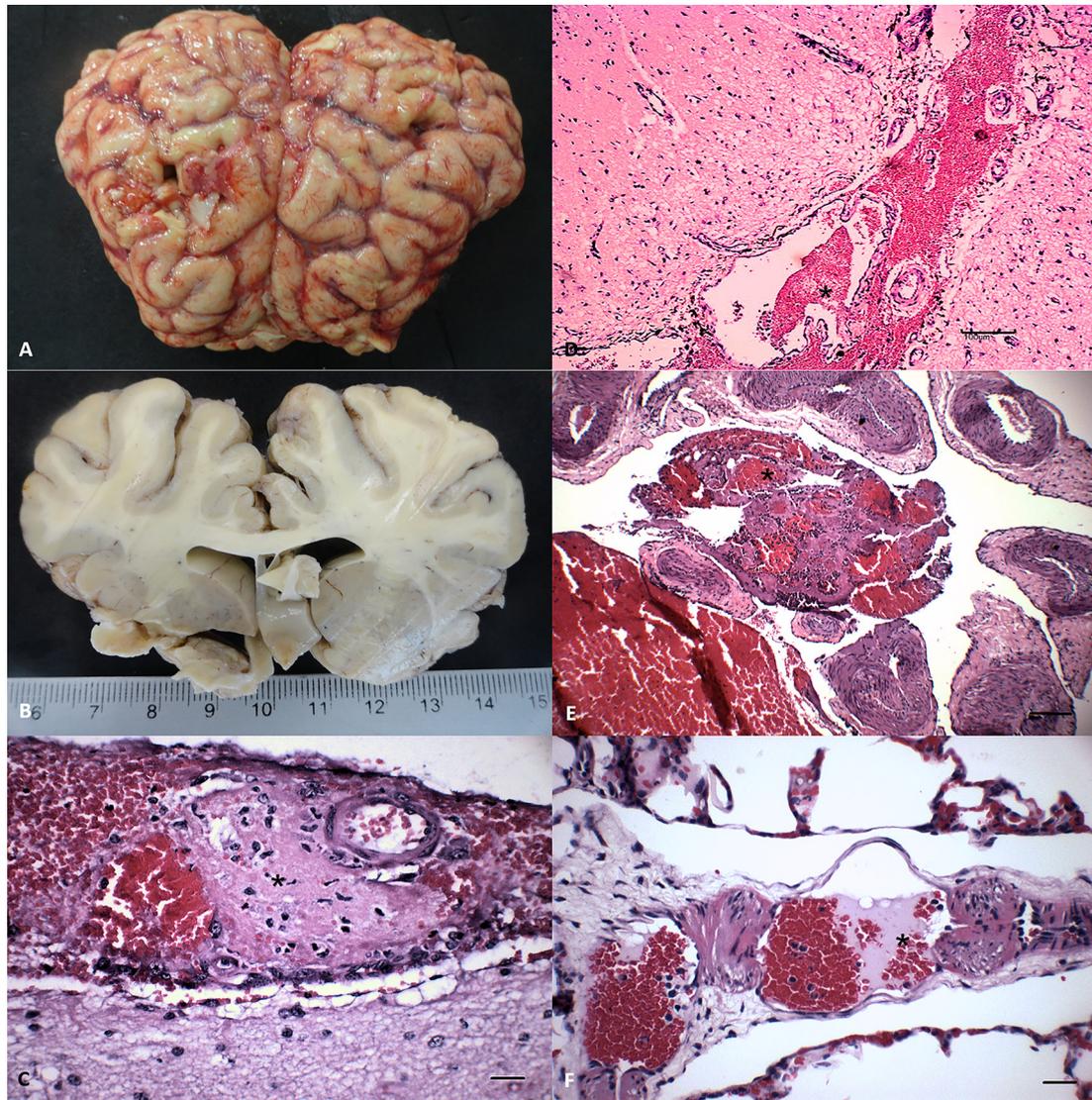


Fig.1. Gross and histopathological features of *Histophilus somni* induced thrombotic meningoencephalitis in cattle from northern Paraná. (A) Congestion of meningeal vessels and hemorrhage with distended gyri and shallow sulci. (B) Edema of the sectioned surface of the cerebrum. There are thrombi (*) at the meninges (C-D), carotid rete mirabile (E), and the lungs (F). (B) Scale in centimeters. (C-F) Hematoxylin and eosin stain. (C and F) Bar = 20µm. (D-E) 100µm.

Table 4. Principal gross findings with the distribution of thrombotic meningoencephalitis and *Histophilus somni* DNA in brain sections of cattle

Animal n ^o	Gross findings	Thrombotic meningoencephalitis					<i>Histophilus somni</i> DNA				
		Brain-stem	Cerebellum	Cerebrum	TNG-CRM	Thalamus	Brain-stem	Cerebellum	Cerebrum	TNG-CRM	Thalamus
1	Congestion of meningeal vessels; myocardial and endocardial petechial, hemorrhage, ulcerativeabomastitis	+	-	+	+	-	+	+	+	+	+
2	Cerebrum; shallow sulci with distended gyri; congestion of meningeal vessels, hydropericardium, hyperemia of intestinal mucosa	+	+	+	+	+	-	-	-	+	-
3	Congestion of meningeal vessels, pulmonary emphysema, and atelectasis, ulcerative abomastitis	+	-	+	+	+	+	-	+	+	+
4	Ascites, cerebrum: shallow sulci with distended gyri, cranioventral consolidation of lungs, enlarged liver, hydrothorax, renal infarction, tongue and oesophagus: ulcerations	+	-	+	+	-	-	+	-	+	+
5	Significant pathological alterations were not observed	+	+	+	+	+	+	-	+	+	+

TNG-CRM = Trigeminal nerve ganglion-carotid rete mirabile complex, + present, - absent.

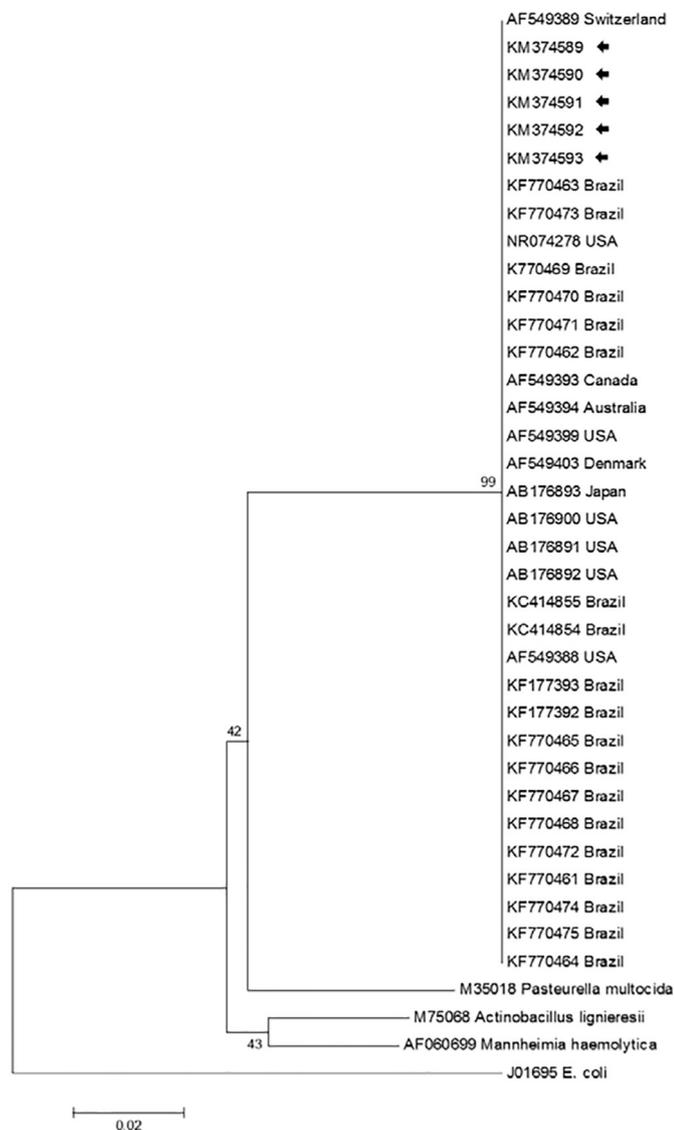


Fig.2. The phylogenetic relationship of selected strains of *Histophilus somni* based on the 16S rRNA gene of the *Pasteurellaceae* family. The isolates from this study are highlighted (arrows); the GenBank accession numbers and country of origin of the sequences used are given. *E. coli* was used as the out-group.

areas, suggest that this bacterium was associated with the neuropathological lesions; the amplification of *H. somni* DNA and sequencing were previously used to identify this pathogen (Angen et al. 1998, Headley et al. 2013b, 2015). Moreover, the negative PCRs and RT-PCRs results with the absence of other histopathological disease patterns suggest that other common pathogens associated with neurological disease of cattle were not involved in the development of the clinicopathological manifestations observed during this investigation. Of extreme importance was the negative PCR assay for OvHV-2, since this is a common cause of neurological disease of cattle from Brazil (Headley et al. 2012, 2013c). Additionally, in the cases from this study, lesions at the carotid rete mirabile were thrombotic and not the characteristic fibrinoid necrosis frequently diagnosed in SA-MCF (Headley et al. 2012, 2013c).

The clinical manifestations, the acute incubation period culminating in spontaneous death or euthanasia, and the reduced number of animals affected at each herd during this study are frequent clinical and epidemiological findings associated with TME (MacDonald et al. 1973, Fecteau & George 2004, George 2009). During this investigation, bacterial isolation was not attempted in any instance since diagnoses of TME were only achieved after histopathological evaluation. It is worthy to mention that the hematological and more specifically, the CSF findings were not indicative of classical bacterial infections of the brain (Scott 2004, Stokol et al. 2009). Although the CBC in cases of TME is nonspecific, the CSF findings normally demonstrate alterations indicative of bacterial disease with an increase in the number of nucleated cells, elevated protein concentrations (Van Dreumel et al. 1970, Fecteau & George 2004, Scott 2004), xanthochromia, and hemorrhage (Scott 2004). Alterations to CSF levels were only observed in animal n^o5 but were indicative of hyperproteinorraquia and lymphocytic pleocytosis, without xanthochromia and hemorrhage. Alternatively, a retrospective study that classified the CSF relative to neurological disease in 101 cattle, observed marked variation in the three cases of TME described, with pleocytosis being neutrophilic, lymphocytic, and histiocytic (Stokol et al. 2009). Interestingly, the biochemical alterations observed in most animals are due to prolonged recumbency. However, the results of the CSF presented in this study are important for the ante-mortem diagnosis and the understanding of TME, since there are few documentations of the alterations associated with CSF in spontaneous cases of neurological histophilosis and the classical representation of CSF alterations in TME is not fully known (Scott 2004).

The distribution of TME was predominant at the brainstem, cerebral cortex, and the TNG-CRM complex of all animals, while these lesions were not consistent at the cerebellum and thalamus. However, *H. somni* DNA was recovered more frequently from the TGN-CRM complex, followed by the thalamus ($n=4$), cerebral cortex, brainstem ($n=3$), and cerebellum ($n=2$). This might suggest that there is no definite correlation between the demonstration of TME in a particular anatomical region of the brain and the amplification of *H. somni* DNA from these areas. Nevertheless, histopathological lesions of TME seem to have predilection for the cerebral cortex (MacDonald et al. 1973, Maxie & Youssef 2007, Zachary 2012) and thalamus (Maxie & Youssef 2007), but can be randomly distributed throughout any part of the brain (MacDonald et al. 1973). These findings are in agreement with the distribution of TME observed during this investigation, and suggest that fragments of multiple anatomical regions of the brain must be collected to obtain an accurate histopathological diagnosis of TME, particularly in non-conventional areas of this disease. Additionally, since histopathological confirmation and the amplification of *H. somni* DNA was constant from the TGN-CRM complex of all animals, this neuroanatomical region, of the brain, particularly the carotid rete mirabile, should always be investigated when TME is suspected.

The identification of *H. somni* DNA from these cases with typical histopathological findings of TME represent the first successfully characterization of this important neurological disease of cattle from Brazil, and definitely extends the geographical distribution of this disease and pathogen. This is because histophilosis was previously described by our group in cattle from southern Brazil (Headley et al. 2013b), while the bacterium has been associated with steers that demonstrated pulmonary distress at a feedlot (Headley et al. 2014a), and in sheep with endometritis (Rizzo et al. 2012). In addition, *H. somni* has been linked to abortions in cattle from different geographical regions of Brazil (Headley et al. 2015). Further, *H. somni* DNA was identified from the bronchialveolar lavage of cattle with and without pulmonary discomfort (Oliveira et al., manuscript in preparation), and in a lamb with omphalitis (Headley et al., manuscript in preparation). Interestingly, vaccination of cattle against *H. somni* is not a routine practice in beef and dairy cattle herds of Brazil, so additional cases of histophilosis are likely to occur nationwide. Consequently, this disease might be a possible threat to the local cattle industry (Headley et al. 2014a), resulting in serious economic losses as have been documented in North America (Saunders et al. 1980). However, since the distribution of *H. somni* in cattle herds of Brazil is unknown, seroepidemiological studies are being implemented to determine the prevalence of this pathogen in beef and dairy herds.

CONCLUSIONS

Histophilus somni DNA was identified from brain fragments of cattle with neuropathological manifestations and histopathological lesions that are consistent with those described in TME of cattle from North America.

Further, sequencing of the *H. somni* 16S rRNA gene from affected brain fragments of all animals confirmed the participation of this pathogen in the lesions herein described.

These results contribute to the documentation of this important emerging infectious disease of cattle from Brazil and South America, complements previous findings of histophilosis by our group (Headley et al. 2013b, 2014a, 2015), and produces novel CSF findings associated with spontaneous cases of TME.

Acknowledgements.- Selwyn A. Headley, Ana Paula F.R.L. Bracarense, Alice F. Alfieri, Júlio A.N. Lisboa, and Amauri A. Alfieri are recipients of the National Council for Scientific and Technological Development (CNPq, Brazil) fellowships. This study was partially funded by a CNPq grant (Proposal #448797/2014-3) and a joint CNPq/Ministry of Agriculture (MAPA) grant (Protocols #578645/2008-4 and #478254/2012-1).

The authors contributed for publication of the article as follows:

SAH and AAA: conceived and designed the study;
SAH, APFRB and WO: were involved in the pathological evaluations and interpretations;
SAH, VHSO, AFA and AAA: participated in the molecular analyses and interpretations;
GRQ and JANL: did the clinical and neurological examinations and visited the farms;
KKMC and JANL: were responsible for the clinical laboratory investigation;
All authors participated in the drafting of the manuscript, read, and approved the final version.

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