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Helicobacter suis:
further characterization of the agent
and its possible impact on the course of Parkinson's disease

Helena Berlamont
July 6, 2021

PROMOTERS

Prof. dr. F. Haesebrouck
Faculty of Veterinary Medicine,
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Prof. dr. A. Smet
Faculty of Medicine and Health Sciences,
University of Antwerp

Prof. dr. R. Vandenbroucke
Faculty of Sciences,
Ghent University

Curriculum Vitae

Helena Berlamont was born on September 19, 1992 in Ghent, Belgium. After successfully completing her secondary education at College O.-L.-V. Ten Doorn in Eeklo in 2010, she started studying Veterinary Medicine at Ghent University, Belgium. In 2016, she obtained her Master's degree in Veterinary Medicine, main subject Research, with magna cum laude.

In October 2016, she started her PhD research at the Department of Pathology, Bacteriology and Avian Diseases at the Faculty of Veterinary Medicine, financed by the Special Research Fund of Ghent University (BOF GOA 01G01014). Her research focused on *Helicobacter suis*, a stomach bacterium naturally colonizing pigs and non-human primates, that can also cause disease in humans. It was a joint PhD with the University of Antwerp, Faculty of Medicine and Health Sciences. Helena also obtained the certificate of the Doctoral Training Program of Life Sciences and Medicine and the certificate of the Interdisciplinary Program in Healthcare Innovation.

Helena Berlamont is (co-)author of several papers in international peer-reviewed journals. She also actively contributed to several (inter)national conferences and symposia.

Where?

Tuesday July 6, 2021, at 16h (4 PM, CEST)

Auditorium A
Faculty of Veterinary Medicine
Ghent University, Campus Merelbeke
Salisburylaan 133, Merelbeke

Due to the current COVID-19 measures, the public defense will be held with limited public, but can be followed via livestream.

The presentation will be given in **Dutch**.

Registration

If you wish to participate in the (video)conference, please send an e-mail to Helena.Berlamont@UGent.be to receive a personal invitation.

Members of the examination committee

Prof. dr. D. Maes
Chairman of the examination committee
Faculty of Veterinary Medicine, Ghent University

Prof. dr. C. Delesalle
Secretary of the examination committee
Faculty of Veterinary Medicine, Ghent University

Prof. dr. J.-P. Timmermans
Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp

Prof. dr. C. Van Steenkiste
AZ Maria Middelares, Ghent

Dr. C. De Witte
Faculty of Pharmaceutical Sciences, Ghent University

Summary thesis

Non-*Helicobacter pylori Helicobacter* (NHPH) species naturally colonize the stomach of animals, but some of them can also infect the human stomach. The most prevalent NHPH species in the human stomach is *Helicobacter suis* (*H. suis*), which naturally colonizes the stomach of pigs and non-human primates. Transmission of *H. suis* from pigs to humans might occur through direct or indirect contact or through the food chain. NHPHs have been detected in 0.2 to 6% of gastric biopsies from human patients with severe gastric complaints. NHPH infections have been associated with gastritis, gastric and duodenal ulcers, and MALT lymphoma in humans. However, due to their fastidious nature, NHPHs are difficult to isolate and cultivate *in vitro*, which has hampered the investigation of their pathogenesis in humans. Besides, diagnosis of NHPH infections is difficult and most techniques used for *H. pylori* diagnosis lack sensitivity and/or are not able to discriminate between different gastric *Helicobacter* species. It is therefore possible that the prevalence of NHPHs in humans is currently underestimated.

In **Chapter 1**, we tried to gain insights into potential virulence factors of *H. suis* and *H. heilmannii* (another zoonotically important cat-/dog-associated NHPH) playing a role in the adherence to the human gastric mucosa. Using RNA sequencing, we investigated bacterial genes that are differentially expressed in a *H. suis* (HS1) and *H. heilmannii* (ASB1) strain when adhered to the human gastric epithelial cell line MKN7 in comparison to non-adhered *H. suis* and *H. heilmannii* bacteria. A list of 134 (83 up-regulated and 51 down-regulated) differentially expressed genes ($p_{adj} \leq 0.01$; fold change ≥ 2) was identified for the adherent *H. suis* strain HS1 and a list of 143 (60 up-regulated and 83 down-regulated) differentially expressed genes ($p_{adj} \leq 0.01$; fold change ≥ 2) was identified for the adherent *H. heilmannii* strain ASB1. Differentially expressed genes of the *H. suis* and *H. heilmannii* strains belonged to multiple functional classes, indicating that adhesion of both strains to human gastric epithelial cells evokes pleiotropic adaptive responses. According to BLASTp analyses, only 2 genes (i.e. the urease accessory protein gene (*UreF*) and ferrochelatase gene) were commonly up-regulated in *H. suis* (HS1) and *H. heilmannii* (ASB1). Four genes (i.e. recombinase A gene (*RecA*), tellurium resistance gene (*TerD*) of *H. heilmannii* with its *H. suis* homolog general stress protein 16U gene, KH domain RNA binding protein gene (*YlqC*) of *H. heilmannii* with its *H. suis* homolog hypothetical protein gene, and heat shock protein gene (*GrpE*)) were commonly down-regulated in both pathogens according to BLASTp analyses. These findings suggest that the porcine *H. suis* strain HS1 and the feline *H. heilmannii* strain ASB1 use distinct pathways when adhering to human gastric epithelial cells. Indeed, several differences between *H. suis* (HS1) and *H. heilmaniii* (ASB1) were found when comparing up- and down-regulated genes implicated in bacterium-host interactions (i.e. *Urease* genes, *NikB*, flagella encoding genes, tumor necrosis factor α inducing protein gene (*Tipα*), *gamma-glutamyl transpeptidase* (*Ggt*), outer membrane protein (OMP) genes, and the gene encoding peptidyl-prolyl cis, trans-isomerase (*ppi*)). Genes that are significantly up-regulated upon adherence to gastric epithelial cells might play a role in gastric pathogenesis and in the ability of *Helicobacter* to have effects beyond the stomach as well.

Mounting evidence suggests a potential role of *H. suis* in Parkinson's disease (PD). Based on the finding of an earlier study whereby the eradication of *H. suis* led to an improvement of both the gastric and neurological symptoms in a PD patient, we hypothesized that a gastric *H. suis* infection might aggravate PD-related symptoms, such as motor dysfunction, and possibly PD-related pathology. To investigate our hypothesis, we used the 6-hydroxydopamine (6-OHDA) PD mouse model. 6-OHDA injection in the brain striatum causes degeneration of dopaminergic neurons, a major

pathological hallmark seen in PD resulting in motor dysfunction. Two animal experiments with *H. suis* infected mice (acute and chronic infection) were performed (**Chapter 2**). Mice were stereotactically injected in the left brain striatum with either 6-OHDA or the vehicle of 6-OHDA at 21 days (experiment 1) or 500 days after intragastric *H. suis* inoculation (experiment 2). Control groups receiving only the growth medium of *H. suis* were included, resulting in 4 groups of mice in both experiments, i.e. 1) control-vehicle, 2) control-6-OHDA, 3) *H. suis*-vehicle, and 4) *H. suis*-6-OHDA. The mice were subjected to several behavior and motor function tests (i.e. traversal beam, pole, footprint analysis, cylinder) before intragastric inoculation (only in experiment 1), before intrastriatal injection, and before euthanasia. All animals were euthanized at 7 days after intrastriatal injection. Tyrosine hydroxylase staining on brain sections showed a lower % loss of dopaminergic neurons in the *H. suis*–6-OHDA groups compared to the control–6-OHDA groups. Correspondingly, just prior to euthanasia, motor function of the *H. suis*–6-OHDA groups was better compared to the control–6-OHDA groups. In both experiments, no significant changes in behavior and motor function between the 4 groups could be demonstrated before intrastriatal injection, suggesting that the gastric *H. suis* infection alone did not result in behavior and/or motor function alterations. Our study shows that a gastric *H. suis* infection protects, at least partially, against 6-OHDA-induced dopaminergic cell loss and motor function impairment in a left unilateral intrastriatal 6-OHDA PD mouse model. This potential protective effect of *H. suis* against 6-OHDA-induced toxicity and motor function impairment needs further elucidation.

There are currently not many diagnostic tools available for reliable diagnosis and species identification of NHPH infections in humans. PCR in combination with sequencing of positive PCR amplicons is currently preferred for detection and differentiation of gastric NHPHs. However, this method is labor-intensive, time-consuming, and expensive. Therefore, we investigated whether matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) might constitute a potential tool for future diagnosis of NHPH infections (**Chapter 3**). This technique is based on the generation of complex fingerprints of specific biomarker molecules by measuring the exact mass/charge ratio of peptides and proteins. Inadequate MALDI-TOF MS identification may occur due to the incompleteness of mass spectrometry databases. The current MALDI Biotyper reference database library only contains 24 *Helicobacter* entries, most of which belonging to the enterohepatic *Helicobacter* species and only 7 belonging to gastric species (i.e. only *H. pylori* strains). Therefore, we identified 93 gastric *Helicobacter* isolates of 10 different NHPH species using MALDI-TOF MS in order to establish a more elaborate *Helicobacter* reference database. While the MALDI Biotyper database was not able to correctly identify any of the isolates, the newly created in-house database correctly identified all individual mass spectra and resulted in 82% correct species identification based on the two highest log score matches (with log scores ≥ 2). However, spectra obtained under different growth conditions, for example dry versus biphasic growth conditions, may differ, and agar medium-related peaks may influence reliability of the obtained results. Our results suggest that MALDI-TOF MS allows rapid differentiation between gastric *Helicobacter* species, provided that an extensive database is at hand and variation due to growth conditions and agar medium-related peaks are taken into account. However, further research to enable its use in clinical practice is needed.

To improve treatment strategies of *H. suis* infections in humans, we determined the *in vitro* susceptibility of 35 *H. suis* isolates (i.e. 20 porcine isolates and 15 non-human primate isolates) to 15 antibiotics (**Chapter 4**). A monomodal distribution of minimal inhibitory concentrations (MICs) was seen for β-lactam antibiotics, macrolides, gentamicin, neomycin, doxycycline, metronidazole, and rifampicin. Presence of a bimodal distribution of MICs indicated that 2 porcine isolates (i.e. HS6 and HS10) did not belong to the wild-type population for fluoroquinolones. This was also the case for 1 porcine isolate (i.e. HS4) for tetracycline, 1 porcine (i.e. HS5) and 2 primate isolates (i.e. HSMm R04052c and HSMm R07055b) for lincomycin, and 1 primate isolate (i.e. HSMm R07055b) for spectinomycin. Single nucleotide polymorphisms (SNPs) were present in the *gyrA* gene of the isolates not belonging to the wild-type population for fluoroquinolones and in ribosomal protein encoding genes of the isolates not belonging to the wild-type population for tetracycline and spectinomycin. MICs of ampicillin, tetracycline, and doxycycline were higher for porcine *H. suis* isolates compared to primate isolates and in these porcine isolates SNPs were detected in genes encoding penicillin binding and ribosomal proteins. Taken together, our study indicates that acquired resistance occasionally occurs in *H. suis* isolates and that porcine isolates may be intrinsically less susceptible to aminopenicillins and tetracyclines than primate isolates. Since human *H. suis* infections have been associated with porcine *H. suis* strains, the intrinsically lower susceptibility to aminopenicillins and tetracyclines, and the occasional presence of acquired resistance should be taken into account when treating *H. suis* infections in human patients.