

HELICOBACTER INFECTION IN PET AND FOOD ANIMALS: OCCURRENCE AND ZOONOTIC POTENTIAL

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Summary

The presence of *Helicobacter* spp. in the stomach of pet and other animal species has been reported worldwide but the documentation of occurrence of the organism in these animals in Malaysia is lacking. This paper discussed the occurrence of helicobacters in cats and dogs (pet animals) as well as in chickens and pigs (food animals) and the zoonotic potential of these organisms in animals that may infections cause in humans. The occurrence of helicobacters in animals in Malaysia from limited available studies is presented.

Keywords: helicobacters, cats, dogs, chickens, birds, zoonotic implications

INTRODUCTION

Helicobacters are gastric spiral- or curved- shaped, gram negative microorganism which have been observed in animals and humans for more than a century. In animals, these microorganisms were first described by Rappin in 1881 in the stomachs of dogs and cats (Cattoli *et al.*, 1996). It was only in 1982 when *Helicobacter pylori* was discovered in diseased gastric tissue of humans and that the subsequent recognition of its prevalence and clinical importance as a cause of gastric ulcers in man, that led to the studies of these organisms in animals with renewed and increased interest.

To date, there are at least 32 species of helicobacters, isolated mainly from the stomachs, intestines and also livers of various animals, including dogs, cats, ferrets, monkeys, pigs, sheep, rats, mice, hamsters, chicken, and birds as well as man (Haesebrouck *et al.*, 2009; Fox, 2002; Fox, 1999; Jalava *et al.*, 1998; Wesley, 1997) (Table 1). These species may be grouped as gastric helicobacter species causing enteric disorders and enterohepatic helicobacter species which are associated with hepatobiliary diseases (Solnick and Schaeur, 2001; Milosavljevic, 2001). In man, *H. pylori* is well known and most importantly in terms of its impact on human health; other helicobacters species that are associated with gastric and hepatic diseases are termed as non-*H. pylori* helicobacters (NHPHs) (Fox, 2002; Milosavljevic, 2001)

The paper discussed the occurrence of helicobacters in pet animals, particularly cats and dogs, avian species namely chickens and pigs and their potential public health significance. Cats and dogs are among the most kept pet animals and chickens and pigs are intensively reared to provide meat which are widely consumed. Although ruminants too are intensively reared for meat production, however information regarding the occurrence of helicobacters in these ruminants are very scarce.

Prevalence of *Helicobacter* infections in cats and dogs

The diagnosis on the presence of helicobacters in mammalian animals is mostly through the use of invasive methods, involving the analysis of gastric biopsy tissues usually obtained during gastric endoscopy. These biopsies are subjected to urease production testing, microbiological culture, gram staining, histological examination with Haematoxylin and Eosin (H&E) or Warthin-Starry silver staining and electron microscopy. There are difficulties in the isolation of helicobacters due to their fastidious nature and also considerable problems in accurately identifying *Helicobacter* spp. using conventional methods, therefore molecular techniques are preferred. These molecular techniques include polymerase chain reaction (PCR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and 16S rRNA gene sequencing. The non-invasive methods involve serology (the measurement of circulating antibodies (IgG)) and urea breath test; the latter test reported as extremely useful for the diagnosis of helicobacteriosis in humans, monkeys, ferrets and pigs and for assessing their response to treatment (Simpson and Burrows, 1997)

Helicobacter felis, a 'spirillum' originally isolated from the cat stomach is also shown to colonize dogs (Otto *et al.*, 1994). To date, there are number of species found to colonize the gastric mucosae of cats and dogs as well as other animals (Table 1). On occasion, cats have been found to harbour *H. pylori* (Handt *et al.*, 1994). *Helicobacter bizzoeronii* (also referred to as *Gastrophillum* or *Helicobacter heilmannii*) and *H. felis* are collectively termed as gastric helicobacter-like organism or GHLOs. Depending on types of detection methods and locations, the presence of helicobacters in gastric biopsy tissues of cats and dogs ranged from 41% and up to 100%. Apart from gastric mucosae, a recent study by Tabrizi *et al.* (2010) found *Helicobacter* spp. in 93% of oral secretions of stray cats while Recordati *et al.* (2007) detected 71% of dogs

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harbour *Helicobacter* spp. DNA in the oral cavity (dental plaque and/or saliva).

Occurrence of *Helicobacter* in food animals

With regard to helicobacters in food animals, reports were mainly available on their occurrence in poultry and pigs. Unlike the helicobacters in cats and dogs, helicobacters in poultry were isolated from intestinal or caecal contents, faeces, livers and carcasses. The samples were isolated using culture media, identified using extensive phenotypic testing and confirmed using molecular techniques as mentioned above. In a number of studies which isolated Campylobacter-like organism (CLOs) from poultry, upon further analyses such as using nucleic acid technique (nucleotide sequence of the 16S rRNA gene) and whole cell protein analysis (SDS-PAGE), identified them as *Helicobacter pullorum* (Stanley *et al.*, 1994; Attabay *et al.*, 1998). CLOs were also found in other animals, such as cats and pigs which were later identified as *Helicobacter* species (Patterson *et al.*, 2003; Shen *et al.*, 2001).

In avian species, which are commonly colonized with thermophilic campylobacters, a technique is needed for rapid detection and differentiation of these organisms from *H. pullorum*; the ability and efficacy of multiplex PCR (Neubauer and Hess, 2003; Miller *et al.*, 2006) and PCR-RFLP analysis of the 16S rRNA gene (Marshall *et al.*, 1999) has been reported. A number of studies isolated *H. pullorum* in chickens, such as Ceelen *et al.* (2006) in Belgium found 34% and 32% *H. pullorum* in caeca and colon respectively, 11% of jejunum and 5% of liver of broiler chickens, Miller *et al.* (2006) in Australia found 27% in broilers in one farm and Zanoni *et al.* (2007) in Italy reported all 60 chickens from 15 farms were positive for *H. pullorum*. The organisms were found in 76% of turkeys (Zanoni *et al.*, 2011).

It is reported that *H. suis* is the main *Helicobacter* species colonizing the stomachs of pigs. Its prevalence in pigs at slaughter age is 60% or more (Haesebrouck *et al.*, 2009). Park *et al.* (2004) examined the stomach samples of 10 pigs of 6-months of age after slaughter and found the 95% infection rate of *Helicobacter* spp. using PCR assay compared to 62.5% when using silver staining. Pigs may also be infected with *H. heilmannii* as high as 100% (Solnick and Schauer, 2001).

Occurrence of *Helicobacter* in cats, dogs and chickens in Malaysia

To date, there is very scanty published data on the prevalence of helicobacters in cats and dogs in the country. In a preliminary study by Ravindran (2002), 80% of the gastric biopsy samples from 30 cats were positive by rapid urease test; while 20% of the gram stained-direct smears of the samples were similar to those described by Lee *et al.* (1988) and Otto *et al.* (1994). On culture, only one sample

(3.3%) showed growth with a thin film watery-like appearance which was positive for *Helicobacter* spp. In another study 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% were presumptively identified as helicobacters and on culture, 36.6% showed growth. Also in this study, upon using the PCR technique, 16.6% of the gastric mucosa samples were positive for *Helicobacter* species.

The occurrence of helicobacters in chickens was reported in a study by Soe Soe Wai *et al.* (2012); *H. pullorum* was isolated from 25% village chickens from five markets and 24.6% in broiler chickens in six farms using culture methods and confirmed by PCR assay.

Public Health Significant

It has been reported that domestic animals may serve as reservoirs for human *Helicobacter* infection. It was discovered that a small subset of human gastritis cases were not caused by *H. pylori* but by other gastric helicobacter-like organism (GHLOs) which were reported to be morphologically identical to those found in animals (Otto *et al.*, 1994). Table 1 shows helicobacters that are zoonotic or has potential as they have been reported to occur in both human and animals (Haesebrouck *et al.*, 2009; Fox, 2002; Solnick and Schauer, 2001; Fox, 1999). In recent years, there has been concern over pet animals, in particular cats and dogs, and pork and chicken meat, as the source of *Helicobacter* infection in man. Four modes of transmission have been proposed for *H. pylori* which are faecal-oral spread, oral-oral spread via salivary secretions, pet-to-human as well as human-to-pet transmission and ingestion of contaminated food and water (Wesley, 1997). In man, *H. pylori* is the major agent of chronic diffuse superficial gastritis, plays causative role in peptic ulcers and is considered a cofactor in the development of gastric cancers (Neiger *et al.*, 1998).

Several suggestions have been made that among pet animals, cats are more likely a potential reservoir of *H. pylori*. Handt *et al.* (1994) cultured *H. pylori* from six young adult cats and reported the possibility that *H. pylori* infected may be a zoonotic disease, with transmission occurring from cats to humans. Fox *et al.* (1999) reported that *H. pylori* was cultured from salivary secretions in 6 of 12 (50%) cats and from gastric fluid samples in 11 of 12 (91%) cats and from faeces in 4 of 5 (80%) cats upon PCR technique and amplifying *H. pylori* 26kDa surface protein. The possibility that *H. pylori* may be transmitted to human from cats may also be brought about the facts that cats are popular pets and there is a significant cat-human contact; also, cats vomit occasionally and continuously grooming

Table 1. Species of *Helicobacter* isolated from cats, dogs and other animals.

Animals	<i>Helicobacter</i> species
Cats	<i>H. felis</i> *, <i>H. pamentensis</i> , <i>H. pylori</i> , <i>H. canis</i> *, <i>H. heilmannii</i> *, <i>H. salomonis</i> *, <i>H. helmannii</i> *, <i>H. bizzozeronii</i> *, <i>H. baculiformis</i> *
Dogs	<i>H. felis</i> *, <i>H. canis</i> *, <i>H. bilis</i> , <i>H. heilmannii</i> *, <i>H. salomonis</i> *, <i>H. rappini</i> *, <i>H. cinaedi</i> *, <i>H. salomoni</i> , <i>H. fennelliae</i> *, <i>H. bizzozeronii</i> *, <i>H. cynogastricus</i> *
Monkeys	<i>H. nemestrinae</i> *, <i>H. cinaedi</i> * (rhesus monkey), <i>H. heilmannii</i> *, <i>H. fennelliae</i> * (macaque)
Hamsters	<i>H. canaedi</i> *, <i>H. cholecystis</i> (liver), <i>H. aurati</i>
Rabbits	<i>H. felis</i> , <i>H. salomonis</i>
Ferrets	<i>H. mustelae</i>
Pigs	<i>H. heilmannii</i> *, <i>H. pametensis</i> , <i>H. suis</i> *
Sheep	<i>H. rappini</i>
Rats	<i>H. muridarum</i> , <i>H. trogontum</i> , <i>H. heilmannii</i> *
Mice	<i>H. hepaticus</i> , <i>H. rappini</i> *, <i>H. bilis</i> , <i>H. heilmannii</i> *, <i>H. rodentium</i> , <i>H. muridarum</i> *
Chickens	<i>H. pullorum</i> *
Wild Birds	<i>H. canadensis</i> *, <i>H. pametensis</i>
Horses	<i>H. equorum</i>

*Reports suggest as zoonotic or has zoonotic potential as they are found in both animals and humans

Source: Haesebrouck et al. (2009); Fox (2002); Solnick and Schauer (2001); Fox (1999).

which may result in oral-oral route of transmission to man (Handt et al., 1994). It also suggested that since ferret shed viable gastric *H. mustelae*, there is a possibility of the presence of *H. pylori* in cats' faeces which man can be exposed to upon cleaning the litter box. A more likely suggestion is that *H. pylori* in cats may well be a reverse zoonosis (anthropozoonosis), whereby humans transmit the organism to cats (Nieger, 2003). *Helicobacter heilmannii* and *H. felis* were reported to colonize a small percentage of humans with gastritis; since the environmental source of these organisms has not been recognize, it is suggested that pets were the source of transmission to man (Fox, 2002). *Helicobacter heilmannii* is said to cause chronic active gastritis in man and animals, such as cats, dogs, pigs and primates, it is caused mild to moderate gastritis. 80-100% of cats, dogs and pigs were reported to be infected with *H. heilmannii* (Dieterich et al., 1998). Dieterich et al. (1998) reported the isolation of *H. heilmannii* in a man who had a 4-years history of recurrent dyspepsia and from his two cats; however, there is the possibility that the cats and their owner became infected from the same source.

It is interesting to note that the overall *H. pylori* prevalence of 49.0% was recorded in man in Malaysia, with the prevalence among the races as follows: Malay 16.4%, Chinese 48.5% and Indians 61.8% (Goh, 1997). According to Goh (1997), the hypothesis of a racial cohort phenomenon is based is based on the presumption that the both Chinese and Indians, being immigrant races, may have 'brought over' the infection from their countries of origin

where the prevalence of *H. pylori* is known to be high; even in Singapore, the Malay population has the lowest prevalence compared to the other two races. Interestingly too, according to Chow et al. (1995), the Chinese immigrants living in Melbourne (Australia) also showed higher prevalence of *H. pylori* infection which was associated with age, birthplace and the use of chopsticks suggesting an oral-oral spread.

Helicobacter pullorum was reported to cause serious gastroenteritis with chronic diarrhoea in man (Fox et al., 2002). The isolation of the organism from diarrhoeic humans and their presence in chicken faeces and carcasses led to suggestion that consumption of chickens may cause *H. pullorum* infection in man (Atabay et al., 1998; Fox et al., 2002). A Swiss study on 387 *Campylobacter* spp. Isolated from human patients with gastroenteritis, found six (1.5%) identified as *H. pullorum*; the authors suggested the possibility of transmission of the organism from poultry to humans (Burnens et al., 1994). Since *H. pullorum* resembles *Campylobacter coli*, it could be overlooked as a cause of human gastroenteritis.

The investigation by Van den Bulck et al. (2005) into the presence of NHPHs in gastric samples of human found that *H. suis* (37%) and *H. salomonis* (21%) to be the most prevalent *Helicobacter* species encountered compared to other *Helicobacter* species and a study reported a high rate (78%) of *H. suis* in German patients. Thus, according to Van den Bulck et al. (2005) pork consumption and direct contact with pigs may constitute a risk factor for human NHPH infection.

In summary, the large number of *Helicobacter* species isolated from animals and that some of these species were also found in human indicate the zoonotic potential of these species of helicobacters. Among these animals, cats and pigs are important in human gastric NHPH infection but other sources need to be investigated. Moreover, the wide range of animal species found to harbour helicobacters not only in their gastrointestinal tracts but also in the oral cavities may increase the possibility of human exposure to these organisms and as such, precautionary measures need to be taken upon handling animals.

REFERENCES

- Atabay, H. I., J.E.L Corry and S.L.W. On (1998). Identification of unusual *Campylobacter*-like isolates from poultry products as *Helicobacters pullorum*. J. Appl. Microbiol., 84: 1017-1024.
- Brunens, A.P., J. Stanley, R. Morgenstren and J. Nicolet (1994). Gastroenteritis associated with *Helicobacter pullorum*. Lancet 344: 1569-1570.
- Cattoli, G., R. Zanoci, C. Bennazi, L. Della Salda, A. Serainno and V. Sanguinetti (1996). Isolation of *Helicobacters felis* from dogs in Italy. In D. Hewell at el (ed) Proceedings of Campylobacters, Helicobacters and Related Organisms. Plenum Press, New York.. 341-343.
- Ceelen, L.M., A. Decostere, K. Van den Bulck, S.L.W. on, M. baele, R. Ducatelle and F. Haesbrouck (2006). *Helicobacter pullorum* in chickens, Belgium. Emerging Infect. Dis. 12: 263 - 267
- Chow, T.F.K., J.R Lambert, M.L. Wahlqvist and B.H.H. Hsu-Hage (1995). *H. pylori* in Melbourne Chinese immigrants: evidence for oral-oral transmission via chopsticks. J. Gastroenterol. Hepatol..10: 562-569.

- Dieterich, C., P. Wiesel, R. Neiger, A. Blum and I. Corthesy-Theulaz (1998) Prevalence of Multiple '*Helicobacter heilmannii*' in a individual suffering from ulcers and in his two cats. J. Clin. Microbiol., 36: 1366-1370.
- Fox, J.G. (1999) Spiral Organisms II: Helicobacter. In Hirsh, D.C. and Zee, C.Y. (Eds). Veterinary Microbiology, Black Well Sci. Inc., New York. Pp. 93-99.
- Fox, J.G. (2002) The non-*H. pylori* helicobacters: their expanding role in gastrointestinal and systematic diseases. Gut, 50: 273-283.
- Goh, K.L. (1997). Prevalence of and risk factor of *Helicobacter pylori* infection in a multi-racial dyspeptic Malaysian population undergoing endoscopy. J. Gastroenterol. Hepatol. 12: S29-S35.
- Haesebrouck, F., F. pasmans, B. Flahou, K. Chiers, M. Baele, T. Meyns, A. Decostere and R. Ducatelle (2009) Gastric Helicobacters in domestic animals and non-human primates and their significance for human health. Clin. Microbiol. Reviews 22 : 202 – 223.
- Handt, L.K., J.G. Fox, F.E. Dewhirst, G.L. Fraser, B.J. Paster, L.L. Yan, H. Rozmiarek, Rufo, R and I.H. Stalis (1994) *Helicobacter pylori* isolated from domestic cat: public health implications. Infect. Immunity, 62: 2367-2374.
- Jalava, K., S.L.W. On, P.A.R. Vandamme, I. Happonen, A. Sukura and M-L. Hanninen (1998) Isolation and identification of *Helicobacter* spp. from canine and feline gastric mucosa. Appl. Environ. Microbiol., 64: 3998-4006.
- Lee, A., S.L. Hazell. J. O'Rourke and S. Kouprach (1988) Isolation of a spiral-shaped bacterium from a cat stomach. Infect. Immunity, 56: 2843-2850.
- Marshall, S.M., P.L. Melito, D.L. Woddward, W.M Johnson, F.G. Rodgers and M.R. Mulvey (1999). Rapid identification of *Campylobacter*, *Arcobacter* and *Helicobacter* isolated by PCR-restriction fragment length polymorphism analysis of the 16S rRNA gene. J. Clin. Microbiol., 37: 4158-4160.
- Miller, K.A., L.L. Blackall, J.K. Mifflin, J.M. Templeton and P.J. Blackall (2006). Detection of *Helicobacter pullorum* in meat chickens in Australia. Aust. Vet. J., 84: 95 - 97
- Milosavljevic, T. (2001). The non-*H. pylori* helicobacters in humans: New development. Arch Gastroenterohepatol. 20 (www.geocities.com/gastroyu/dec2001/Tomica.htm)
- Neiger, R., C. Dieterich, A. Burnens, A. Waldvogel, I. Corthesy-Theulaz, F. Halter, B.Lauterberg and A. Schmassmann (1998). Detection and prevalence of *Helicobacter* infection in pet cats. J. Clin. Microbiol., 36: 634-637.
- Neubauer, C. And M. Hess (2003). Detection and identification of *Campylobacter*-like bacteria from poultry samples as *Helicobacter pullorum*. Proceedings 5th World Congress Foodborne Infections and Intoxications, Vol. IV: 1023-1024.
- Nur Zaliza, A. (2004) Isolation and identification of *Helicobacter* sp. in Canine and Feline Gastric Mucosa. Final Year Project under Doctor of Veterinary Medicine programme , Faculty of Veterinary Medicine, Universiti Putra Malaysia.
- Otto, G., S.H. Hazell, J.G. Fox, C.R. Howlett, J.C. Murphy nad J.L O'Rourke (1994) Animal and public health implication of gastric colonization of cats by *Helicobacter*-like organisms. J. Clin. Microbiol., 32: 1043-1049.
- Park, J-H., S-H. Seok, S-A. Cho, M-W. Baek, H-Y. Lee, D-J. Kim and J-H. Park (2004). The high prevalence of *Helicobacter* sp. in porcine pyloric mucosa and its histopathological and molecular characteristics. Vet. Microbiol., 104: 219 – 225.
- Petersen, R.F., W. Abu Al-Soud, J. Waldenstrom, T. Wadstrom and S.L.W. On (2003). *Helicobacter pullorum/H. Canadensis*-like bacteria in pigs. Abstracts of 12th International Workshop on Campylobacter, Helicobacters and Related Organism in International J.Med. Microbiol., 293: 90.
- Ravindran, M. (2002). Detection of *Helicobacter* in Cats. Final Year Project under Doctor of Veterinary Medicine programme , Faculty of Veterinary Medicine, Universiti Putra Malaysia.
- Recordati, C., V. Gualdi, S. Tosi, R.V. Facchini, G. Pengo, M. Luini, K.W. Simpson and E. Scanziani (2007). Detection of *Helicobacter* spp. DNA in the oral cavity of dogs. Vet. Microbiol., 119: 346 – 351.
- Shen, Z., Y. Feng, F.E. Dewhirst and J.G. Fox (2001). Coinfection of enteric *Helicobacter* spp. in and *Campylobacter* spp. in cats. J. Clin. Microbiol., 39: 2166-2172.
- Simpson, K.W. and C.F. Burrows (1997). Gastritis, ulcers and *Helicobacter* spp. in humans, dogs and cats. Waltham Focus, 7: 2-6.
- Soe Soe Wai, A.A. Saleha, Z. Zunita, L. Hassan and A. Jalila (2012). Occurrence of co-infection of *Helicobacter pullorum* and *Campylobacter* spp. in broiler and village (indigenous) chickens. Pakistan Vet. J., 32 : 503 – 506.
- Solnick, J.V. and D.B. Schauer (2001). Emergence of diverse *Helicobacter* species in the pathogenesis of gastric and enterohepatic diseases. Clin. Microbiol. Reviews, 14: 59-97.
- Stanley, J., D. Linton, A.P. Burnens, F.E. Dewhirst, S.L.W. On, A. Porter, R.J. Owen and M. Costas (1994). *Helicobacter pullorum* sp. nov.-genotype and phenotype of a new species isolated from poultry and from human patients with gastroenteritis. Microbiol., 140: 3441-3449.
- Tabrizi, A.S., Sh. Jamshidi, A. Oghalaei, T.Z. Salehi, A.B. Eshkaftaki and M. Mohammadi (2010). Identification of *Helicobacter* spp. in oral secretions vs gastric mucosa of stary cats. Vet. Microbio., 140 : 142 - 146
- Van den Bulck, K., A. Decostere, M. Baele, A. Driessen, J-C. Debongnie, A. Burette, M. Stolte, R. Ducatelle and F. Haesebrouck (2005). Identification of Non-*Helicobacter pylori* spiral organisms in gastric samples of humans, dogs and cats. J. Clin. Microbiol., 43: 2256 - 2260
- Wesley, V.W. (1997) *Helicobacter* and *Arcobacter*: potential human foodborne pathogens? Trends in Food Sci. Technol., 8: 293-298.
- Zanoni, R.G., M. Rossi, D. Giacomucci, V. Sanguinetti and G. Manfreda (2007). Occurrence and antibiotic susceptibility of *Helicobacter pullorum* from broiler chickens and commercial laying hens in Italy. Int. J. Food Microbiol., 116 : 168 -173
- Zanoni, R.G., S. Piva, M. Rossi, F. Pasquali, A. Lucchi, A. De Cesare and G. Manfreda. (2011). Occurrence of *Helicobacter pullorum* in turkeys. Vet. Microbiol., 149: 492 - 496