The dog as an animal model for gastric Helicobacter spp. infection

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Abstract
The gastric mucosa of dogs is often colonized by non-Helicobacter pylori helicobacters (NHPH), while H. pylori is the predominant gastric Helicobacter species in humans. The colonization of the human stomach by H. pylori is highly dependent on the recognition of host glyccan receptors. Our goal was to define the canine gastric mucosa glycophenotype, to evaluate the capacity of different gastric Helicobacter species to adhere to the canine gastric mucosa and correlate these data with those obtained in dogs spontaneously infected with the different NHPH. This study provides an important contribution to the epidemiology of Helicobacter spp. infection and to the molecular mechanisms underlying dog-related Helicobacter species adhesion.

INTRODUCTION
Since the discovery of Helicobacter pylori in the early eighties and its association with gastric pathology, research on the Helicobacter genus has increased tremendously [1]. In addition to H. pylori, other helicobacters commonly denominated non-H. pylori Helicobacters (NHPH) are found to colonize the human stomach and have been associated with gastric disease in humans [2-5]. NHPH are of zoonotic significance and the dog remains a natural reservoir for many species [6]. The predominant gastric Helicobacter spp. in dogs are H. felis, H. bizzozeronii, H. heilmannii sensu stricto, H. salomonis, H. cynogastricus and H. baculiformis [6]. Although H. pylori is well accepted as an important human pathogen and is believed to be the primary cause of important gastric diseases, the pathogenic significance of gastric Helicobacter species in dogs is controversial. Nevertheless, dogs may constitute a source of infection for their owners [6]. On the other hand, H. pylori has occasionally been identified in the canine stomach [7]. Helicobacter species adhesion to the gastric mucosa is a critical early step in the colonization process, which occurs through recognition of specific glyccan receptors expressed by the host epithelial cells [8]. Gastric Helicobacter species have a host species preference, but may occasionally cross this host species barrier [6]. The molecular mechanisms underlying this partial host-adaptation are not known, but may be related to differences in glycosylation profile of the gastric mucosa.

MATERIALS AND METHODS
In vitro studies
With the aim of understanding the mechanistic process associated with the colonization of NHPHs frequently found in the stomach of dogs, the glycosylation profile both in antral and body compartments of canine gastric mucosa was assessed, with focus on the expression of histo-blood group antigens, namely type 1 and type 2 Lewis antigens [8]. Additionally, the in vitro binding capacity of FITC-labelled H. pylori and NHPH to canine gastric mucosa was also evaluated in cases representative of the canine glycosylation pattern, as previously described [8]. The following bacteria strains were used: H. pylori 17875/Leb (a spontaneous mutant which expresses both BabA and SabA adhesins but does not show sialylated dependent binding); the H. pylori 17875babA1A2 (a BabA adhesin mutant strain which expresses a functional SabA adhesin); H. heilmannii (ASB1.4); H. felis (CS1), H. salomonis (R1053) and H. bizzozeronii (R1051). Evaluation was estimated by the number of adhered bacteria under 100x magnification, analyzing at least five different fields per case and quantified using the ImageJ software (NIH, USA). Statistical analysis was performed using GraphPad (GraphPad Inc., USA). p values < 0.05 were considered as statistically significant.

In vivo studies
Gastric mucosa samples of 69 dogs (45 male and 24 female, ranging in age from 3 months to 15 years) were evaluated. Tissues were fixed in 10% neutral buffered formalin and embedded in paraffin wax. Gastric histopathological alterations were evaluated according to the World Small Animal Veterinary Association (WSAVA) guidelines [9]. The presence of Helicobacter spp. organisms was assessed with haematoxylin-eosin (HE),...
modified Giemsa (MG) stain, immunohistochemistry (IHC) with a polyclonal antiserum specific for H. pylori (RBK012; ZytoMed, German) and through polymerase chain reaction (PCR), as previously described [12]. A dog was classified as Helicobacter spp. positive whenever one of these methods gave a positive result. Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Portugal). p values < 0.05 were considered as statistically significant.

RESULTS

In vitro studies

Canine gastric mucosa lacks type 1 Lewis antigens and presents an extensive expression of type 2 Lewis structures and A antigen, both in the surface and glandular epithelium [10]. Regarding Helicobacter spp. adhesion, H. heilmannii s.s. presented the highest adhesion scores in the canine antral mucosa whereas the Saba-positive H. pylori strain showed the highest adhesion in the body region followed by H. heilmannii s.s.. The other NHPH presented a lower in vitro adhesion rate compared to H. heilmannii s.s. In descending order, H. felis was the second most adherent bacteria, followed by H. bizzozeronii and then H. salomonis and this tendency was observed in both stomach compartments [10].

In vivo studies

Canine NHPH infection was determined by four methods (HE, MG, IHC and PCR) and a prevalence of 87.0% was detected. NHPH infection was significantly accompanied by mild to moderate intraepithelial lymphocyte infiltration and mild to moderate gastric epithelial injury (p < 0.05). A clear relationship between gastritis and Helicobacter infection in dogs could not be established [11]. In 51.5% of the positive samples, only one Helicobacter species was identified while mixed infections were detected in 48.5%. H. heilmannii-like organisms were the most commonly found (66.7%) and H. salomonis was the second most prevalent species (51.5%) although it was mainly found in association with other NHPH (42%) rather than alone (9.1%). In the body area, the most frequently identified species was H. salomonis (44%) whereas in the antrum the most prevalent species was H. heilmannii-like (57.7%) [11].

DISCUSSION AND CONCLUSIONS

The expression pattern of type 1 and type 2 Lewis antigens in the canine stomach revealed a different distribution when compared to the human gastric mucosa [10]. The absence of type 1 Lewis antigens expression is explained by the lack of expression of enzymes with α1,4 fucosyltranferase activity. The demonstration that the glycosylation profile of canine gastric mucosa is different from human gastric mucosa suggests that alternative glycan receptors may be involved in Helicobacter spp. adhesion and may explain the differences between the prevalence of gastric helicobacters in both mammalian species. Furthermore, Helicobacter pylori and NHPH strains display different abilities to adhere to canine gastric mucosa.

As previously reported, the prevalence of Helicobacter spp. in the canine stomach is high [3]. According to the results of the in vitro binding assays, H. heilmannii s.s. was the species of NHPH group that showed the highest adhesion to the canine gastric mucosa. In agreement, H. heilmannii s.s. was also the NHPH species more prevalent in spontaneous infected dogs in our geographical location [10, 11]. The H. heilmannii s.s. adhesion value obtained in vitro was higher in the antrum than in the body compartment of the canine gastric mucosa. This finding was further corroborated by our in vivo data which identified this as the most prevailing species in the antral portion of the stomach.

Interestingly, the second most prevalent species found in the Portuguese dogs was H. salomonis, mainly in association with other NHPH. However, in the in vitro binding assays this was the species that adhered less to the canine stomach. Given these results and the high percentage of mixed infections comprising H. salomonis it can be suggested that, in the gastric context, the colonization capacity of this specific species may be enhanced when associated with other NHPH. With the present investigation it was concluded that the differences in canine gastric mucosa glycosylation profile play a role in the host adaptation of the gastric Helicobacter species and indicate that adhesion of NHPH involves different mechanisms, probably mediated by proteins with alternative receptor specificity yet to be characterized. Furthermore, despite the high prevalence and worldwide distribution of NHPH, geographic variations partially account for the prevalence of a specific Helicobacter species in the dog stomach.

REFERENCES