

Non-*Helicobacter pylori* in feline gastrointestinal neoplasia.

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INTRODUCTION

Although the microbiota is intimately involved in gastrointestinal (GI) homeostasis and immune balance, several studies in animal models and humans highlighted bacterial-induced lower grade smouldering stomach and gut inflammations, as optimal local environments, inducing malignant transformation. While *Helicobacter pylori* (*H. pylori*) is causally linked to the development of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma in humans, other distinct *Helicobacter* species, designated non-*H. pylori helicobacters* (NHPH) have been detected in gastric and intestinal mucosa of several animal species, including cats. Over the last two decades, bacterial mucosal colonisation, particularly involving NHPH, has been highlighted as a potential oncogenic factor in feline gastric lymphoma and poorly differentiated large intestinal adenocarcinoma.

The group of NHPH that mainly colonizes the GI mucosa of cats includes *H. felis*, *H. bizzozeronii*, *H. salomonis*, *H. cynogastricus*, *H. baculiformis*, and *H. heilmannii sensu stricto*. *H. heilmannii*, seems to be particularly involved in gastrointestinal feline oncogenesis.

AIM

Our main goal is to assess the occurrence of *Helicobacter* spp. in distinct regions of the feline gastrointestinal tract and to correlate it with putative histopathological alterations.

Our specific objective in this work was to validate simple molecular diagnostic techniques to identify and list the presence of specific *Helicobacter* species in feline gastrointestinal (GI) tract.

METHODOLOGY

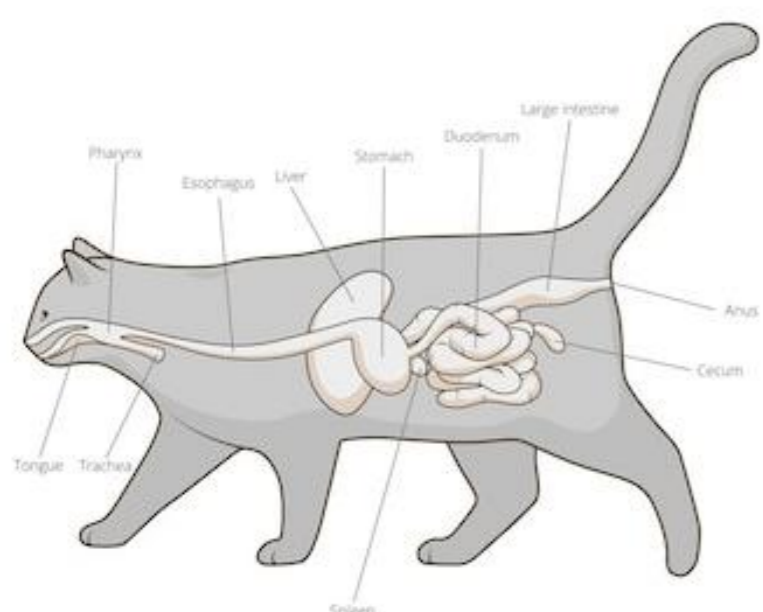
1. Selection of feline formalin fixed paraffin-embedded samples.
2. DNA extraction from 25 paraffin-embedded tissue.
3. Identification of *Helicobacter* spp. by PCR.
4. Sequencing of PCR-positive samples.
5. Comparison of sequenced PCR products with GenBank/EMBL/DDBJ databases using the Basic Local Alignment Search Tool (BLAST).

Biological samples

Selected samples

- Samples were retrieved from the DNATECH Veterinary Laboratory's archive, from cases sent to the laboratory for routine histopathological diagnosis, during the year of 2020 and 2021.
- 249 cases of cats with a previous histopathological diagnosis of gastrointestinal lymphoma or other gastrointestinal disease were selected.
- Formalin fixed paraffin embedded (FFPE) tissue from any portion of the GI tract were obtained in case of involvement of both stomach and intestine.

Digestive system of the cat



104 cases of Gastrointestinal Lymphoma

149 with other Gastrointestinal Disease (neoplastic and non-neoplastic)

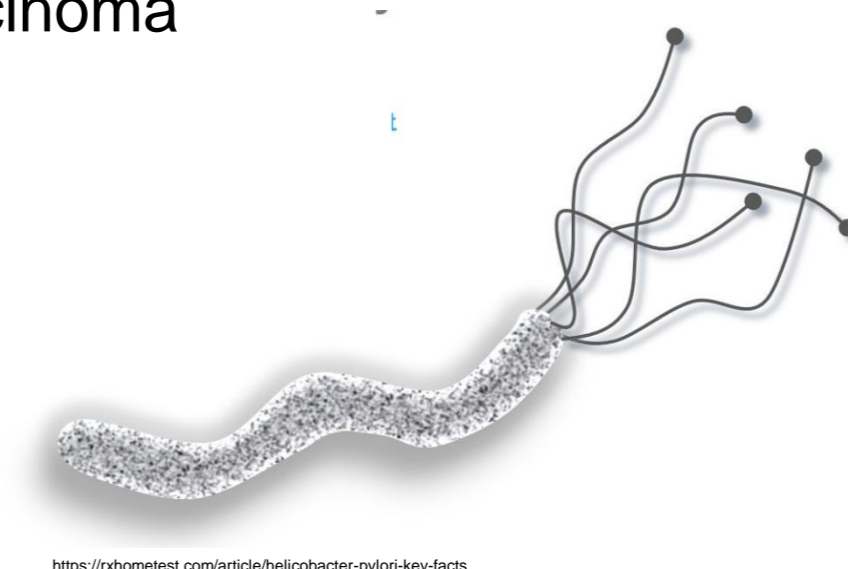
Sample characterization

Gastrointestinal lymphomas	
Gastric Lymphomas	7
Intestinal Lymphomas	94
Gastrointestinal Lymphomas	3
Neoplastic gastrointestinal diseases	
Gastrointestinal Adenocarcinoma	18
Intestinal Adenoma	1
Intestinal Angiosarcoma	1
Intestinal Leiomyosarcoma	1
Intestinal Mast Cell Tumor	1
Non- Neoplastic gastrointestinal diseases	
Colitis	12
Enteritis	86
Gastritis	25

RESULTS: *Helicobacter* species identification

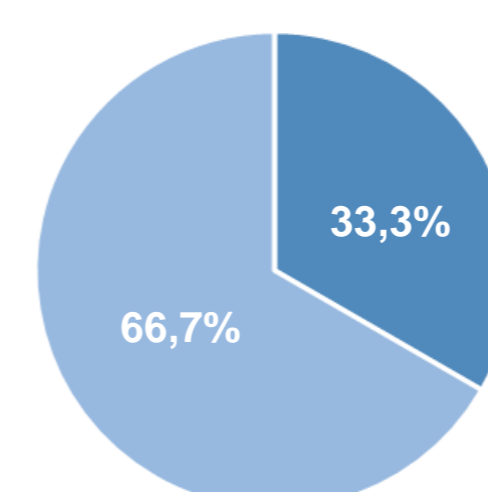
DNA was extracted from 25 FFPE blocks, corresponding to 18 cases:

- 5 Gastric Lymphomas (2 high grade)
- 8 Intestinal Lymphomas (7 high grade, 1 low grade)
- 3 High grade Gastrointestinal Lymphomas
- 1 Gastric Adenocarcinoma
- 1 Gastritis



Helicobacter spp. in feline gastrointestinal samples

Helicobacter spp. were identified by PCR in 33,3% of analyzed samples.



- 1 High grade Gastric Lymphoma
- 1 High grade Intestinal Lymphoma
- 3 High grade Gastrointestinal Lymphomas (in stomach samples)
- 1 Gastritis

■ PCR-positive cases ■ PCR-negative cases

3 samples were Giemsa-stained positive. *Helicobacter* spp. PCR only amplified DNA in 1 sample.

Non-*Helicobacter pylori* colonizes feline GI tract

DNA sequencing allowed the identification of specific NHPH in each sample.

- *H. heilmannii* was the most frequent species, identified in 4 samples
- *H. ailurogastricus* was identified in 1 sample
- *H. felis* and *H. bizzozeronii* were identified in the same sample

CONCLUSION

Our preliminary results confirm the occurrence of NHPH in distinct regions of the feline GI tract. However, the PCR sensitivity must be improved and more samples will be needed in order to establish a correlation between specific *Helicobacter* species and feline gastrointestinal manifestations.