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OCCURRENCE OF *HELICOBACTER* IN CATS

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SUMMARY

The presence of *Helicobacter* spp. in the stomach of cats has been reported worldwide but the documentation on the occurrence of these organisms in cats in Malaysia is lacking. This preliminary study aimed to determine the occurrence of helicobacters in cats in the country. *Helicobacter*-like organisms were detected by rapid urease testing and Gram staining in gastric biopsy samples in 80% (24 of 30) and 20% (6 of 30) of samples, respectively. Culture was positive for one cat (3.3%) and identified as *Helicobacter* sp. Further study is needed to determine the infections in these pet animals.

Keywords: *Helicobacter*, cats, gastric biopsies

INTRODUCTION

One of the first animal species identified as a member of Helicobacter was *Helicobacter felis*, a 'spirillum' originally isolated from a cat stomach and shown to also colonise dogs (Otto *et al.*, 1994). Currently a number of species are found to colonise the gastric mucosa of cats and dogs . On occasions, cats have been found to harbour *H. pylori* (Handt *et al.*, 1994). *Helicobacter bizzozeronii* (also referred to as Gastrospirillum or *Helicobacter heilmannii*) and *H. felis* are collectively referred to as gastric helicobacter-like organisms or GHLOs. The occurrence of helicobacters in cats and dogs has been reported worldwide, ranging from 13.6% to 100% in cats (Jalava *et al.*, 1998; Papasouliotis *et al.*, 1997) and 51% to 90.7% in dogs (Jalava *et al.*, 1998; Eaton *et al.*, 1996).

To date, there is no published data on the prevalence of helicobacters in cats in the country. In recent years, concerns have been expressed over pet animals, in particular cats and dogs, to be the source of Helicobacter infection in man.

STUDY CONDUCTED AND DISCUSSION

In our preliminary study, gastric biopsy samples were obtained from 30 cats at a local animal shelter; two samples were taken from each cat, at the gastric corpus and antrum sites. Twenty-four (24) or 80% of the gastric biopsy samples were positive on rapid urease test and only those which turned pink within 4 hours were examined further. In almost all the previous studies, in order to detect Helicobacter sp., gastric biopsies were subjected to urease testing, microbiological culture and gram staining, with some studies including histological evaluation using H&E or Warthin-Starry silver stain. According to Simpson and Burrows (1997), "evaluation of urease production by biopsies is commonly used as a rapid screening that relies on urease-producing bacteria in gastric biopsies (most likely Helicobacter spp.) releasing ammonia from urea in the test solution and causing a pH change in an indicator solution; the time taken for a colour change to occur is thought to be related to the number of bacteria present in the biopsy." Six or 20% of the Gram stained-direct smears of the samples were observed to show tightly coiled, spiral shaped bacteria, similar to those described by Lee *et al.*, (1988) and Otto *et al.* (1994) as typical of Helicobacter sp. The surface of the gastric mucosa was washed aseptically, scraped and cultured on Columbia blood agar with *H. pylori* supplement (OXOID) added. The agar plates were then incubated at 37°C under microaerophilic conditions for 3-5 days. On culture, only one sample (3.3%) showed growth with a thin film watery-like appearance; it was positive for *Helicobacter* spp. Eaton *et al.* (1996) reported pinpoint colonies of Helicobacter on culture. The biochemical tests carried out were catalase production, nitrate reduction, alkaline phosphatase hydrolysis, indoxyl acetate hydrolysis, growth at 42°C and resistance to cephalothin.

The failure to culture helicobacters is not surprising as helicobacters are fastidious in growth requirements. A similar finding was reported by Nieger *et al.* (1998): their culture was positive for one cat only although 78% (45 of 58) and 79% (46 of 58) of the biopsy samples were positive on rapid urease test and Gram staining respectively. This was also indicated by Jalava *et al.* (1998) in their study who stated that 56.4% of biopsies in which helicobacters were indicated such as by rapid urease test, failed to give positive results on culture. Eaton *et al.* (1996) recovered helicobacters from only 6 of 39 gastric biopsy samples from dogs. Thus, in most cases the helicobacters were not culturable. This could possibly be due to the organisms being viable but not culturable (VNC) when exposed to environmental stresses, such

as in the presence of oxygen, exposure to antimicrobial agents. According to Papasouliotis *et al.* (1997), the lack of growth on Helicobacter selective media suggestthat the GHLOs observed were neither *H. pylori* nor *H. felis*

In another Malaysian study done by Nur Zaliza (2004) on the gastric biopsy samples of 30 cats and dogs, it was reported that 66.0% were positive by rapid urease test, 16.6 % by Gram staining of the direct smear samples and on culture, 36.6% showed growth. One of the reasons for the high isolation rate upon culture in this study was probably because the animals were fasted for 12 hours before gastric biopsies were carried out whereas in our study, the animals were not fasted. This could have resulted in the presence of high numbers of contaminating bacteria in the cultures which inhibited the growth of helicobacters.

The use of more sophisticated and expensive techniques, such as, electron microscopy (scanning and transmission) and molecular techniques which include PCR, 16S rRNA sequencing, whole-cell protein profiling and AFLP analysis can certainly detect the presence and identify accurately the species of helicobacter in the gastric tissues; such techniques have been reportedly used to identify helicobacters in faecal materials.

Further work is required on a larger number of cats and also dogs, possibly from different localities and of different health status, such as those clinically healthy, those with gastrointestinal problems, and cats and dogs kept as pets or who are stray animals, and to use techniques that can identify Helicobacter to species level as well as to culture them.

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