

# Evidence of Zoonotic Transmission of *Helicobacter canis* Between Sheep and Human Contacts

Maha A. Sabry, Khaled A. Abdel-Moein, and Aya Seleem

## Abstract

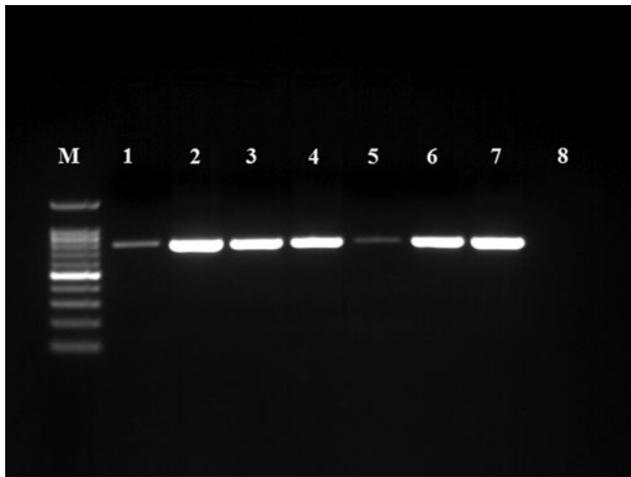
*Helicobacter* species are newly emerging bacteria with great public implications but till now its epidemiology is not fully understood; so, this study was conducted to investigate the possible role of ruminants in the epidemiology of these pathogens. For this purpose, fecal samples were collected from 149 animals (76 sheep, 33 goats, 21 cattle, and 19 buffaloes) and stool specimens from 10 animal caretakers in intimate contact with the examined animals. All samples were examined for the presence of *Helicobacter* species through detection of *Helicobacter* genus specific 16S rRNA using PCR. Then, all positive *Helicobacter* spp. amplicons were sequenced to recognize their species through BLAST analysis at GenBank. The overall prevalence of *Helicobacter* spp. was 14.8% while the distribution among the different animals was 26.3%, 3%, 4.8%, and 0% in sheep, goats, cattle, and buffaloes respectively. *Helicobacter canis* was the predominant species and detected only in sheep (21%) and goats (3%). Moreover, *Helicobacter winghamensis* and *Helicobacter canadensis* were also detected in sheep but not in other animals, whereas the only positive bovine sample was identified as *Helicobacter bovis*. On the other hand, 4 out of 10 humans were positive for *Helicobacter* spp. and all sequences were identified as *H. canis*. The sequences identity matrix and phylogenetic analysis of *H. canis* sequences from humans and sheep contacts revealed that one human sequence was identical to that of sheep and making sister group clade, which prove the zoonotic transmission of this pathogen between sheep and human contacts. However, our findings highlight sheep as a potential reservoir for *H. canis*, further researches are needed to address the potential role of sheep in the food-borne transmission of such emerging pathogen.

**Keywords:** *Helicobacter canis*, reservoir, sheep, zoonosis

## Introduction

THE DISCOVERY OF *Helicobacter pylori* in 1982 by Warren and Marshall and its association with gastric and duodenal ulcers is not only led to change in the medical theories that had established for many years ago (Warren and Marsall 1983) but also, resulting in the designation of a new genus *Helicobacter*. Although, *H. pylori* keeps its position to be the first and the most important species in this genus, the advances in the molecular techniques and use of genus specific primers lead to the expansion of genus *Helicobacter* and now it comprises a wide range of species that inhabit stomach, intestine, liver, and many other tissues to produce a wide range of diseases in both humans and animals (Whary and Fox 2004). Because of the difficulty in the isolation of most *Helicobacter* spp. and may be impossible in some ones (Fox 2002), the epidemiology of *Helicobacter* spp. is still not well

known. Animals have their adapted *Helicobacter* spp., which inhabit their stomach and intestinal tracts and may produce disease when moved to humans but this subject is not yet fully investigated (O'Rourke et al. 2001), while the presence of animal reservoirs for many *Helicobacter* spp. were established even for *H. pylori*, which is formally thought to be only human pathogen (Momtaz et al. 2014). Moreover, people in intimate contact with sheep show very high *H. pylori* prevalence, a result that magnifies the role of animals in transmission of *Helicobacter* infection for humans in certain settings (Dore et al. 1999, Papież et al. 2003). Nevertheless, studies investigated the role of animals in the epidemiology of human pathogenic helicobacters are scarce whereas many non-pylori helicobacters are emerging to cause diseases in humans (O'Rourke et al. 2001, Liu et al. 2015). Therefore, this study was carried out to investigate the possible role of ruminants in the epidemiology of human pathogenic *Helicobacter* spp.



**FIG. 1.** Occurrence of *Helicobacter* spp. among examined animals and humans Lane M: DNA ladder; lanes 1–7: positive samples for *Helicobacter* spp. showed specific bands at 764 bp; lane 8: negative control.

to explore the zoonotic potential of *Helicobacter* spp. and accordingly prompt control of such emerging pathogen.

**Materials and Methods**

Fecal samples were collected from 76 sheep, 33 goats, 21 cattle, and 19 buffaloes (apparently healthy, randomly selected from different farms in Giza and Sharkia governorates, Egypt), all samples were obtained from rectum of the examined animals using sterile gloves and placed in sterile cups. Moreover, stool specimens were gathered from 10 animal caretakers (apparently healthy) in intimate contact with the examined animals through their voluntary contribution in the study. All samples were transported in icebox to laboratory of Department of Zoonoses, Faculty of Veterinary Medicine, Cairo University for further processing.

*Molecular detection of Helicobacter spp*

All examined animals and humans were tested for the presence of *Helicobacter* spp. in their feces through direct detection of *Helicobacter* genus specific 16S rRNA gene by PCR.

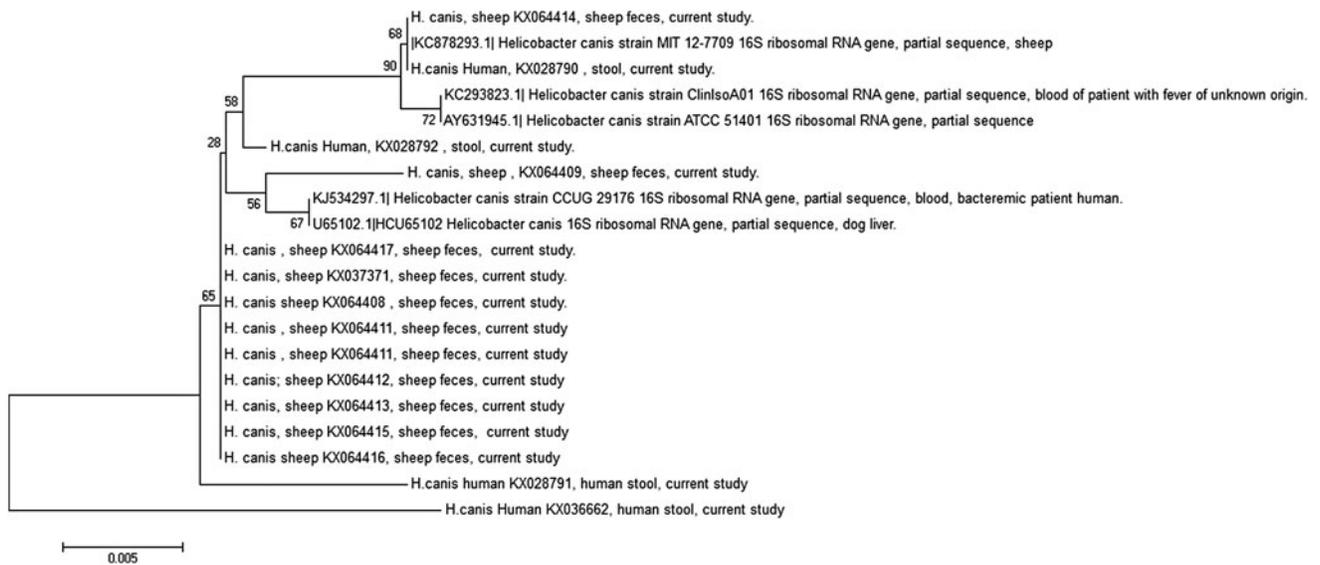
**Extraction of DNA.** DNA was extracted from fecal and stool samples of examined animals and humans using QIAamp DNA stool mini kit (Qiagen) according to the procedure of the kit. The extracted DNA was stored at  $-20^{\circ}\text{C}$  until use.

**Polymerase chain reaction.** PCR was done upon the extracted DNAs to detect 16S rRNA gene specific for genus *Helicobacter* using the following pair of primers:

Forward: 5' GGCTATGACGGGTATCCGGC 3' and reverse 5' GCCGTGCAGCACCTGTTTTTC 3'. The primers were synthesized by (Metabion) according to (Moyaert et al. 2008). The amplification reaction was carried out using HotStarTaq Master Mix kit (Qiagen). The thermal profile of the reaction was initial denaturation at  $95^{\circ}\text{C}$  for 15 min, 45 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $65^{\circ}\text{C}$  for 30 s; extension at  $72^{\circ}\text{C}$  for 30 s. Then final extension for 10 min at  $72^{\circ}\text{C}$ . Afterward, the amplicons were entered electrophoresis step and specific bands were noted at 764 bp (Fig. 1) (Moyaert et al. 2008).

**Gene sequencing for 16S rRNA gene.** PCR products of positive samples were purified using GeneJET™ PCR purification kit (Thermo) and the sequencing step was conducted with Big Dye Terminator V3.1 Cycle sequencing Kit (Applied Biosystems). The obtained sequences were identified through BLAST analysis with sequences available at GenBank. [http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)

**Sequences identity matrix and phylogenetic analysis of *Helicobacter canis* from humans and sheep contacts.** All



**FIG. 2.** Phylogenetic tree constructed by neighbor-joining method with boot-strap test (replicates 500). The analysis was conducted based on partial sequence of 16S rRNA gene of 20 *Helicobacter canis* sequences. The analysis was done by MEGA6 version (6.06).

TABLE 1. PREVALENCE OF *HELICOBACTER* SPECIES AMONG THE EXAMINED ANIMALS AND HUMANS

Species	Number examined	Numbers positive for <i>Helicobacter</i> spp. (%)	Identification	n (%)
Sheep	76	20 (26.3)	<i>Helicobacter canis</i> <i>Helicobacter winghamensis</i> <i>Helicobacter canadensis</i>	16 (21) 2 (2.6) 2 (2.6)
Goats	33	1 (3)	<i>Helicobacter canis</i>	1 (3)
Cattle	21	1 (4.8)	<i>Helicobacter bovis</i>	1 (4.8)
Buffaloes	19	0 (0)	0	0
Total animals	149	22 (14.8)	—	—
Human	10	4 (40)	<i>H. canis</i>	4 (40)

human sequences were aligned against *Helicobacter canis* sequences from contact sheep and against the most similar sequences available at GenBank to study the identity and evolutionary history of these sequences. Clustral W Multiple alignment and sequences identity matrix were done with using (BioEdit) software whereas phylogenetic tree was constructed by neighbor-joining method using MEGA6 software version (6.06) (Fig. 2).

#### Nucleotide sequence accession numbers

The nucleotide sequences of 16S rRNA of *H. canis* determined in this study have been deposited in GenBank under the following accession numbers:

*H. canis* from humans: KX028790-KX028792; KX036662.

*H. canis* from sheep: KX037371; KX064408-KX064417.

#### Results

Of 149 examined animals, 22 yielded *Helicobacter* spp. with an overall prevalence 14.8%. The occurrence of *Helicobacter* spp. among different animal species was 26.3%, 3%, 4.8%, and 0% in sheep, goats, cattle, and buffaloes, respectively (Table 1). The results of 16S rRNA sequencing of the amplicons identified most of detected *Helicobacter* spp. to be *H. canis* with the following preva-

lence among different animal species 21%, 3% for sheep and goats respectively while none of cattle yielded *H. canis*. Moreover, *Helicobacter canadensis* and *Helicobacter winghamensis* were also identified in sheep with a prevalence of 2.6% for each, whereas the bovine sequence was identified as *Helicobacter bovis* (Table 1). On the other hand, all human *Helicobacter* sequences were identified as *H. canis* (Table 1). The sequences identity matrix of *H. canis* from both human and sheep contacts and 16S rRNA sequence of *H. canis* strain ATCC 51401 were displayed in (Table 2).

#### Discussion

In the recent years genus *Helicobacter* has gained ground in the medical community as it stands behind many gastrointestinal complaints in humans. The results of this study revealed the occurrence of *Helicobacter* spp. in the feces of the examined animals in an overall prevalence 14.8% whereas the prevalence rates among different animal species showed that the vast majority of positive results were derived from sheep (26.3%) rather than the other examined animals. Furthermore, the sequencing results of 16S rRNA revealed the predominance of *H. canis* (21%) rather than other *Helicobacter* spp. (*H. canadensis*, *H. winghamensis*) (2.6% for each) among examined sheep. However, *H. canis* was first recognized in dogs and cats (Stanley et al. 1993, Foley et al.

TABLE 2. SEQUENCES IDENTITY MATRIX OF THE OBTAINED HUMAN *H. CANIS* SEQUENCES AND THOSE FROM SHEEP CONTACTS

	Human KX028790	Human KX028791	Human KX028792	Human KX036662	H. canis ATCC strain 51401
Human KX028790	ID	0.981	0.988	0.962	0.998
Human KX028791	0.981	ID	0.985	0.962	0.979
Human KX028792	0.988	0.985	ID	0.970	0.986
Human KX036662	0.962	0.962	0.970	ID	0.960
Sheep1 KX037371	0.988	0.988	0.994	0.971	0.986
Sheep3 KX064408	0.988	0.988	0.994	0.971	0.986
Sheep4 KX064409	0.981	0.981	0.986	0.964	0.979
Sheep5 KX064410	0.988	0.988	0.994	0.971	0.986
Sheep6 KX064411	0.988	0.988	0.994	0.971	0.986
Sheep7 KX064412	0.988	0.988	0.994	0.971	0.986
Sheep8 KX064413	0.988	0.988	0.994	0.971	0.986
Sheep 9 KX064414	1.000	0.981	0.988	0.962	0.998
Sheep10 KX064415	0.988	0.988	0.994	0.971	0.986
Sheep11 KX064416	0.988	0.988	0.994	0.971	0.986
Sheep12 KX064417	0.988	0.988	0.994	0.971	0.986

1999), a recent study by Swennes et al. (2014) isolated *H. canis* from sheep to shed light on sheep as a possible reservoir for *H. canis*. The results of our study augment this concept and underscore sheep as a potential reservoir for *H. canis* and may play a pivotal role in its epidemiology through shedding of the pathogen in its feces to contaminate milk, animal carcass, and may be transmitted directly through fecal-oral route to human contacts. Interestingly, the results of human samples provide concrete evidence on the zoonotic transmission of *H. canis* between sheep and human contacts as out of 10 animal caretakers, 4 were positive for *Helicobacter* spp. while all human sequences were identified as *H. canis*. All *H. canis*-positive humans were in intimate contact with 11 positive sheep and lived in the same farm. On the molecular basis, one human sequence was identical to one ovine sequence and making a sister group as revealed by phylogenetic tree and 100% identity as appeared in sequence identity matrix, whereas other human sequences showed high genetic relatedness with the animal ones. To the best of our knowledge, this is the first record of zoonotic transmission of *H. canis* between sheep and human contacts, which highlights the possible role of sheep in the epidemiology of *H. canis*. Moreover, the sequences identity matrix and the phylogenetic analysis of the obtained *H. canis* from sheep and human contacts with other published *H. canis* sequences showed high level of identity and some sequences were grouped in the same clade with those previously recorded in serious human cases such as bacteremia in patient with fever of unknown origin (Abidi et al. 2013) and bacteremia in patient with multifocal cellulitis a matter that magnifies the public health burden of our findings. Additionally, we could identify both emerging enteropathogenic *Helicobacter* spp.; *H. canadensis* and *H. winghamensis* from sheep feces, which are responsible for gastroenteritis in humans (Melito et al. 2001, Waldenstrom et al. 2003, Loman et al. 2009), raising the probability of sheep to be a zoonotic reservoir for such pathogens. Despite the large number of helicobacters that could be identified in sheep feces, we only detected *Helicobacter* sp. in one goat and identified as *H. canis*. Nonetheless, to the best of our knowledge it is the first time *H. canis* was detected in the feces of goats. On the other hand, *Helicobacter* sp. was detected only once in cattle and recognized as *H. bovis* while none of the examined buffaloes yielded positive results. In conclusion, the detection of *H. canis* in sheep feces in such high prevalence expands this pathogen host and identifies sheep as a potential reservoir for *H. canis*. Moreover, this study records a zoonotic transmission between sheep and human contacts, which is new evidence in the epidemiology of such pathogen.

#### Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Khaled A. Abdel-Moein  
Department of Zoonoses  
Faculty of Veterinary Medicine  
Cairo University  
Cairo 12211  
Egypt

E-mail: khal\_105@cu.edu.eg; khal\_105@yahoo.com