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Short communication

Campylobacter jejuni, *Campylobacter coli*, and cytolethal distending toxin (CDT) genes in common teals (*Anas crecca*).

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A B S T R A C T

To evaluate the presence of *Campylobacter* spp. and related *cdt* genes, cloacal swabs were collected from 70 common teals (*Anas crecca*) and analyzed by culture methods and polymerase chain reaction. In addition, *C. jejuni* were examined also for the presence of *wlaN* gene. This is believed to be the first report of *Campylobacter* spp. in common teal and our results confirm the very common occurrence of *C. jejuni* (n = 40) and *C. coli* (n = 13) in waterfowls. Furthermore, the *cdt* genes were frequently present in both *C. jejuni* and *C. coli* isolated. Moreover, seven *C. jejuni* isolates carried also the *wlaN* gene which is presumably involved in the expression of ganglioside mimics in Guillian-Barré syndrome.

***Key words*:** *Campylobacter jejuni*; *Campylobacter coli*; common teal; *cdt* genes; *wlaN* gene.

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1. Introduction

Infection by *Campylobacter* spp., in particular *C. jejuni* and *C. coli*, is considered to be the most prevalent cause of bacterial-mediated diarrhoeal disease worldwide. Although disease is generally mild and self-limiting, severe post-infectious complications such as Gullain-Barrè syndrome may occur (Humphrey et al., 2007).

Many studies have provided strong evidence that various mammals and several avian species may serve as reservoir for this microorganism (Newell and Fearnley, 2003; Gargiulo et al., 2008). However, poultry are considered the main carrier of *Campylobacter* spp. and serve as major sources of infection to human. In fact, the consumption of inadequately cooked poultry meat and its incorrect handling are the main source of infection for humans (Lee and Newell, 2006).

Several virulence factors are considered to be important for the induction of gastroenteritis, such as resistance to bile salts (Lin et al., 2003), invasion of epithelial cells (Russell et al., 1993) and cytholethal distending toxin (CDT) production (Konkel et al., 2001). In particular, CDT is encoded by three linked genes termed *cdt*A, *cdt*B and *cdt*C (Samosornsuk et al., 2007). CDT causes eukaryotic cells to arrest in the G2/M phase of the cell cycle, preventing them from entering mitosis, leading to cell death (Zilbauer et al., 2008). Furthermore, in the last decade, it was identified a gene termed *wlaN* which is presumably involved in the expression of ganglioside mimics in Guillian-Barré syndrome (Linton et al., 2000).

Little is known about the prevalence of *Campylobacter* spp. in wild birds; it has been frequently isolated by waterfowl (Nonga and Muhairwa, 2010) although current scientific knowledge on the presence of this microorganism in common teal (*Anas*

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crecca) are limited. To address this lack of information, the present study was undertaken with the aim to evaluate the prevalence of *Campylobacter* spp. in common teal (*Anas crecca*), and related cytholethal distending toxin genes as well as to evaluate the presence of *wlaN* gene.

2. Materials and Methods

2.1. Sampling

Cloacal swabs were collected from 70 adult common teals (*Anas crecca*) during the wintering period (i.e. January 2008). This sample size was calculated by the formula proposed by Thrusfield (1995) using the following values: study population (about 1500 teals), expected prevalence (5%), confidence interval (95%) and desired absolute precision (5%). The sampling was performed in the wetland areas of the WWF Oasis of Serre-Persano located on the southern side of the Campania region (southern Italy). The corners of the collection area were located at 40° 36' 7.07" N and 15° 8' 9.85" E. The birds were captured, in different roosts, by mist-nets and tunnel traps. Each common teal was individually sampled using a sterile cloacal swab, marked by ring and then released. Marking procedures were performed by Association for Ornithological Studies of Southern Italy (ASOIM) during the regional monitoring for avian influenza under the national program of surveillance for avian influenza. Bird-handling procedures were performed according to the Office of Animal Care and Use guidelines.

2.2. Isolation of bacteria

Cloacal swab samples were inoculated into *Campylobacter* selective enrichment broth (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 42°C for 48 h under microaerobic conditions provided by CampyGen (Oxoid). Subsequently, each sample

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was streaked onto *Campylobacter* blood-free selective agar (CCDA; Oxoid). After incubation at 42°C for 48 h under microaerobic conditions, the plates were examined for typical *Campylobacter* colonies. Suspected colonies were sub-cultured on sheep blood agar (Oxoid) and finally incubated for 24 h at 42°C. Under phase contrast microscopy, colonies comprising curved or spiral motile rods were presumptively identified as *Campylobacter* spp. and submitted to a multiplex polymerase chain reaction (PCR).

2.3. Polymerase Chain Reaction (PCR)

The extraction and purification of DNA from isolated colonies on sheep blood agar was performed using a Bactozol kit (Molecular Research Center, Inc., Cincinnati, Ohio, USA) as described previously (Khan and Edge, 2007). The specific detection of the *Campylobacter* genus was based on PCR amplification of the *cad*F gene using oligonucleotide primers *cad*F2B, and *cad*R1B as described by Konkel et al. (1999). All DNA extracts were also examined, by a triplex PCR, for the presence of *C. jejuni*, *C. coli* and *C. lari* species using oligonucleotide primers ICJ-UP and ICJ-DN, ICC-UP and ICC-DN, ICL-UP and ICL-DN, respectively, as previously described (Khan and Edge 2007). The PCR conditions were as described by Khan and Edge (2007) and the products were separated by electrophoresis on 1.5% agarose gels (Gibco–BRL, Milan, Italy), stained with ethidium bromide and visualized under UV light. PCR amplified without the DNA was used as negative control, whereas three reference *Campylobacter* strains, *C. jejuni* ATCC 29428, *C. coli* ATCC 33559, and *C. lari* ATCC 43675, obtained from LGC Promochem (LGC Promochem, Teddington, UK), were used as positive controls. Furthermore, positive samples for *C. jejuni* and *C. coli* were also examined for the presence of the cytolethal distending toxin genes (*cdt*A, *cdt*B, *cdt*C,

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and *cdt* cluster) using the primers and the procedures described by Bang et al. (2003). Finally, *C. jejuni* positive samples were also examined for the presence of the *wlaN* gene according to Talukder et al. (2008).

3. Results

Out of 70 common teals examined, 42 (60.0%; 95% confidence interval (CI) = 47.6-71.3%) were positive to *Campylobacter* spp. As proved by triplex PCR for the presence of *C. jejuni*, *C. coli* and *C. lari* species, some of species isolated were recovered from mixed infections. Specifically, *C. jejuni* were found in 40 out of 42 (95.2%; 95% CI = 82.6 - 99.2%) positive samples and *C. coli* were found in 13 out of 42 (30.9%; 95% CI = 18.1 – 47.2%) positive samples. In contrast, *C. lari* were consistently not found.

With respect to CDT, all *C. jejuni* isolates carried *cdt*A, *cdt*B, *cdt*C, and *cdt* cluster genes and all *C. coli* isolates carried *cdt*B and *cdt* cluster genes except three *C. coli* strains that not carried both *cdt*A and *cdt*C genes. Regarding the *wlaN* gene investigated for *C. jejuni*, seven strains carried *wlaN* gene. The results of the present study are summarized in table 1.

4. Discussion

The food-borne pathogen *Campylobacter* is a leading cause of gastrointestinal human infection in many industrialized countries, and particularly *C. jejuni* is the most common species implicated (Friedman et al., 2000). Avian species are considered the main reservoir of this microorganism; in fact, it was isolated from both domestic and wild birds but never from the common teal (Van Dyke et al., 2010). A recent study conducted in Tanzania by Nonga and Muhairwa (2010) isolated *Campylobacter* spp.

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from ducks with a prevalence of 80.0% (*n* = 72/90) where the isolation rate of *C. jejuni* was significantly higher (81.9%) than that of *C. coli* (18.1%). Moreover, a study conducted in Turkey by Aydin et al. (2001) isolated *Campylobacter* spp. from geese with a prevalence of 100% (*n* = 40/40) where the majority was identified as *C. jejuni*. Furthermore, a study conducted in United Kingdom by Colles et al. (2008) isolated *Campylobacter* spp. from geese with a prevalence of 50.4% (*n* = 167/331) where the majority was identified as *C. jejuni*.

The 60.0% prevalence rate, inferred from the percentage of cloacal samples positive for *Campylobacter* spp. where once again the majority was identified as *C. jejuni*, among common teals reported in the present study was in line with those rates reported from other waterfowls described above. To our knowledge, this is the first report on the prevalence of *Campylobacter* spp. in common teal and our results confirm the waterfowls as a carrier of this pathogen.

With respect to CDT, our results confirm the high prevalence of the *cdt*A, *cdt*B, and *cdt*C genes in *C.jejuni* which is consistent with the results of previous studies (Pickett et al., 1996; Martínez et al., 2006). Regarding *C. coli*, interestingly, not all isolates carried *cdt*A and *cdt*C genes even though all *C. coli* isolates were positive to PCR using *cdt* cluster primers. These findings are in agreement with Ripabelli et al. (2010) who reported that several mismatches are present in the sequences of PCR primers used in this study and *cdt* genes of *C. coli*. Furthermore, a recent study by Kabir et al. (2011) suggested deletions within *cdt*A, *cdt*B and *cdt*C sequences used for several multiplex PCR. However, as reported by the above cited authors a combination of *cdt* cluster, *cdt*A, *cdt*B, and *cdt*C gene-based multiplex PCR provides more accurate and reliable results for the specific identification of *C. jejuni* and *C. coli*. Several

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publications have reported on the frequency of *cdt* genes among *Campylobacter* isolates from different sources (Bang et al., 2001; Bang et al., 2003; Van Deun et al., 2007). Little is known about the frequency of these toxin genes among *Campylobacter* isolated from waterfowls. Therefore, the results presented here suggest that the *cdt* genes may frequently be present in both *C. jejuni* and *C. coli* also isolated from common teals.

Noteworthy is the detection of the *wlaN* gene carried by *C. jejuni* isolated from common teals as it is presumably involved in the expression of ganglioside mimics in Guillian-Barré syndrome (Linton et al., 2000). Interestingly, the prevalence (17.5%) of this gene in common teal displayed in the present study was similar to that from poultry meat (23.8 %) and to that from human clinical samples (25.0 %) reported by Datta et al. (2003).

In conclusion, therefore, the results presented here underline the high prevalence of *Campylobacter* spp. in common teals and, because this waterfowl lives in wetland, the environmental sources such as water can be contaminated from common teal faeces and the pathogens can be widely disseminated. Thus, *Campylobacter* spp. may easily be transmitted to other waterfowls and humans via this environmental source and/or by close contact. In fact, Varslot et al. (1996) described two water-borne outbreaks of *C. jejuni* infections in humans affecting 1000 persons in central Norway. These epidemics were reported to be caused by contamination of drinking water by the stools of pink-footed geese. The microorganisms from the faeces of the geese were transmitted to the population via untreated drinking water, causing disease in 50% of the population. In addition, in the Italian environment presented here, the common teals have very close contact with other animals and with humans which during the hunting season utilize this

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bird as a game bird. Finally, common teals, as migratory birds, may spread *Campylobacter* spp. for wide-ranging enhancing, thus, their carrier role.

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Table 1

Detection of *C. jejuni* and *C. coli*, related *cdt* genes (*cdt*A, *cdt*B, *cdt*C, *cdt* cluster), and *wlaN* gene from 70 common teals (*Anas crecca*).

Species[a]	No. of isolates	No. of PCR positive with				
		*cdt*A	*cdt*B	*cdt*C	*cdt* cluster	*wlaN*
C. jejuni	40	40	40	40	40	7
C. coli	13	10	13	10	13	/

[a] Some of species isolates were detected from mixed infections.

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