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Epidemiology of *Helicobacter* Infection in Wild Rodents in the Xinjiang-Uygur Autonomous Region of China

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Abstract. *Helicobacter* species were detected in the feces of wild rodents captured in Qiemo and Ruoqiang in the Xinjiang-Uygur autonomous region of China by polymerase chain reaction and 16S rRNA partial sequence analysis. Forty-four wild rodents, including one Przewalski's gerbil (*Brachiones przewalskii*), three Northern three-toed jerboas (*Dipus sagitta*), one long-eared jerboa (*Euchoreutes naso*), 34 midday gerbils (*Meriones meridianus*), two short-tailed bandicoot rats (*Nesokia indica*) and three great gerbils (*Rhombomys opimus*), were examined. Epidemiological studies indicated that *Helicobacter* spp. were detected in all genera tested; that *H. hepaticus*, *H. apodemus*, *H. canadensis*, and *H. winghamensis* were widespread in wild rodents; and that the dominant *Helicobacter* species in rodents differed depending not only on the order or genus of the animal but also on the animal's habitat. *H. bilis*, *H. pylori*, *H. rodentium* and "*H. suncus*" were not detected in any animals. It appears that the wild rodents tested in this study are not a reservoir of *H. pylori* infection.

Since *Helicobacter pylori* was isolated from humans in 1983 [11], many *Helicobacter* species have been reported not only in wild animals [1, 9] but also in laboratory animals [3, 7, 8, 10].

We previously reported that the dominant *Helicobacter* species in the intestinal flora of laboratory mice were *H. hepaticus* and *H. rodentium*, while that of Mongolian gerbils (*Meriones unguiculatus*) was *H. hepaticus* [4].

In this study, to investigate epidemiological relationships among wild and laboratory rodents, we clarified the current status of *Helicobacter* infection in wild rodents using the polymerase chain reaction (PCR) based on 16S rRNA and partial 16S rRNA gene sequence analysis for the prospective identification of *Helicobacter* species in fecal samples of rodents in the Xinjian-Uygur autonomous region of China.

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Materials and Methods

Animals. Forty-four animals in total, including one *Brachiones przewalskii*, one *Dipus sagitta*, 23 *Meriones meridianus* and two *Nesokia indica* from Qiemo (38° 13′ N, 85° 53′ E), and two *Dipus sagitta*, one *Euchoreutes naso*, 11 *Meriones meridianus* and three *Rhombomys opimus* from Ruoqiang (39°03′ N, 88° 00′ E) in the Xinjian-Uygur autonomous region of China were captured on September 3 to 9, 2002, and feces were sampled from these animals.

RNA extraction and reverse transcription (RT)-nested PCR. Extraction of RNA and RT-PCR were performed as described previously [4] using Pyrobest DNA polymerase (Takara Bio, Shiga, Japan). For detection of *H. pylori*, PCR primers (5′ GTT GGA GGG CTT AGT CTC T 3′) and (5′ TTA GAG TTC TCA GCA TAA CCT 3′) were used. Length of the amplified products with *Helicobacter* genus-specific primers was estimated as 350 bp, and those with *Helicobacter* species-specific primers as 419 bp for *H. bilis*, 405 bp for *H. hepaticus*, 328 bp for *H. pylori*, 324 bp for *H. rodentium* and 358 bp for "*H. suncus*".

Cloning, sequencing of the PCR products, and analysis of the sequences. The PCR products amplified with *Helicobacter* species specific primers were purified using a QIAEX II Gel extraction kit (Qiagen, Tokyo, Japan), and sequenced using the same primers as those

Table 1. Detection of *Helicobacter* species from feces of wild rodents by PCR and sequence analysis

Animal species	Animals from	No. of animals	No. of positive samples by PCR (%)						No. of positive samples by sequence analysis (%)					
			Helicobacter spp.	*H. bilis*	*H. hepaticus*	*H. pylori*	*H. rodentium*	*H. suncus*	*H. apodemus*	*H. canadensis*	*H. ganmani*	*H. pametensis*	*H. winghamensis*	"*Helicobacter* sp. MIT94-022"
Brachiones przewalskii	Qiemo	1	1 (100%)	–	1 (100%)	–	–	–	–	–	–	–	1 (100%)	–
Dipus sagitta	Qiemo	1	–	–	–	–	–	–	–	–	–	–	–	–
	Ruoqiang	2	2 (100%)	–	–	–	–	–	1 (50%)	2 (100%)	–	–	–	–
Euchoreutes naso	Ruoqiang	1	1 (100%)	–	–	–	–	–	1 (100%)	1 (100%)	–	–	–	–
Meriones meridianus	Qiemo	23	22 (96%)	–	8 (35%)	–	–	–	2 (9%)	–	13 (57%)	–	–	–
	Ruoqiang	11	8 (73%)	–	5 (46%)	–	–	–	–	–	–	–	3 (27%)	2 (18%)
Nesokia indica	Qiemo	2	1 (50%)	–	–	–	–	–	1 (50%)	–	–	1 (50%)	–	–
Rhombomys opimus	Ruoqiang	3	2 (67%)	–	–	–	–	–	–	–	–	–	2 (67%)	–

–, not detected.

used in the nested amplification. Sequencing was performed using ABI 377 (PE Applied Biosystems, Foster City, CA).

The nested PCR products amplified with *Helicobacter* genus-specific primers were cloned into a plasmid vector, pCR-Blunt II-TOPO vector, using the Zero Blunt TOPO PCR Cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The nucleotide sequences of the recombinant plasmids were determined by the dye terminator method with a DYEnamic ET dye terminator kit (Amersham Biosciences, Piscataway, NJ.). Sequence reactions were prepared using MegaBACE1000 (Amersham Biosciences, Piscataway, NJ.). The sequences were compared with a DNA database using the DDBJ (DNA Data Bank of Japan) homology search system.

Results and Discussion

Results are summarized in Table 1. *Helicobacter* species were detected in 37 (84%) animals including all genera of animals tested. *H. hepaticus* was detected by PCR in *Brachiones przewalskii*. By sequence analysis of PCR products amplified with *Helicobacter* genus-specific primers, sequences which showed high homology (98.8%) with *H. winghamensis* (GenBank accession No. AF246988) were obtained. In *Dipus sagitta* captured in Ruoqiang, sequences which showed high homology with *H. apodemus* (AY009192) (98.9%) and *H. canadensis* (AF262037) (99.4%) were detected. *H. apodemus* (99.7%) and *H. canadensis* (99.4%) were also detected in *Euchoreutes naso*. Among 23 *Meriones meridianus* captured in Qiemo, *Helicobacter* species were detected in 22 (96%) samples including 8 (35%) *H. hepaticus* positive samples. In the 22 *Helicobacter* species positive samples, sequences which showed high homology with *H. apodemus* (100%) and *H. ganmani* (AF000222) (100%) were detected. Among 11 *Meriones meridianus* obtained in Ruoqiang, *Helicobacter* species were detected in 8 (73%) samples including 5 (46%) *H. hepaticus* positive samples. The sequences of PCR products amplified with *Helicobacter* genus-specific primers showed high homology with *H. winghamensis* (98.6%) and *Helicobacter* species MIT94-022 (AF225550) (100%). From *Nesokia indica*, sequences which showed high homology with *H. apodemus* (99.4%) and *H. pametensis* (AF302105) (99.4%) were detected in PCR products amplified with *Helicobacter* genus-specific primers. *H. winghamensis* sequence (homology: 98.6%) was detected in *Rhombomys opimus*.

All the sequences of PCR products amplified with *H. hepaticus*-specific primers were completely (100%) the same as that of *H. hepaticus* (L391222) (data not shown).

The present results show that only *H. hepaticus*, among *Helicobacter* species such as *H. hepaticus*, *H. bilis* and *H. rodentium* widespread in laboratory animals, was detected in the animals tested. It was found in two of the six genera: *Brachiones* and *Meriones*. The fact that the Mongolian gerbil, used worldwide as a laboratory animal, belongs to same genus as *Meriones meridianus*

suggests that the genera *Mus* and *Meriones* may be natural hosts of *H. hepaticus*.

The distance between Qiemo and Ruoqiang is approximately 250 km and it was considered that the animals in each area could not easily come into contact with each other. *Helicobacter* species were detected from all genera of animals captured in both areas. In spite of the fact that *H. apodemus* and *H. ganmani* were the dominant *Helicobacter* species in *Meriones meridianus* obtained from Qiemo, *H. winghamensis* and *Helicobacter* species "MIT94-022" were the dominant *Helicobacter* species in *Meriones meridianus* obtained from Ruoqiang. These results suggest that not only the order or genus of the animal but also its habitat is important for the composition of *Helicobacter* flora in these animals.

Primers for detection of *H. pylori* in this study were selected from the sequence of *H. pylori* 16S rRNA (positions 793–809 and 1100–1120 in the sequence of GenBank accession no. M88157). *H. pylori* was also not detected using another primer set reported by Engstrand et al. [2] (data not shown). *H. pylori* is one of the most common pathogens in humans although the routes of transmission are not clear. Waterborne [5] and fecal-oral spread [6] are possible routes of *H. pylori* transmission. It has been reported that *H. pylori* can be detected from domestic cats, and *H. pylori* can colonize in the stomach of Mongolian gerbils after experimental inoculation [6], but our data suggested that zoonotic transmission is a rare route of *H. pylori* infection.

In this study, we proved that *Helicobacter* species were epidemic not only in laboratory animals but also in wild rodents. *H. hepaticus*, *H. apodemus*, *H. canadensis* and *H. winghamensis* in particular were dominant in wild rodents, but *H. pylori* was not observed. The habitat of the animals affects their *Helicobacter* flora.

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