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Daniel Dourado Guerra Segundo

HELICOBACTER SPP. NA SALIVA E MUCOSA GÁSTRICA DE CÃES DOMÉSTICOS COM E SEM VÔMITO CRÔNICO NA REGIÃO CENTRAL DO RIO GRANDE DO SUL

Santa Maria, RS
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Dissertação apresentada ao Curso de Pós-Graduação em Medicina Veterinária, Área de Cirurgia e Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Medicina Veterinária**

Orientador: Prof. Dr. Saulo Tadeu Lemos Pinto Filho

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Saulo Tadeu Lemos Pinto Filho, Prof. Dr. (UFSM)
(Presidente/Orientador)

Paula Cristina Basso, Dra. (UFSM)

Anelise Bonilla Trindade-Gerardi, Profa. Dra. (UFRGS)

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RESUMO

HELICOBACTER SPP. NA SALIVA E MUCOSA GÁSTRICA DE CÃES DOMÉSTICOS COM E SEM VÔMITO CRÔNICO NA REGIÃO CENTRAL DO RIO GRANDE DO SUL

AUTOR: DANIEL DOURADO GUERRA SEGUNDO
ORIENTADOR: SAULO TADEU LEMOS PINTO FILHO

Helicobacter pylori são bactérias espiraladas frequentemente detectadas no estômago de humanos e que estão atribuídas à patogênese de doenças do trato digestório superior. Em animais domésticos, a presença de *Helicobacters* não-*Helicobacter pylori* (HNHP) também é frequente e tem sido alvo de diversos estudos devido à sua importância zoonótica. Este estudo teve o objetivo de investigar a ocorrência de *Helicobacter* spp. em amostras de saliva e mucosa gástrica de cães. Além disso, foram avaliadas a densidade de colonização bacteriana gástrica, presença de lesões gástricas e buscamos identificar os principais grupos filogenéticos de *Helicobacter* spp. em cães domésticos da região central do Rio Grande do Sul. Este estudo incluiu a coletas de amostras de 20 cães e as análises empregas foram a citologia, histopatologia, reação em cadeia da polimerase, teste rápido da urease e análise filogenética. A *Helicobacter* spp. foi presente na mucosa gástrica de 17 e na saliva de 3. Todos os cães assintomáticos foram positivos para a *Helicobacter* spp., enquanto 2 cães com vômitos crônicos foram negativos. Quanto à densidade de colonização bacteriana gástrica, 3 dos cães apresentaram escore médio, 6 escore moderado e 8 animais apresentaram escore severo. Infiltrado linfocítico-plasmocítico, foi a principal lesão gástrica observada. Entretanto, a presença da *Helicobacter* e a sua densidade, pareciam não estar relacionadas às lesões gástricas encontradas. As amostras possuíram uma alta identidade nucleotídica com sequências notavelmente semelhantes entre algumas espécies de HNHP como a *H. heilmannii s.s.*, *H. salomonis*, *H. felis* and *H. bizzozeronnii*. Nestes cães estudados, alta densidade bacteriana, taxa de ocorrência e predominância de HNHP de importância zoonótica foram encontradas no estômago, com uma baixa ocorrência de *Helicobacter* spp. na saliva. A saliva de cães domésticos, mesmo daqueles sem colonização gástrica, pode causar infecção por *Helicobacter* em humanos e outros animais.

Palavras-Chave: Brasil. Cães. Mucosa Gástrica. *Helicobacter*. Saliva.

SUMMARY

HELICOBACTER SPP. IN SALIVA AND GASTRIC MUCOSA OF DOMESTICS DOGS WITH AND WITHOUT CHRONIC VOMITING IN CENTRAL REGION OF RIO GRANDE DO SUL

AUTHOR: DANIEL DOURADO GUERRA SEGUNDO
ADVISER: SAULO TADEU LEMOS PINTO FILHO

Helicobacter pylori is a spiral-shaped bacterium frequently detected in the stomach of humans and plays a role in the pathogenesis of upper digestive tract diseases. In domestic animals, non-*H. pylori Helicobacter* species (NHPH) have also been discovered, which are frequently studied because of their zoonotic importance. This study aimed to investigate the occurrence of *Helicobacter* spp. in saliva and gastric samples from dogs. Furthermore, we evaluated the gastric bacterial colonisation density, presence of gastric lesions and sought to identify the main phylogenetic groups of the *Helicobacter* spp. isolated from domestic dogs in the central region of Rio Grande do Sul. This study included 20 dogs and the analysis employed were cytology, histopathology, polymerase chain reaction, rapid urease testing and phylogenetic analysis. *Helicobacter* spp. was present in the gastric mucosa of 17 of the dogs and saliva of 3 dogs. All asymptomatic dogs were positive for *Helicobacter* spp., whereas 2 dogs with chronic vomiting were negative. Density analysis of the gastric bacterial colonisation revealed that 3, 6 and 8 dogs had a mild, moderate and severe infection, respectively. Lymphocytic–plasmacytic infiltrates were the primary type of gastric lesions observed. However, the presence of *Helicobacter* and the density appeared to be unrelated to the gastric lesions found. The samples possessed a high nucleotide identity with remarkably similar sequences among some of the species of NHPH such as *H. heilmannii s.s.*, *H. salomonis*, *H. felis* and *H. bizzozeronnii*. In the dogs, increased density, occurrence rate and predominance of NHPH of zoonotic importance were found in the stomach with a lower occurrence of *Helicobacter* spp. in the saliva. The saliva of domestic dogs, even of those without gastric colonisation, can cause *Helicobacter* infection in humans and other animals.

Keywords: Brazil, Dogs, Gastric Mucosa, *Helicobacter*, Saliva

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1 INTRODUÇÃO

1.1 ETIOLOGIA

As *Helicobacter* ssp. são bactérias gram-negativas, microaerófilas, móveis, de formato espiralado (VANDAMME; GOOSSENS, 1992; LECOINDRE et al., 2000; CLYNE; DOLAN; REEVES, 2007) e que possuem o tropismo pela mucosa gástrica de humanos, cães, gatos e diversas outras espécies de mamíferos (NEIGER et al., 1998; FOX; FOX, 2012). Segundo alguns autores, a última caraterística supracitada, é decorrente da alta adaptação ao ambiente adverso do estômago, que favorece a sua presença e multiplicação no baixíssimo pH encontrado no lúmen deste órgão. Os mesmos pressupõem, que isto tornou-se possível devido ao mecanismo de sobrevivência oriundo da sua evolução genética, no qual a permite captar fontes de ureia por difusão através de canais UreI ativados pelo baixo pH estomacal, que uma vez adentrada no citoplasma bacteriano, é convertida em amônia e CO2 pela ação das ureases citoplasmáticas, consequentemente elevando o pH e permitindo o seu desenvolvimento e proliferação neste nicho gástrico (WEEKS et al., 2000; CLYNE; DOLAN; REEVES, 2007; FOX, 2012). Em pH neutro, a UreI não é ativada, a fim de evitar a alcalinização do ambiente gástrico, visto que o elevado pH alcalino é letal à bactéria (CLYNE; LABIGNE; DRUMM, 1995). Além disto, estas bactérias possuem enzimas que desestabilizam a estrutura oligomérica da mucina presente na camada muco protetora gástrica (WINDLE et al., 2000), permitindo a sua penetração, proteção dos ácidos gástricos e movimentação no interior desta estrutura, sendo um segundo mecanismo que permite a fuga do baixo pH (CLYNE; DOLAN; REEVES, 2007).

A primeira espécie do gênero *Helicobacter* ssp. a ser submetida com sucesso à cultura, identificada e nomeada, foi a *Helicobacter pylori* (*H. pylori)*, obtida de biópsias gástricas de pacientes humanos com úlceras pépticas (MARSHALL; WARREN, 1984). Anos mais tarde, outra bactéria espiralada, isolada da mucosa gástrica humana e com características morfológicas diferenciadas da *H. pylori* e semelhantes à bactérias espiraladas encontradas na mucosa gástrica de animais domésticos, foi documentada (DENT et al.,1987), passando a ser denominada como *Gastrospirillum hominis* (MCNULTY et al., 1989) e posteriormente por consenso, nomeada como *Helicobacter heilmannii* (*H. heilmannii*) (SOLNICK et al., 1993). Na década seguinte, após estudos filogenéticos utilizando 16S rRNA como gene alvo, foi descoberto que a *H. heilmannii* pertencia à uma gama de diferentes espécies de *Helicobacter* spp., sendo estas divididas em duas subclassificações, denominadas como "*H. heilmannii* tipo 1 e tipo 2"

(HAESEBROUCK et al., 2009). O tipo 1 sendo composto unicamente pela *Helicobacter suis* (*H. suis*), isolada do estômago de suínos (DE GROOTE et al., 1999; O'ROURKE et al., 2004), enquanto que o tipo 2, composto pela *Helicobacter felis* (*H. felis*), *Helicobacter bizzozeronii* (*H. bizzozeronii*), *Helicobacter salomonis* (*H. salomonis*), *H. heilmannii s.s.*, *Helicobacter cynogastricus* (*H. cynogastricus*) e *Helicobacter baculiformis* (*H. baculiformis*), obtidas de isolados da mucosa gástrica de cães e gatos (O'ROURKE et al., 2004; HAESEBROUCK et al., 2009). Com o propósito de organizar as nomenclaturas, este grande grupo de bactérias incluídas nas sub-classificações das *H. heilmannii* tipos 1 e 2, passaram a ser denominados como *Helicobacter heilmannii sensu lato* (*H. heilmannii s.l.*), para se referir às espécies de *Helicobacter ssp.* não-*H. pylori* (HNHP) (HAESEBROUCK et al., 2011).

1.2 EPIDEMIOLOGIA

Cães e gatos são hospedeiros naturais de espécies de HNHP, sendo comumente identificadas a *H. felis*, *H. bizzozeronii*, *H. salomonis*, *H. heilmanni* s.s., *H. bílis*, *H. rappini* (THOMSON et al., 1994; EATON et al., 1996; HANNINEN et al., 1996; JALAVA et al., 1999; LEE et al., 1998; ASL et al., 2010) e *H. pylori* (FOX, 1995; JANKOWSKI et al., 2016). Segundo os estudos de alguns autores, estas bactérias apresentam a prevalência de 67-86% em cães com vômitos crônicos e de 61-100% em assintomáticos, havendo uma maior predominância em cães sem sinais clínicos, fato que leva à suposições discordantes sobre significância patológica destas bactérias na espécie canina (2 et al., 1993; HERMANNS et al., 1995; LECOINDRE et al., 2000; HWANG; HAN; YOUN, 2002; SIMPSON, 2005; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013). Embora alguns autores cogitem que esta menor ocorrência em animais sintomáticos destes estudos, estejam relacionadas ao uso recente antibióticos antecedendo a coleta de amostras gástricas (NEIGER; SIMPSON, 2000).

Frente à estas suposições e centenas de estudos existentes, atualmente ainda é um amplo mistério a significância desta infecção e a sua relação ao desencadeamento dos mecanismos patológicos das gastrites em animais domésticos (NEIGER et al., 1998; LECOINDRE et al., 2000; NEIGER; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013).

A *H. pylori*, que tem como hospedeiro natural o homem, já foi eventualmente identificada em cães e gatos (FOX et al., 1996; DUBOIS, 1998; NEIGER et al., 1998; HAESEBROUCK et al., 2009; ABDI et al., 2014; KUBOTA-AIZAWA et al., 2017). Modelos experimentais de felinos naturalmente infectados por *H. pylori* durante um longo período,

apresentaram o desenvolvimento de gastrites crônicas, incluindo alterações microscópicas como gastrite atrófica, aumentos nos níveis de proliferação e apoptose (STOLTE et al., 1994; FOX et al., 1995; ESTEVES et al., 2000; SIMPSON et al., 2001). Desta forma revelando o potencial que a *H. pylori* possui para desencadear ações patogênicas em animais domésticos (STOLTE et al., 1994; FOX et al., 1995; ESTEVES et al., 2000; SIMPSON et al., 2001) e surgindo a possibilidade de ser uma antroponoses (infecção animal decorrente de um patógeno humano) (EL-ZAATARI et al., 1997).

Diante de algumas evidências, sugere-se que o modo de criação dos animais exerce uma grande influência na ocorrência de *Helicobacter* spp., visto que populações caninas que vivem em grande aglomeração ou confinamento (biotérios), apresentaram 99-100% de prevalência comparado à cães domésticos (EATON et al., 1996; SOUZA et al., 2004; LANZONI et al., 2011; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013). Supõe-se que o mecanismo de transmissão possa ocorrer pela via oral-oral e fecal-oral, pois a sua detecção por análises moleculares já foi obtida por meio de amostras salivares e fecais (RECORDATI et al., 2007; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013; JANKOWSKI et al., 2016).

1.3 PATOGÊNESE

Na medicina humana, devido à grandes avanços de estudos acerca da *Helicobacter pylori*, já é concretizado que os mecanismos de patogenicidade deste agente estão atrelados à citocinas pró-inflamatórias como interleucina (IL)- 1b, IL-6, IL-8 (NOACH et al., 1994), proteínas de membrana, lipopolissacarídeos, fatores de virulência CagA e VacA, secreções de fosfolipases, mucinase e hemolisinas MONTEIRO, 1995; TORRES; BACKERT, 2008; BACKERT et al., 2016). Além da indução de gastrites auto-imunes por meio do estímulo da produção de anticorpos específicos para antígenos de Lewis, que comumente constituem estruturas das células parietais do epitélio gástrico (MONTEIRO, 1995; TORRES; BACKERT, 2008; BACKERT et al., 2016).

Entretanto, diferente da *H. pylori* na espécie humana, a patogenicidade das HNHP em cães ainda é um amplo mistério, devido as discordâncias existentes entre diferentes estudos nestas espécies. Aonde em alguns não foram observadas diferenças significativas quanto a presença destas bactérias ou grau de densidade de infecção em cães sintomáticos e assintomáticos (NEIGER et al., 1998; LECOINDRE et al., 2000; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013; SUÁREZ-ESQUIVEL et al., 2017) e em contrapartida, em outros

havendo relações significativamente positivas (HERMANNS et al., 1995; AMORIM et al., 2015; KUBOTA-AIZAWA et al., 2017).

Alguns autores sugerem que a patogenicidade destas bactérias dependa da espécie, virulência da cepa, e a capacidade de colonizar e criar um nicho ecológico no estomago do hospedeiro (LECOINDRE et al., 2000). Já outros pesquisadores, acreditam que as HNHP atuem simplesmente como um agente patobionte (CHOW; TANG; MAZMANIAN, 2011).

Em observações histológicas da mucosa gástricas de cães e gatos infectados com *Helicobacter* spp., são frequentemente relatados infiltrados linfoplasmocíticos e eventualmente infiltrados neutrofílicos, eosinofílicos, múltiplos folículos linfoides (GEYER et al, 1993; OTTO et al., 1994; HERMANNS et al., 1995; HAPPONEN et al., 1996; DAY et al., 2008; TAKEMURA et al., 2009; FOX, 2012; AMORIM et al., 2015; SUÁREZ-ESQUIVEL et al., 2017), redução da produção de mucina, aumento da proliferação epitelial, degeneração, nidificação e atrofia das glândulas gástricas (TAKEMURA et al., 2009; FOX, 2012).

Entretanto, devido à estas intensas discordâncias, a recomendação para os clínicos é que antes de se ter em mente a *Helicobacter* spp. como causa primária da alteração gastrointestinal em cães e gatos infectados, é necessário excluir outras possíveis causas (intolerância alimentar, doença intestinal inflamatória ou parasitismo) para então ser instituída uma terapia antimicrobiana (NEIGER; SIMPSON, 2000; SIMPSON, 2005).

1.4 SINAIS CLÍNICOS

Os sinais clínicos relacionados à animais domésticos infectados pelas *Helicobacter ssp.*, são episódios de vômitos (contendo muco, fluídos gástricos ou bile), diarreia crônica e em casos mais infrequentes melena, anemia, febre, perda de peso e grave emaciação (HERMANNS et al., 1995; FOX, 2012).

1.5 DIAGNÓSTICO

Os meios mais comuns e aplicados para a detecção da *Helicobacter* spp., têm sido a avaliação histopatológica da mucosa gástrica, utilizando corantes de Whartin-Starry, giemsa ou hematoxilina e eosina, citologia por imprint das amostras gástricas utilizando corantes de Giemsa e o kit de teste para urease (LECOINDRE et al., 1997; KANJI; SUEMATSU; TAKAHASHI, 1998; NEIGER et al., 1998; NEIGER; SIMPSON, 2000; LECOINDRE et al., 2000; TABRIZI et al., 2010; ASL et al., 2010; FOX, 2012). A análise histopatológica é referência nos métodos de estudo diagnóstico em animais, pois é capaz de oferecer 100% de especificidade e 90% de sensibilidade (LECOINDRE et al., 2000). A análise citológica por imprint da amostra gástrica é um método menos dispendioso e que oferece concordância de 100% com a histopatologia, além de permitir o uso da mesma amostra para avaliação histopatológica (MISRA et al., 1998; ÇUBUKÇU et al., 2000). O kit de teste para urease é simples, rápido e oferece cerca de 70%-90% de sensibilidade (JENKINS, 1997; NIEGER et al., 1998).

As análises de isolamento e cultura de alguns subtipos como *H. heilmannii s.s*, *H. felis* e outras *Helicobacter ssp.*, demonstram ser muito laboriosas e onerosas a fim de se obter o êxito, uma vez que necessitam de ambientes (5 % de oxigênio, 10% de carbono e 85% de nitrogênio), meios de culturas, antimicrobianos e temperaturas especiais para garantir seu crescimento (JALAVA et al., 1998; FOX, 2012). Desta forma, impõe muitas dificuldades, pois se expostas à tensão de oxigênio de 20% em uma pressão atmosférica normal, as bactérias entram em colapso e sucumbem (JALAVA et al., 1998; FOX, 2012). Em um estudo com 60 culturas gástricas preparadas de caninos, exclusivamente em 17 foi possível se obter crescimento, porém, em somente uma destas foi possível ser realizado o isolamento (ASL et al., 2010). Em outro estudo com culturas gástricas de felinos, 58 culturas foram preparadas e somente um isolamento foi possível de ser realizado (NIEGER et al., 1998). Devido à estas grandes dificuldades, as técnicas de cultura e isolamento não têm sido muito empregadas em estudos e tão pouco na rotina clínica (JALAVA et al., 1998).

A microscopia eletrônica permite a distinção de somente algumas espécies, portanto, não sendo um método fidedigno (NEIGER; SIMPSON, 2000), visto que as *Helicobacter* spp. eventualmente modificam sua morfologia natural decorrente de condições ambientais (JALAVA et al., 1998; FAWCETT; GIBNEY; VINETTE, 1999). Contudo, a tentativa de diferenciações fidedignas de espécies de *Helicobacter* spp., são obtidas somente por métodos

moleculares como a análise filogenética e por meio da PCR utilizando primers espécie-específicos (NEIGER et al., 1998; NEIGER; SIMPSON, 2000; SIMPSON, 2005).

1.6 TRATAMENTO

Em humanos, o tratamento para erradicação para *H. pylori* tem obtido inúmeros sucessos, graças a diversos testes clínicos avaliando uma ampla variedade de drogas antimicrobianas (NEIGER; SIMPSON, 2000). A terapia comumente utilizada baseia-se no emprego associativo de um (metronidazol, amoxicilina ou claritromicina) ou dois (claritromicina + amoxicilina) fármacos antimicrobianos, associados à um antagonista de canais H2 ou bloqueador de bombas de prótons, durante 2 ou 3 semanas (LECOINDRE et al., 2000; NEIGER; SIMPSON, 2000; FOX, 2012). Os fármacos empregados em animais domésticos, são adaptações de terapias humanas profiláticas à *H. pylori* (LECOINDRE et al., 1998). Estudos *in vitro* demonstraram a suscetibilidade da *H. bizzozeronii*, *H. salominis* e *H. felis* à drogas antimicrobianas como a claritromicina, ampicilina amoxicilina ou tetraciclina durante 2 semanas (VAN DEN BULCK et al., 2005). Resistência adquirida ao metronidazol têm sido observadas em isolados de *H. bizzozeronii* e *H. felis*, desta forma, sendo desaconselhada a possibilidade do uso deste fármaco nas terapias empregadas (VAN DEN BULCK et al., 2005; KONDADI et al., 2013).

Em cães, protocolos terapêuticos envolvendo a terapia tripla com amoxicilina, metronizadol e famotidina forneceram cura clínica em mais de 90% dos cães de uma população de 63 animais com sinais gastrointestinais. Entretanto, neste estudo não houve a descrição de reavaliação gastroscópica ou de algum teste de detecção após a terapia, para comprovar a erradicação bacteriana (DENOVO; MAGNE, 1995). O uso da amoxicilina, claritromicina e omeprazol durante 21 dias, forneceu erradicação completa em 15 cães naturalmente infectados (MIRZAEIAN et al., 2013). Outro estudo empregando um protocolo semelhante, porém com o uso da lansoprazol ao invés do omeprazol durante 7 dias, forneceu eficácia de eliminação bacteriana em 100% dos animais. Entretanto, 60 dias após o tratamento, houve reinfecção destes animais (ANACLETO et al., 2011). Contudo, apesar destes resultados supracitados, ainda são escassos estudos *in vivo* em cães e gatos que envolvam protocolos de terapia antimicrobianas contra as HNHP, havendo carências de dados que forneçam novas opções de combinações

terapêuticas, períodos de administração e taxas de recidivas de infecção (FOX, 2012; MIRZAEIAN et al., 2013).

1.7 SAÚDE PÚBLICA

Em humanos, as HNHP têm prevalência de 0,5% em países desenvolvidos (O'ROURKE, GREHAN; LEE, 2001) e de 6,2-15% em países em desenvolvimento (ZHANG et al., 1998; LIU et al., 2015). Desta forma, países com menor desenvolvimento socioeconômico, tendem a ter maior prevalência de pessoas infectadas pelas HNHP (O'ROURKE, GREHAN; LEE, 2001; CHOW; TANG; MAZMANIAN, 2011). *H. felis, H. bizzozeronii* e *H. salomonis* com a ausência da *H. pylori*, já foram detectadas em 48,5% (n= 48/101) de amostras obtidas de mucosa gástricas de humanos apresentando históricos de gastrites crônicas (DE GROOTE et al., 2005). Assim como em outros diversos estudos, as HNHP também já foram documentada em pacientes humanos apresentando quadros de úlceras pépticas e/o carcinomas gástricos, que seriam alterações características pela infecção por *H. pylori* (FLEJOU; DICMANDE; MOLAS, 1990; HEILMANN; BORCHARD, 1991; SOLNICK et al., 1993; MORGNER et al., 1995; HAESEBROUCK et al., 2009; LIU et al., 2015) Frente a estes resultados e dentre outros similarmente já encontrados, diversos pesquisadores alegam prováveis implicações zoonóticas relacionadas às HNHP (STOLTE et al., 1994; THOMSON et al., 1994; MEINING; KROHER; STOLTE, 1998; LECOINDRE et al., 2000; NEIGER; SIMPSON, 2000; NEIGER; JALAVA et al., 2001; DE GROOTE et al., 2005; VAN DEN BULCK et al., 2005; DE BOCK et al., 2007; BENTO-MIRANDA & FIGUEIREDO, 2014; MLADENOVA-HRISTOVA; GREVOKA; PATEL, 2017).

Alguns autores sugerem que esta relação patológica das HNHP aos seres humanos esteja atrelada à aproximada relação evolutiva e às similaridades genômicas com a *H. pylori* (GUENEAU; GOËR, 2002), visto que as HNHP abrigam genes que expressam fatores de virulência e de versatilidade metabólica que também são encontrados na *H pylori* (ØVERBY et al., 2016). O que pode explicar a sua capacidade de adaptar-se ao ambiente gástrico humano e induzir doenças gástricas assim como a *H. pylori*, realçando a natureza zoonótica destas bactérias (SCHOTT et al., 2011).

Em populações humanas e de carnívoros domésticos, à falta de higiene e saneamento básico, o confinamento com alta densidade populacional e a promiscuidade, podem ser os

principais fatores predisponentes na transmissão destas bactérias dos animais ao homem e vice-versa (LEE et al., 1991; VICENT, 1996). Pois a exposição às fezes e saliva de cães infectados por HNHP, são supostamente fontes de transmissão ao homem, uma vez que já se foi identificada a presença destas bactérias nestas excreções (NEIGER; SIMPSON, 2000; RECORDATI et al., 2007; EKMAN; FREDRIKSON; TROWALD-WIGH, 2013; JANKOWSKI et al., 2016).

2 ARTIGO

TRABALHO A SER SUBMETIDO PARA PUBLICAÇÃO

Periódico: Zoonosis and Public Health

(INSS 1863-2378)

HELICOBACTER SPP. IN SALIVA AND GASTRIC MUCOSA OF DOMESTICS DOGS WITH AND WITHOUT CHRONIC VOMITING IN CENTRAL REGION OF THE RIO GRANDE DO SUL

Helicobacter spp. in saliva and gastric mucosa of domestics dogs with and without chronic vomiting in central region of the Rio Grande do Sul

Running title: *Helicobacter* spp. in dogs

Daniel D. Guerra Segundo[1], Saulo T. L. Pinto Filho[2]

[1]Universidade Federal de Santa Maria, Programa de Pós-Graduação em Medicina Veterinária, Santa Maria, Rio Grande do Sul, Brasil

[2]Universidade Federal de Santa Maria, Departamento de Clínica de Pequenos Animais, Santa Maria, Rio Grande do Sul, Brasil

Correspondence to: Daniel Dourado Guerra Segundo
Universidade Federal de Santa Maria, Programa de Pós-Graduação em Medicina Veterinária, Av. Roraima, n.1000, Bairro Camobi, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brasil
E-mail: danielguerradourado@gmail.com

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SUMMARY

Helicobacter pylori is a spiral-shaped bacterium frequently detected in the stomach of humans and plays a role in the pathogenesis of upper digestive tract diseases. In domestic animals, non-*H. pylori Helicobacter* species (NHPH) have also been discovered, which are frequently studied because of their zoonotic importance. This study aimed to investigate the occurrence of *Helicobacter* spp. in saliva and gastric samples from dogs. Furthermore, we evaluated the gastric bacterial colonisation density, presence of gastric lesions and sought to identify the main phylogenetic groups of the *Helicobacter* spp. isolated from domestic dogs in the central region of Rio Grande do Sul. This study included 20 dogs and the analysis employed were cytology, histopathology, polymerase chain reaction, rapid urease testing and phylogenetic analysis.

Helicobacter spp. was present in the gastric mucosa of 17 of the dogs and saliva of 3 dogs. All asymptomatic dogs were positive for *Helicobacter* spp., whereas 2 dogs with chronic vomiting were negative. Density analysis of the gastric bacterial colonisation revealed that 3, 6 and 8 dogs had a mild, moderate and severe infection, respectively. Lymphocytic–plasmacytic infiltrates were the primary type of gastric lesions observed. However, the presence of *Helicobacter* and the density appeared to be unrelated to the gastric lesions found. The samples possessed a high nucleotide identity with remarkably similar sequences among some of the species of NHPH such as *H. heilmannii s.s.*, *H. salomonis*, *H. felis* and *H. bizzozeronnii*. In the dogs, increased density, occurrence rate and predominance of NHPH of zoonotic importance were found in the stomach with a lower occurrence of *Helicobacter* spp. in the saliva. The saliva of domestic dogs, even of those without gastric colonisation, can cause *Helicobacter* infection in humans and other animals.

Keywords: Brazil, Dogs, Gastric Mucosa, *Helicobacter*, Saliva

IMPACTS

- Increased density, occurrence rate and predominance of NHPH of zoonotic importance were discovered in the stomach of domestic dogs in the central region of Rio Grande do Sul.
- Previous studies on dogs have reported that the occurrence of *Helicobacter* spp. in saliva of dogs in this region was low, despite the presence of bacteria in the stomach.
- However, a dog that was negative for *Helicobacter* spp. in the gastric mucosa was found to be positive in the saliva by polymerase chain reaction. This finding was previously only reported in cats.

1 INTRODUCTION

Helicobacter spp. are spiral-shaped mobile Gram-negative bacteria (Simpson, 2005) with tropism for the gastric mucosa of humans and animals (Haesebrouck et al., 2009). *Helicobacter pylori* (*H. pylori)* was the first species isolated from the *Helicobacter* genus by Australian researchers Marshall and Warren in 1983 (Marshall & Warren, 1984). Further research has shown that humans are the natural host of *H. pylori* and have established this species as the primary aetiology of peptic ulcers and gastric neoplasms (Dunn, Cohen & Blaser,

1997). Years later, in samples of gastric mucosa from humans, another spiral-shaped bacteria with similar morphological features to *H. pylori* was documented, and this microbe was found to be remarkably similar to the spiral-shaped bacteria found in the gastric mucosa of domestic animals (Dent, McNulty, Wilkinson & Gear, 1987). This new spiral bacteria was later classified by Solnick et al. (1993) as *Helicobacter heilmannii*. However, phylogenetic studies were later employed utilising the 16 rRNA target gene, and these studies found instead that this bacteria belonged to a range of several *Helicobacter* species isolated from domestic and wild animals, such as *H. felis, H. bizzozeronii, H. salomonis, Helicobacter heilmannii sensu stricto* (*H. heilmannii s.s.*), *H. bílis*, *H. cynogastricus*, and *H. baculiformis* (O'Rourke et al., 2004). Therefore, in order to organise this large group of morphologically undifferentiated bacteria into a single term, they were consensually denominated as *non-Helicobacter pylori Helicobacter* (NHPH) (Haesebrouck et al., 2011).

NHPH became the target of recent studies because an association was found with upper digestive tract illnesses in humans (Jankowski et al., 2016) of zoonotic importance (Mladenova-Hristova, Grevoka & Patel, 2017). Dogs and cats are the natural hosts of NHPH and harbour this bacteria in their gastric mucosa, gut, and oral cavity (Recordati et al., 2007; Tabrizi et al., 2010; Ekman, Fredriksson & Trowald-Wigh, 2013; Jankowski et al., 2016). Thus, gastric juice, saliva, and faeces are possible sources of transmission for this bacteria to infect humans (Recordati et al., 2007; Tabrizi et al., 2010; Ekman, Fredriksson & Trowald-Wigh, 2013; Jankowski et al., 2016). In dogs and cats, the main species of NHPH identified were *H. heilmannii s.s, H. bizzozeronii, H. salomonis, H. felis, and H. canis* (2010; Ekman, Fredriksson & Trowald-Wigh, 2013; Jankowski et al., 2016).

Chronic inflammation of the gastric mucosal tissue and peptic ulcers are the main clinical alterations described in humans (Jankowski et al., 2016; Mladenova-Hristova, Grevoka & Patel, 2017). In 1994, these bacteria were classified by the International Agency for Research on Câncer (IARC) as risk factor I for the development of gastric carcinomas and mucosal-associated lymphoid tissue (Smith et al., 2012). In human populations, NHPH has a prevalence of 0.5% in developed countries (O'Rourke et al., 2001) and 6.2%–15% in underdeveloped countries (Zhang et al., 1998; Liu et al., 2015). Thus, countries with lower socioeconomic development tend to have a higher prevalence of infected people with NHPH (Chow, Tang & Mazmanian, 2011). However, information regarding the importance of domestics dogs as reservoirs for this bacteria and the data related to the number of NHPH occurrences in the canine population of these countries has not yet been explicated (Polanco et al., 2011).

Due to the zoonotic implications of NHPH, compounded by the high density of domestic animals, and sanitary problems affecting Brazil, it is important to elucidate this information to assist with future studies of public health. Moreover, geographic variation could affect the prevalence of the *Helicobacter* species (Jankowski et al., 2016). Thus, the aim of this study was to isolate *Helicobacter spp.* from saliva and the gastric mucosa of domestic dogs. Moreover, this study documented the gastric bacterial colonisation density, the number of gastric lesions present, and sought to identify the main phylogenetic groups of *Helicobacter* spp. found in dogs from the central region of Rio Grande do Sul.

2 MATERIAL AND METHODS

2.1 Animals

This study collected saliva and gastric mucosal samples from 20 client-owned domestic dogs. They included 6 males and 14 females, ranging from 7 months to 17 years of age. Ten of these dogs appeared clinically normal (all females), and 10 (6 males and 4 females) had a history of chronic vomiting. None of the dogs received effective eradication protocol for gastric *Helicobacter* spp. infection, employing the combination of amoxicillin and clarithromycin or metronidazole and proton pump inhibitor (omeprazole or lansoprazol) or famotidine (Anacleto et al., 2011; Mirzaeian, Sarchahi, Shojaee Tabrizi & Derakhshandeh, 2013; Kubota et al., 2013; Kubota-Aizawa et al., 2017).

2.2 Sample Collection

Following a fasting period of 12 hours, all dogs were anesthetically induced with propofol (4mg/kg) and held in an anaesthetised state with isoflurane and 100% oxygen. Saliva collection was performed with a sterile swab prior to the endoscopic procedure, and the samples were stored in sterile normal saline and frozen at – 80ºC until further processing.

The collection of gastric biopsies was performed with a Karl Storz flexible endoscope (Karl Storz GmbH & Co.KG., Tuttingen, Germany) and 2.3 mm diameter biopsy forceps (Changzhou Jiuhong Medical Instrument Co. Ltd., Changzhou, China). Following the mucosal evaluation, four biopsy samples were collected from the body and gastric antrum. Impression smears of the two gastric biopsy samples from the body and antrum were prepared on an air-dried slide. Then impression smears from the same samples were placed into 2 tubes (one tube

for the body samples and the other for the antrum samples) containing 10% formalin until further processing. One sample of each gastric zone was submitted for the rapid urease test. For molecular analysis, one sample from each gastric zone was stored in a tube containing sterile normal saline and frozen at – 80ºC until further processing.

2.3 Rapid Urease Test (RUT)

RUT was employed with a Urease test kit (RenyLab, Barbacena, Brazil). Following collection, the biopsy samples for each gastric zone were placed together into a test tube and evaluated for 60 minutes. Colour transformation from yellow to pink within 60 minutes was considerate positive; while, no colour transformation within 60 minutes was considered negative.

2.4 Cytology

Following the impression smears of the biopsy samples, the slides corresponding to both gastric zones were stained with a quick panoptic stain. The gastric bacterial colonisation densities (evaluated 10 fields at x 400 and considered the most concentrated field) were recorded as follows (0) No bacteria; (1= mild) less than 10 bacteria per field; (2= moderate) 10-50 bacteria per field and; (3= severe) more than 50 bacteria per field (Tabrizi et al., 2010; Amorim et al., 2015).

2.5 Histopathology

The formalin-fixed gastric samples for the body and the antrum were sectioned and stained with haematoxylin and eosin and then processed routinely. The gastric samples were evaluated by using the World Small Animal Veterinary Association (WSAVA) gastrointestinal standardisation visual analogue scale (Day et al., 2008). Where the attributed scores are based on the gastric lesion severity and recorded as follows: (0) lesion not observed; (1) mild lesion; (2) moderate lesion; and (3) severe lesion. The values obtained in the evaluation of the 3 random fields for each gastric zone were calculated and expressed with the mean of the gastritis severity score.

2.6 DNA Extraction and Polymerase Chain Reaction Assay (PCR)

DNA was extracted from the saliva and gastric biopsies with the DNeasy Blood and Tissue Kit (Qiagen N.V., Hilden, Germany), following the manufacturer's instructions. Total DNA was extracted for each sample and submitted to PCR testing which used the following oligonucleotide primers (F-5′- AAC GAT GAA GCT TCT AGC TTG CTA-3′; R-5′- GTG CTT ATT CST NAG ATA CCG TCA T-3′), which amplified a fragment of 399 base pairs (bp) for the 16S rRNA gene of the *Helicobacter* spp. (Germani et al., 1997). PCR testing involved 1x buffer of PCR containing $MgCl_2$, 10 mM of dNTPs (0,2 mM of each), 10 pmol of each primer, 1 U of Taq polymerase enzyme (Promega, Madison, Wisconsin, USA) and qsp water; following these conditions the initial denaturation (95°C per 5 minutes), followed by 32 cycles at 94°C–30 s; 62°C–30 s to annealing of the initiators and 72°C–30 s for chain extension; and final extension for 3 minutes at 72°C. The amplified product was analysed by electrophoresis in an agarose gel 1% (60V, 1 hour and 30 minutes), using GelRed® (Biotium, California, USA) and visualised by a transilluminator with ultraviolet light. In all of the amplifications, the positive control was obtained from the gastric mucosal samples from a dog known positive for *Helicobacter* spp. and the negative controls were ultrapure water samples.

2.7 Sequencing and Phylogenetic Analysis

The amplification products for PCR were submitted in duplicate for nucleotide sequencing via the Sanger method using Prism 3500 Genetic Analyzer equipment (Life Technologies, California, USA). The consensus sequence from the Staden Package program was used to start the nucleotide sequences (Staden, 1996). Phylogenetic analysis utilised the consensus sequence for each of the amplified samples and the nucleotide sequences of the *Helicobacter* spp. obtained from the GenBank database (www.ncbi.nlm.nih.gov). The sequences were edited and aligned using the BioEdit Alignment Editor software suite, version 7.0.5.3 (Hall, 1999). The phylogenetic tree was constructed using the MEGA X program (Kumar, Stecher, Li, Knyaz & Tamura, 2018).

2.8 Statistical Analyses

Statistical analyses were then performed using the statistical software Action Stat Pro (Estatcamp, São Carlos, Brazil). Non-parametric testing of Kruskal–Wallis was employed to compare the bacterial colonisation density scores between of symptomatic and assympstomtic dogs, and between regions of the body and antrum. The Spearman test was used to evaluate the correlation between the scores of the histologic gastric lesions and the gastric bacterial colonisation density scores in both of the gastric zones. Fisher's exact test was used to determine if the NHPH infection was associated with gastric lesions in the histopathology findings. The significance level used was $P < 0.05$ for all statistic evaluations.

2.9 Ethics Statement

This study was approved by the Animal Ethics Committee of Universidade Federal de Santa Maria (Approval number 2827081018) and all experiments were performed in accordance with national guidelines and regulations for the care and use of laboratory animals established by National Council for Animal Experimentation Control (CONCEA), Brazil.

3 RESULTS

3.1 Detection of *Helicobacter* spp. and gastric bacterial colonisation density (Table 1)

In accordance with RUT and cytology, 85% (17/20) of the dogs were positive, respectively. Detection of the 16S rRNA, specific to the *Helicobacter* genus, was positive 15% in saliva (3/20) and 65% (13/20) in the gastric mucosa.

Cytology results for the presence of NHPH for all of the positive dogs (Figure 1), 17.6% (3/17) showed a mild score, 35.3% (6/17) a moderate score, and 47.1% (8/17) a severe score (note-only the gastric zone with major scores were observed). The dogs with chronic vomiting group results found that the gastric bacterial colonisation density was absent in 30% (3/10) of the dogs, 10% mild score (1/10), 40% moderate score (4/10), and 20% severe score (2/10). All asymptomatic dogs were positive for the presence of *Helicobacter* spp., 20% of the dogs showed a mild score (2/10), 20% moderate score (2/10), and 60% a severe score (6/10). There were no statistically significant differences in comparison of the gastric bacterial colonization

density scores between of symptomatic and asymptomatic dogs (P =0.06) and in comparison of scores between of the body and the antrum (*P* = 0.8) (this compared only the dogs with *Helicobacter* in both gastric zones).

The occurrence of NHPH in this dog population was 90% (18/20). The presence of bacteria in the saliva and gastric mucosa occurred in 15% (3/20) and 85% (17/20), respectively. In the gastric mucosa, the PCR detection of *Helicobacter* spp. occurred in 60% (6/10) of dogs with chronic vomiting and 70% (7/10) of asymptomatic dogs. Only one dog was positive for NHPH in the saliva and negative in the gastric mucosa for all tests.

3.2 Findings of the Upper Digestive Endoscopy and Histopathology

Endoscopic evaluation of 3 dogs without *Helicobacter* gastric presence showed macroscopic alterations, such as erythema (2/3), oedema (1/3), petechiae (1/3), superficial irregularities (1/3) and vascular pattern visibility (1/3). Moreover, 17 dogs with *Helicobacter* gastric presence showed the following macroscopic alterations observed petechiae (4/17), erythema (2/17), oedema (2/17), and vascular pattern visibility (2/17) (Figure 2). Based on the WSAVA standards, for the 3 dogs without *Helicobacter* gastric presence, only one showed gastric lesions while the 17 dogs with *Helicobacter* gastric presence, 13 showed gastric lesions, 11 mild gastritis, and one moderate gastritis (Table 2). The gastric lesion patterns found in the 20 dogs were lymphocytic–plasmacytic infiltrates (13/20), neutrophilic infiltrates (7/20), eosinophilic infiltrates (4/20), fibrosis, and mucosal atrophy (3/20), gastric lymphofollicular hyperplasia (3/20), and intraepithelial lymphocytes (2/20) (Table 3) (Figure 3).

Although the number of lymphocytic–plasmacytic infiltrates was high in the animals with *Helicobacter* gastric presence (Table 2); statistically, this was not associated with a known pattern of gastric lesions ($P > 0.05$). In addition, there was no correlation between the scores of histologic lesions with gastric bacterial colonisation density scores in both gastric zones ($P = 0.11$) (Table 3).

3.3 Nucleotide Identity and Phylogenetic Analysis

The identity of the nucleotides between the sequences obtained, varied by 96.1 to 100%. The identity was high too when these sequences were compared with sequences of *H. heilmannii* (HM625818; 96.4 to 98.8%) *H. salomonis* (U89351; 96.8 to 99.2%), *H. felis* (AY686607; 96.4 to 99.2%) and *H. bizzozeronnii* (NR026372; 96.4 to 99.6%). The identity of

the nucleotide changed from 89.1% to 91% when compared with the sequences of *H. pylori* (U00679) samples (Figure 4).

4 DISCUSSION

The occurrence of *Helicobacter* spp. (90%) in the dogs of this study was higher than that of the dogs from Japan (35%) (Kubota-Aizawa et al., 2017), South Korea (78.4%) (Hwang, Han & Youn, 2002), Denmark (76.7%) (Wiinberg et al., 2005), Germany (82%) (Hermanns, Kregel, Breuer & Lechner, 1995) and Portugal (87%) (Amorim et al., 2015). However, our results were lower than dogs of Poland (96.7%) (Jankowski et al., 2016), Iran (95%) (Asl et al., 2010), Venezuela (95%) (Polanco et al., 2011) and Costa Rica (95%) (Suárez-Esquivel, Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo, 2017). These results demonstrated the high level of geographic variation for this bacteria in domestic dog populations, regardless of socioeconomic level. Interestingly, these results were different than those observed in human populations, whose occurrence of *Helicobacter* spp. increased according to the socioeconomic underdevelopment of the region (O'Rourke, Grehan & Lee, 2001; Chow, Tang & Mazmanian, 2011). However, additional data from developed and under developed countries are required in order to correlate the NHPH prevalence in populations of domestic dogs from different geographic regions (Polanco et al., 2011; Amorim et al., 2015) as the majority of research thus far, has been condensed into just a few countries.

The occurrence of NHPH was significantly higher in the asymptomatic dogs in this study, and this result was in accordance with the results of other studies (Simpson, 2005; Amorin et al., 2015). Ten of the animals at first presentation appeared clinically healthy; however, they were found via testing to be infected with high levels of NHPH, and subclinical gastritis was present during upper digestive endoscopy and histopathology; as previously described by other authors (Ekman, Fredriksson & Trowald-Wigh, 2013; Amorin et al., 2015; Suárez-Esquivel, Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo, 2017). The positive dogs for NHPH using cytology revealed that 17.6% showed a mild score, 35.3% a moderate score, and 47.1% a severe score. Thus, more than half of the subject dogs were reservoirs of a high colonisation density for NHPH.

During the upper digestive endoscopy, visibly explicit macroscopic alterations in the gastric mucosal of dogs were observed, regardless of whether they presented as clinically healthy. The main alterations observed were petechiae and erythema and; however, none of the dogs showed the presence of erosions or ulcerations that were described by Suárez-Esquivel,

Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo (2017). Furthermore, some of the gastroscopy findings failed to match the histological findings, for instance, when macroscopic lesions were not observed, the microscopic evaluation exhibit alterations; however, these findings are not abnormal for NHPH results (Carpenter & Talley, 1995). Therefore, the diagnosis of gastritis should not be based solely upon gastroscopy visualisation, as biopsy collection is indispensable to confirm the diagnosis of gastritis and for the determination of the lesion pattern (Redéen, Peterson, Jönsson & Borch, 2003).

The main histologic changes observed in the dogs of this study were the presence of lymphocytic–plasmacytic infiltrates, which were commonly observed when NHPH was present (Amorin et al., 2015; Suárez-Esquivel, Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo, 2017). Nevertheless, NHPH factors regarding the gastric presence and gastric bacterial colonisation density were not found to be correlated with the gastric lesions and severity scores, and this result was similar to other authors' results (Polanco et al., 2011; Ekman, Fredriksson & Trowald-Wigh, 2013; Suárez-Esquivel, Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo, 2017). Furthermore, the type of lesion patterns found in this study were not confined to NHPH infection, and could also be caused by the host immune response to parasites, dietary antigens, or bacterial components (Simpson, Neiger, DeNovo & Sherding, 2000). Moreover, the virulence and pathogenicity could vary between the different *Helicobacter* species and strains within an NHPH infection (Joosen et al., 2013). Thus, future taxonomic studies are required in order to distinguish these species, their specific virulence factors (Joosen et al., 2013; Suárez-Esquivel, Alfaro-Alarcón, Guzmán-Verri & Barquero-Calvo, 2017), and to determine if NHPH is a pathobiont of dogs, which would explain their clinically healthy presentation (Chow, Tang & Mazmanian, 2011).

This study was the first conducted in Brazil and investigated the presence of NHPH in the saliva of dogs, and the results revealed divergent findings from that of other studies. Commonly, a high prevalence of 71.1% to 100% of *Helicobacter* spp. was detected in the saliva of canine populations and documented (Recordati et al., 2007; Ekman, Fredriksson & Trowald-Wigh, 2013); however, the present study observed an occurrence significantly lower 15% (3/20). Another novel finding was that one dog was positive for NHPH in the saliva and negative in the gastric mucosa, which has not been reported by another study in a canine population. This observation has been previously described in cats (Tabrizi et al., 2010). Thus, it is probable that dogs with a gastric absence of *Helicobacter* spp. could harbour this bacteria in the oral cavity. Some authors have suggested that the oral cavity could be a potential niche of *Helicobacter* spp. colonisation (Recordati et al., 2007). Suggesting the possibility of canine

saliva as a potential transmission source of NHPH to humans and other domestic animals (Recordati et al., 2007; Tabrizi et al., 2010; Jankowski et al., 2016).

The *Helicobacters* found by cytology on the impression smears showed NHPH morphology; however, it was not possible to distinguish the different species by this evaluation. Because when observed with an optical microscope, NHPH exhibited indiscriminate morphology, lengths, and widths (Jalava et al., 1997; Robíc et al., 2007) that eventually led to modification of their natural morphology (Jalava et al., 1998; Fawcett, Gibney & Vinette, 1999).

Furthermore, the phylogenetic analysis also failed to determine the species of the *Helicobacter* detected. However, we could affirm that the nucleotide identity between the sequences was extremely high and very similar to *H. heilmannii s.s*, *H. salomonis*, *H. felis,* and *H. bizzozeronnii*. These species of *Helicobacter* are important as they are considered to share close evolutionary relationships and genomic similarities with *H. pylori* (Schott, Kondadi, Hänninen & Rossi, 2011; Øverby, Murayama, Matsui & Nakamura, 2016). This might explain the capacity of these NHPH to readily adapt to the gastric environment of humans and to induce gastric diseases, highlighting the zoonotic nature of these bacterial species (Schott, Kondadi, Hänninen & Rossi, 2011).

Notably, as the human population increases their contact with domestic animals, a directly proportional increase in NHPH infected people could result relative to the socioeconomic underdevelopment of the country they inhabit (O'Rourke, Grehan & Lee, 2001). Furthermore, saliva and faeces of dogs and cats with NHPH may be potential sources of infection for human populations (Tabrizi et al., 2010; Ekman, Fredriksson & Trowald-Wigh, 2013). Thus, this is what motivated the authors to initiate and develop this study, as we were faced with a growing canine population, while poor sanitary conditions are still a reality in our country. Due to the zoonotic importance of NHPH bacteria, future cohort studies evaluating the prevalence of *Helicobacter* spp. in the Brazilian human population that could be directly associated with their pets, would be extremely relevant in order to elucidate whether NHPH is a concern to public health. Currently in Brazil has a high mortality rate associated with gastric cancer, it was the third-largest cause of death for men, and the fifth among women (Instituto Nacional do Câncer, 2015) and the prevalence and mortality rates due to peptic ulcers have increased as the population age increases (Oliveira et al., 2008).

5 CONCLUSION

The present study demonstrated a low occurrence of *Helicobacter* spp. in the saliva, and a high occurrence, predominance, and density of zoonotic importance in the stomach of domestic dogs of the central region of Rio Grande do Sul. Saliva of the domestic dogs may be a transmission reservoir of NHPH, even in animals without bacterial colonisation. The *Helicobacter* gastric presence and gastric colonisation density failed to show a relationship or correlation with the gastric lesions pattern observed during histopathology.

CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest.

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TABLES

TABLE 1 Detection of *Helicobacter* spp., in different tests evaluated

Animal No.	Chronic Vomiting	Saliva	Gastric mucosa			
				Score Cytology		RUT
		16S rRNA		Body	Antrum	
1	P	+	-	0	0	N/R
2	P	-	-	0	0	N/R
3	P	+	+	2	2	+
4	P	-	-	0	0	-
5	P	+	+	1	3	+
6	P	-	-	2	2	+
7	P	-	+	3	3	+
8	P	-	+	2	1	+
9	A	-	+	1	3	+
10	A	-	+	2	1	+
11	A	-	+	3	3	+
12	A	-	+	2	N/R	+
13	A	-	-	1	3	+
14	A	-	+	3	3	+
15	A	-	+	1	N/R	+
16	P	-	+	2	1	+
17	A	-	+	3	1	+
18	A	-	-	1	N/R	+
19	A	-	-	3	3	+
20	P	-	+	1	1	+
	Total	3/20	13/20	17/20	14/20	17/20

P, present; A, absent; RUT, Rapid Urease Test; N/R, not realized; -, negative; +, positive; 1, mild score; 2, moderate score; 3, severe score.

TABLE 2 Findings of histologic gastric lesion on infected and non-infected dogs (mean of both gastric zones)

Histologic gastric lesion (Day et al., 2008)	Total (n=20)	Infected (n=17)	Non-infected (n=3)
Mild gastritis	12	11	1
Moderate gastritis	1	1	0
Fibrosis and mucosal atrophy	3	2	1
Lymphocytic-plasmacytic infiltrate	13	12	1
Neutrophilic infiltrate	7	6	1
Eosinophilic infiltrate	4	3	1
Gastric lymphofollicular hyperplasia	3	3	0
Intraepithelial lymphocytes	2	2	0

TABLE 3 Findings of histologic gastric lesion and gastric bacterial colonisation density for each gastric zone (body and antrum)

Gastric zone	Histologic gastric lesion (Day et al., 2008)	gastric bacterial colonisation density (Scores)			
		(0)	(1)	(2)	(3)
	Fibrosis and mucosal atrophy	0	0	0	1
	Lymphocytic-plasmacytic infiltrate	1	3	2	4
Body	Neutrophilic infiltrate	1	2	1	3
	Eosinophilic infiltrate	1	1	0	1
	Gastric lymphofollicular hyperplasia	0	0	1	2
	Intraepithelial lymphocytes	0	0	0	2
	Fibrosis and mucosal atrophy	1	0	1	0
	Lymphocytic-plasmacytic infiltrate	1	2	1	2
Antrum†	Neutrophilic infiltrate	1	0	0	0
	Eosinophilic infiltrate	1	1	0	0
	Gastric lymphofollicular hyperplasia	0	0	0	0
	Intraepithelial lymphocytes	0	0	0	0

† Samples of 6 dogs were not viable for histological evaluation

FIGURES

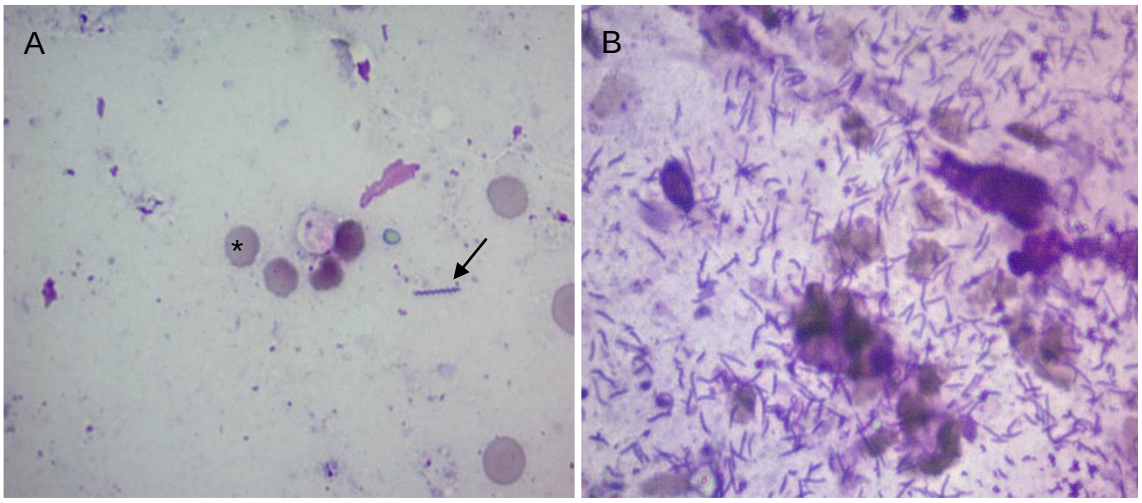

FIGURE 1 Impression smears of the gastric biopsies samples obtained of antrum; (A) cytology showed the presence of a *Helicobacter* spp. (arrow) between of degenerating gastric mucosa epithelial cells (asterisk). (B) cytology showed high density of *Helicobacter* spp. and the presence of degenerating gastric mucosa epithelial cells (quick panoptic stain, x1000).

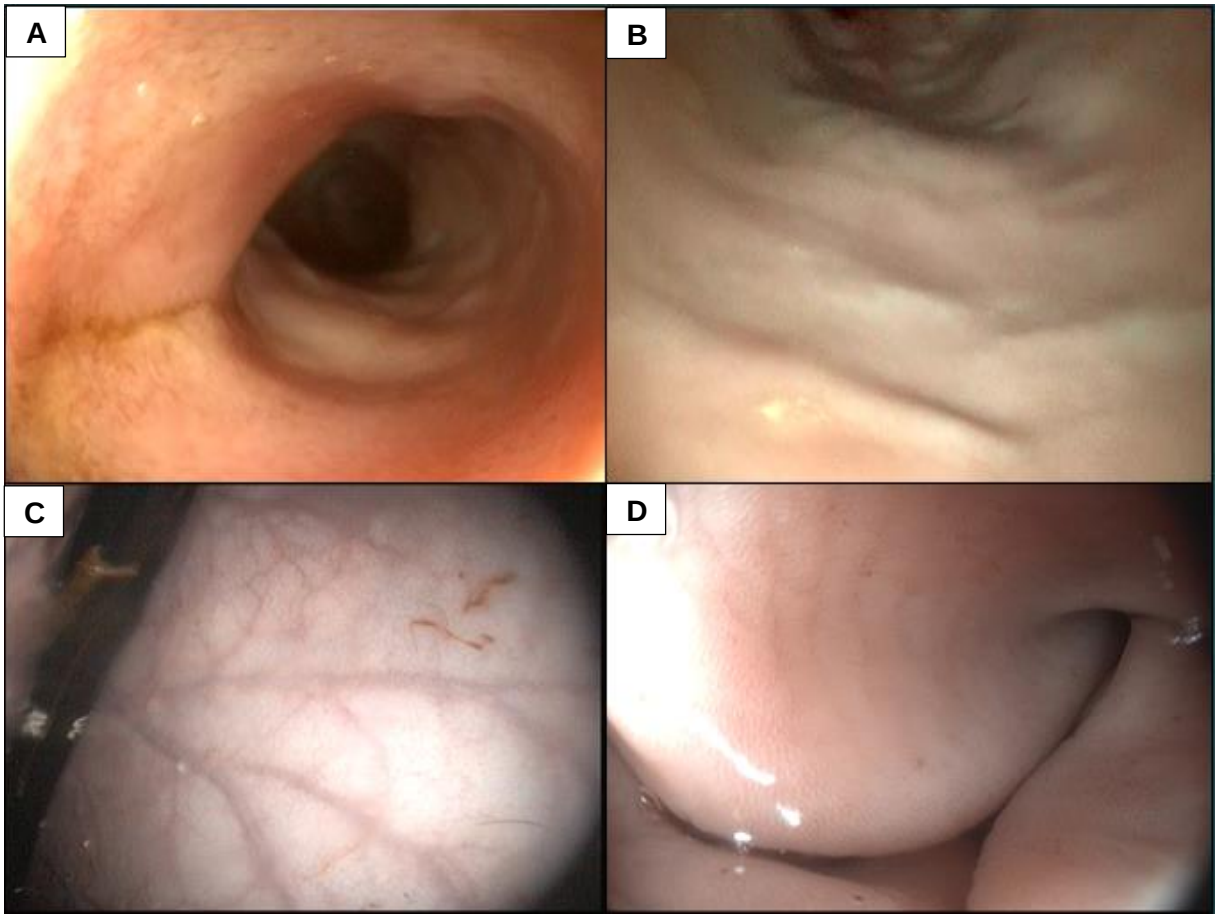

FIGURE 2 Findings of upper digestive endoscopy; (A) Erythema. (B) Surface irregularities. (C) Visibility of vascular pattern. (D) Discrete petechiae.

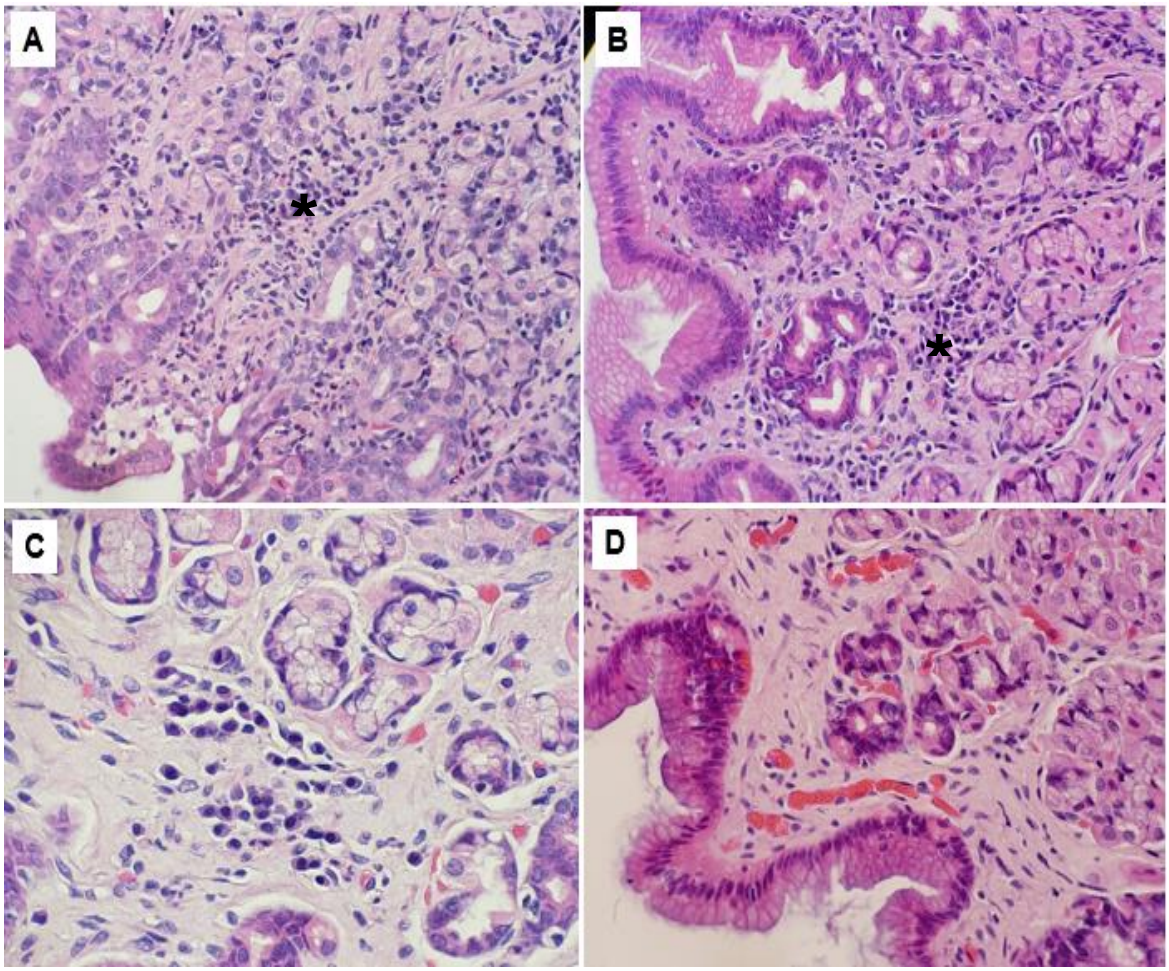

FIGURE 3 Gastric mucosa (body A, B and C; antrum, D) biopsy sections; (A) Lamina propia with high quantity of polymorphonuclear cells (asterisk), mainly eosinophilic infiltrate. (B) Lamina propia showed high quantity of mononuclear cells (asterisk), with predominance of plasmocytes. (C) Approximate view of figure C. (D) Image of body without alteration of morphology and no infiltration of inflammatory cells. (Haematoxylin and eosin stained, x400).

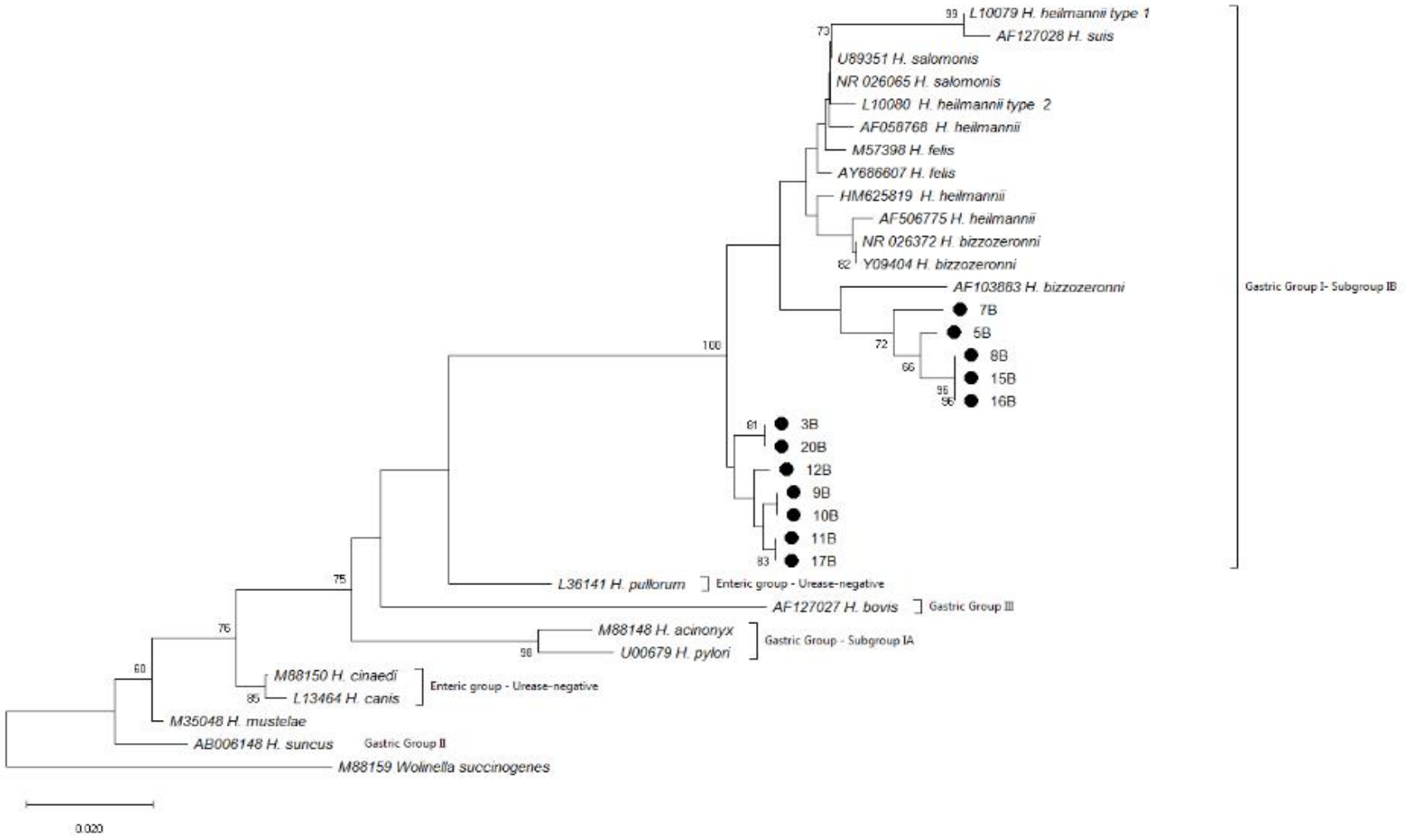

FIGURE 4 Phylogenetic tree based in nucleotide sequence of 16S rRNA gene of *Helicobacter* spp. The tree was build employing the Neighbor-Joining method with *boostrap* of 1000 replicates. The evolutionary distances were computed using of Kimura-2 method. The tree was build with MEGA X software. Values above 60% are showed. The sequences obtained of this study are highlighted with a black circle; nºB, animal number/gastric biopsy

3 CONCLUSÃO

Conclui-se que existe uma elevada densidade de *Helicobacter* spp. de importância zoonótica no estômago de cães domésticos da região central do Rio Grande do Sul. A saliva de cães domésticos pode ser um reservatório de transmissão de *Helicobacter* spp., mesmo em animais sem colonização bacteriana gástrica. A presença da infecção gástrica não apresenta correlação com as lesões gástricas quando observadas nas análises histopatológicas.

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