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Original article

Helicobacter felis and *Helicobacter bizzozeronii* induce gastric parietal cell loss in Mongolian gerbils

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Abstract

Non-*pylori* helicobacter infections are associated with gastritis, gastric ulcers and MALT lymphomas in man. Approximately 50% of these are caused by helicobacters commonly found in dogs and cats, including *Helicobacter felis*, *Helicobacter bizzozeronii* and *H. salomonis*. In contrast to *Helicobacter pylori,* the virulence mechanisms of these species are unknown. In this study the virulence of *H. felis*, *H. bizzozeronii* and *H. salomonis* was investigated in Mongolian gerbils. Female SPF gerbils were inoculated intragastrically with *H. felis*, *H. bizzozeronii* or *H. salomonis* and sacrificed 3 weeks later. Fundus and antrum samples were taken for bacterial detection by PCR. A longitudinal strip covering all stomach regions was taken for histology. Gastric colonization, inflammation, apoptosis, loss of parietal cells and cell proliferation were assessed. Controls and *H. salomonis* inoculated gerbils were negative in PCR. *H. felis* and *H. bizzozeronii* inoculated animals were positive. *H. felis* inoculated animals showed loss of parietal cells extending from the limiting ridge into the fundus. A high cell proliferation rate was noticed in the mucosal area devoid of parietal cells. A dense band of apoptotic cells and large numbers of *Helicobacter* bacteria were seen at the transition zone between affected and normal parietal cells. In *H. bizzozeronii* infected gerbils, this was less pronounced. Focal apoptotic loss of gastric epithelial cells was spatially associated with the presence of bacteria especially in *H. felis* and to a lesser extent in *H. bizzozeronii* infected gerbils. This loss of cells may lead to intestinal metaplasia.

Keywords: Helicobacter felis; *Helicobacter bizzozeronii*; *Helicobacter heilmannii*; Apoptosis; Mongolian Gerbil; Gastric inflammation; Parietal cells

1. Introduction

Helicobacter spp. are spiral, flagellated, gram-negative bacteria with worldwide prevalence, which colonize the gastrointestinal tract of human beings and animals. *Helicobacter pylori* in humans are associated with gastritis, gastric ulcerations [1], gastric adenocarcinoma and lymphoma of mucosal-associated lymphoid tissue (MALT lymphoma) [2]. In a small percentage of cases (0.2–0.6%), other bacteria, morphologically distinct from *H. pylori*, are found in the stomach of people suffering from gastritis [3]. These bacteria also belong to the *Helicobacter* genus. Due to their similar morphology and unculturable status, they were collectively and provisionally named *"Helicobacter heilmannii"* [4]. *"H. heilmannii"* is not only found in humans but also in a wide range of animal hosts such as cats [5], dogs [6], pigs and cattle. Through 16S rDNA sequence analysis, two different types of *"H. heilmannii"* could be identified in human gastric biopsies. Type 1 is phylogenetically closely related if not identical to "*Candidatus* H. suis", a bacterium found in the stomach of pigs [7]. Type 2 closely affiliates to helicobacters encountered in the stomach of cats and dogs, namely *Helicobacter felis, H. bizzozeronii* and *H. salomonis* [8]. Andersen and colleagues [9] were the first to actually culture an *"H. heilmannii"*-like strain from the stomach of an infected person, which was later identified as *H. bizzozeronii* [10]. Recent research concerning the molecular identification of non-*H.*

Abbreviations: cagPAI, *cag* pathogenicity island; *H.*, *Helicobacter*; HE, hematoxylin and eosin; MALT, mucosal-associated lymphoid tissue; PAS, periodic acid schiff; PCR, polymerase chain reaction; SPF, specific pathogen-free.

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pylori spiral organisms in human gastric samples showed "*Candidatus* H. suis" and *H. salomonis* as the most prevalent ones. *H. felis* and H. *bizzozeronii* were present, though less common in human gastric samples [11]. It has been postulated that *"H. heilmannii"* is indeed zoonotic in nature [12], but solid proof of this hypothesis is still lacking.

H. pylori strains may differ in virulence. More virulent strains can cause damage to gastric epithelial cells that has been attributed to the presence or absence of certain virulence genes [13]. The virulence mechanisms of non-*H. pylori* gastric helicobacters however are hitherto largely unknown nor is it clear whether the same differences in terms of virulence are present among these organisms. The Mongolian gerbil is used to study *H. pylori* induced stomach ulcers and gastric carcinogenesis [14,15]. The gerbil model appears to be more susceptible to *H. pylori*-induced gastric carcinogenesis [16] and inflammatory changes may progress more rapidly and aggressively than in other laboratory animal models or in man [14,17]. Court et al. were the first to describe the successful colonization of Mongolian gerbils with a *H. felis* strain [18]. *H. bizzozeronii* and *H. salomonis* however were not included. In the present study, we used a Mongolian gerbil model to examine and compare the virulence of *H. felis, H. bizzozeronii* and *H. salomonis* and their effect on the oxyntic mucosa.

2. Materials and methods

2.1. Animals

Twenty-three specific pathogen-free (SPF) female Mongolian gerbils (Elevage Janvier, Le Genest St Isle, France) of 6 weeks old were barrier-maintained in a room with controlled environment and housed in pairs in filtertop cages on autoclaved (121 °C, 15 min) wood shavings. They were fed an autoclaved diet containing 18% protein (Teklad Global Rodent Diet, Harlan NL, Horst, the Netherlands) and received autoclaved water ad libitum. The experimental protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University.

2.2. Bacterial strains and inocula

The bacterial strains used in this study were *H. bizzozeronii* CCUG 35545, *H. salomonis* CCUG 37845 (both kindly provided by P. Vandamme) and *H. felis* ATCC 49179 (kindly provided by R. Ferrero). They were grown on brain–heart infusion (BHI; Oxoid Ltd., Basingstoke, UK) agar to which Skirrow supplement (Oxoid Ltd), vitamins (Vitox®, Oxoid Ltd), amphotericin B (Fungizone®, Bristol-Myers Squibb, New York, USA) and 10% horse blood were added. The plates were incubated (37 °C, 48 h) in flushing jars under microaerophilic conditions, created by evacuating 80% of the normal atmosphere and introducing a gas mixture of 84% N_2, 8% H_2 and 8% CO_2. The bacteria were harvested and the final concentration was adjusted to an optical density of 1.0 at 660 nm corresponding to approximately 10^8 cfu/ml [19].

2.3. Experimental procedure

Ten animals were inoculated three times (with 48 h time interval) with 0.4 ml *H. felis* ATCC 49179 suspension. The same procedure was conducted with *H. bizzozeronii* CCUG 35545 and *H. salomonis* CCUG 37845, but this time five animals were used for each bacterial strain. Inoculation was performed intragastrically using a ball-tipped gavage needle. Three gerbils were inoculated with sterile culture medium and served as uninfected controls. All the animals were killed 3 weeks after the first inoculation using isoflurane anaesthesia followed by cervical dislocation. The stomach was resected and samples were taken for polymerase chain reaction (PCR) analysis and histological examination as described below.

2.4. PCR analysis

Samples of fundus and antrum (ca. 4 mm^2) from controls, *H. felis, H. bizzozeronii* and *H. salomonis* inoculated animals were taken for DNA extraction (DNeasy Tissue Kit, Qiagen, Hilden, Germany) and multiplex PCR analysis as described elsewhere [20]. This PCR test allows the discrimination between *H. felis*, *H. bizzozeronii* and *H. salomonis*, based on the transfer RNA intergenic spacers of *Helicobacter* species and the urease gene of *H. felis* (Table 1). Briefly, PCR reactions were performed in a volume of 10 µl containing a final primer concentration of 0.1 µM of each of the fluorescently (TET™, NED™, HEX™; Applied Biosystems, Foster City, USA) labeled oligonucleotides, 0.1 µM of each of the unlabeled primers, 40 µM of each deoxynucleoside triphosphate (Amersham Pharmacia Biotech, Little Chalfont, England), 3 mM $MgCl_2$, Polymerase Taq platinum (Invitrogen Life Technologies, Carlsbad, USA) 0.03 U/µl, and

Table 1
Oligonucleotide primers used in the multiplex PCR

Primer and label	Sequence	Source or reference (nt positions)[a]	Target sequence
T3B (TET)	5′ AGG TCG CGG GTT CGA ATC C	[21]	tRNA genes
HT135R-tail	5′ tail- ACC AAC TGG GCT AAG CGA CC	[20]	tRNA genes
Bi1F (HEX)	5′ AAC CAA YAG CCC CAG CAG CC	*H. felis* urease, nt 936–955	Urease gene *H. bizzozeronii*
Bi2R	5′ TGG TTT TAA GGT TCC AGC GC	*H. felis* urease, nt 1309–1290	Urease gene *H. bizzozeronii*
Fe1F (NED)	5′ TTT GGT GCT CAC TAA CGC CCT C	*H. felis* urease, nt 966–987	Urease gene *H. felis*
Fe3R	5′ TTC AAT CTG ATC GCG TAA AG	*H. felis* urease, nt 1403–1382	Urease gene *H. felis*

[a] Nucleotide (nt) positions are based on *H. felis* urease X69080.