Identification of enteric Helicobacter in avian species

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Summary

The presence of enteric Helicobacter species was investigated in poultry (n = 130) and in pet and ornamental birds (n = 50) using a PCR sequencing method which permits the differentiation of many Helicobacter species derived from animal tissues. All samples were of Italian origin, except for 21 Guinea fowl from a French flock. About 80% of poultry (chickens, laying hens, Guinea fowl) were positive to Helicobacter DNA. H. pullorum was most frequently (62.1%) identified whereas H. pylori and 3 H. sp. hamster B strains were seen in only 3 cases each. Pet and ornamental birds were all negative. H. canadensis was found in all Guinea fowl from a French farm. This is the first report on the occurrence of this bacterium in poultry.

Keywords: Helicobacter canadensis, Helicobacter pullorum, PCR-DNA sequencing, avian species, Italy

Introduction

Helicobacter are a group of bacteria that were at first included in the Campylobacter genus and have been reclassified as a separate genus in 1989 (Goodwin et al., 1989). Since then this group has rapidly expanded from the first two species, H. pylori and H. mustelae, to over 24 species which have been isolated from the gastrointestinal tract of humans and several animal species. In the last few years particular attention has been given to the enteric Helicobacter which have proven to cause inflammatory lesions and septicemia in man. Since some of these bacteria have been found in domestic (H. pullorum, Burnens et al., 1996) and wild birds (H. canadensis, Waldenström et al., 2003), the issue of a zoonotic risk has been raised. H. pullorum is an enterohepatic spirillum which was first described by Stanley et al. (1994) and was isolated from faeces of humans affected by gastroenteritis (Burnens et al., 1994; Steinbreuckner et al., 1997; Ceelen et al., 2005) and in the bile of humans affected by primary sclerosing cholangitis (Fox et al., 1998; Nilsson et al., 2000). In animals, H. pullorum has been isolated from the caecal contents and carcasses of slaughtered chickens (Burnens et al., 1996; Atabay et al., 1998; Ceelen et al., 2006).

H. canadensis was identified as a new species and described as an emerging pathogen by Fox in the year 2000. This organism was later isolated from patients with enteritis and patients with bacteraemia (Tee et al., 2001). So far in animals, it has only been found in wild geese (Waldenström et al., 2003) and recently in swine (Inglis et al., 2006). As a result of the increasing number of reports on the identification of Helicobacter recovered from bird and human specimens, the aim of the present study is to investigate the occurrence of enteric Helicobacter species in industrial farm poultry and pet and ornamental birds.

Animals, Material and Methods

Animals and specimen collection

One hundred and eighty birds, including 130 individuals obtained from industrial poultry farms (Group A) and 50 ornamental birds (Group B), were tested between 2004 and 2005 (Tab. 1). Group A consisted of samples randomly chosen from 4 categories of poultry, which had been slaughtered between the 8th and
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Table 1: Subdivision of samples in group A (farm poultry) and group B (pet and ornamental birds)

<table>
<thead>
<tr>
<th>Group</th>
<th>Avian Species</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Gallus domesticus</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Leghorn chickens</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Laying hens</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Numida meleagris</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Guinea fowl</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Agapornis, Ara,</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Cacatua</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pea fowl</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Pavo cristatus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Struthio camelus</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Ephippiorhynchus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mycteris</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Casuarius cassius</td>
<td>1</td>
</tr>
</tbody>
</table>

64th week of life, broilers and laying hens, respectively. The intestines were taken from a poultry slaughter immediately after evisceration.

Group B consisted of 33 dead parrots collected from 3 breeding centres and 17 ornamental birds from a bird park. The parrots were hosted in groups of about 20 individuals in aviaries whereas pet and ornamental birds lived in a private park, in a vast net bounded area where visitors could enter. These animals underwent a necropsy and intestines were collected. All samples examined were of Italian origin, except for 21 Guinea fowl birds from a French flock.

DNA extraction

Intestines were collected and individually packed in plastic bags, delivered to our laboratory and tested separately. The caeca were dissected, rinsed with sterile phosphate buffered saline solution (Sigma Chemical Co, St. Louis, MO) and cut up using sterile scissors. Approximately 25 mg of the caecum were collected in sterile vials and stored at −20°C until DNA extraction. Because of the lack of caeca in parrots, DNA was extracted from the duodenum. Genomic DNA was extracted using a commercial kit (DNeasy Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. Enteric tissue extracts were first amplified with universal primers for the 16S rRNA gene to demonstrate the presence of amplifiable DNA (Gramley et al., 1999).

PCR and DNA sequencing

In order to detect the presence of Helicobacter in avian samples, PCR procedure was performed as described by Beckwith et al. (1997). Genus-specific primers (5′-TAT GAC GGG TAT CCG GC-3′; 5′-ATT CCA CCT ACC TCT CCC A-3′) were designed from regions of the 16S rRNA gene, which is conserved among members of the Helicobacter genus. PCR products were purified using Qiagen and 18 for H. pullorum (similarity of 98%–99% with AJ876521) and 18 for H. canadensis (similarity of 98%–99% with H. canadensis AY323505). H. sp. hamster B (similarity of 99% with AF072333) and H. pylori (similarity of 97%–98% with H. pylori AY304558) were detected in 3 samples each.

Results

The presence of amplifiable DNA was observed in 174 out of 180 samples and six samples were negative (3 from Guinea fowl birds, 2 from broiler chickens, 1 from a hen), therefore they were excluded from further tests. A total of 100 out of 124 animals in Group A (80.6%) were positive for Helicobacter genus-PCR, whereas pet and ornamental birds in Group B (0%) were all negative. Sequencing of PCR products showed that 76 samples were positive for H. pullorum (similarity of 98%–100% with H. pullorum AY323505) and 18 for H. canadensis (similarity of 98%–99% with H. canadensis AY323505). H. sp. hamster B (similarity of 99% with AF072333) and H. pylori (similarity of 97%–98% with H. pylori AY304558) were detected in 3 samples each.
In particular, *H. pullorum* was detected in 19 out of 37 broiler chickens (51.3%), 22 out of 24 laying hens (91.7%), 25 out of 30 leghorn chickens (83.3%), 11 out of 15 Italian Guinea fowl birds (73.3%). *H. canadensis* was found in all Guinea fowl from a French farm. Two *H. pylori* and 3 *H. sp. hamster B* were found in leghorn chickens and one *H. pylori* in an Italian Guinea fowl bird. *H. pullorum* and *H. canadensis* strains identified in this study fall within the group of closely related organisms. As shown in the phylogenetic study, *H. canadensis* strains isolated from Guinea fowls are identical to those from wild geese and have the same difference as human strains (Fig 1). *H. pullorum* resulted identical to both avian and human strains used to create a phylogenetic tree, in accordance with Fox et al. (2000).

**Discussion**

Detection of species such as *H. pullorum* and *H. canadensis* is becoming increasingly important since these organisms are recognised as human and/or animal pathogens. Their phenotypic identification is complex because of fastidious growth characteristics and atypical biochemical reactions of some strains (On, 1996). Furthermore, they share culture characteristics with *Campylobacter* species and might easily be mistaken for these bacteria (Atabay et al., 1998; Melito et al., 2000).

Therefore, data regarding the presence of enteric *Helicobacter* in birds might be underestimated in favour of *Campylobacter* species. In this study, we report a simple PCR sequencing method, which allows the differentiation of many *Helicobacter* species directly from animal tissue. The protocol consists of one set of primers that amplifies a fragment within the region of 16S rRNA gene followed by sequencing of amplified products. This method is a useful assay to identify *Helicobacter* species in birds and should also be expanded to other animals.

From the sequences obtained, we identified 4 different species: *H. pullorum*, *H. canadensis*, *H. sp. hamster B* and *H. pylori*. Interestingly, this technique allows us to differentiate *H. pullorum* from *H. canadensis*, a closely related species. Phylogenetic analysis showed that *H. canadensis* identified in French Guinea fowl was homologous to strains isolated from wild birds (Waldénström et al., 2003), but slightly different from human strains.

*H. pullorum* was found in 77 poultry samples (62.1%). This percentage is higher than other studies (60%, 33.6%, 4%; 10, 11, 2 respectively), and shows that this bacterium is common in Italian poultry farms. Our study reveals a statistically significant difference (p < 0.01) between laying hens (91.7%) and broiler chickens (51.3%), probably because layers have a longer life and thus more likely to become infected.

Moreover, other helicobacters (*H. pylori*, *H. sp. hamster B*) were found in poultry, but such a low prevalence (6.0%) might simply be an index of environmental contamination by other animals or man.

No pets or ornamental birds were positive for *Helicobacter* spp. Since these birds live in a habitat that is completely different from poultry, it might be reasonable to presume that different housing systems may result in different levels of exposure to *Helicobacter*.

So far, in avian species *H. canadensis* has only been isolated in wild geese (Waldénström et al., 2003) and this is the first report on domestic birds (Guinea fowls). Given the potential zoonotic of this bacterium (Fox, 2000; Waldénström et al., 2003), finding *H. canadensis* in farm birds might play a significant role in public health due to frequent human contact with poultry and the consumption of undercooked meat.

It will now be important to investigate the role of *H. canadensis* in human infection.

In conclusion, we observed a high prevalence of *H. pullorum* in poultry farms and, were able to detect for the first time, *H. canadensis* in domestic birds. These
data suggest that farm poultry plays an important role as a reservoir for enteric helicobacters. However, further studies on the epidemiology and pathogenicity are needed before poultry may be considered as a significant source of human infection and therefore a public health challenge.

**References**


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**Identification d’Helicobacters dans l’intestin de différents oiseaux**

On a étudié la présence de diverses souches d’Helicobacters chez la volaille (m = 130) ainsi que chez des oiseaux de volière et d’ornement (m = 50) au moyen d’une PCR qui permet la caractérisation exacte des diverses souches. A l’exception de 21 pintades provenant de France, tous les échantillons venaient d’Italie. Chez environ 80% des volailles (poules pondeuse, poulets d’engraissement, pintades), de l’ADN d’Helicobacters a pu être mis en évidence et H. pullorum étant le plus fréquent (62,1%). H. pylori ainsi que H. sp. hamster B n’étaient présents que dans 3 cas. Toutes les autres espèces d’oiseaux étaient négatives quant à Helico- bacter. Chez toutes les pintades françaises ont a pu mettre en évidence pour la première fois H. cana- densis.

**Identification di Helicobacter enterici in specie avarii Riassunto**

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