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1 **Short communication**

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

4

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14 ABSTRACT

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

24

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

27

28

29 1. Introduction

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

34 Many studies have provided strong evidence that various mammals and several
35 avian species may serve as reservoir for this microorganism (Newell and Fearnley,
36 2003; Gargiulo et al., 2008). However, poultry are considered the main carrier of
37 *Campylobacter* spp. and serve as major sources of infection to human. In fact, the
38 consumption of inadequately cooked poultry meat and its incorrect handling are the
39 main source of infection for humans (Lee and Newell, 2006).

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

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

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

56 **2. Materials and Methods**

57 *2.1. Sampling*

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

71 *2.2. Isolation of bacteria*

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

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

82 2.3. Polymerase Chain Reaction (PCR)

83 The extraction and purification of DNA from isolated colonies on sheep blood
84 agar was performed using a Bactozol kit (Molecular Research Center, Inc., Cincinnati,
85 Ohio, USA) as described previously (Khan and Edge, 2007). The specific detection of
86 the *Campylobacter* genus was based on PCR amplification of the *cadF* gene using
87 oligonucleotide primers *cadF2B*, and *cadR1B* as described by Konkel et al. (1999). All
88 DNA extracts were also examined, by a triplex PCR, for the presence of *C. jejuni*, *C.*
89 *coli* and *C. lari* species using oligonucleotide primers ICJ-UP and ICJ-DN, ICC-UP and
90 ICC-DN, ICL-UP and ICL-DN, respectively, as previously described (Khan and Edge
91 2007). The PCR conditions were as described by Khan and Edge (2007) and the
92 products were separated by electrophoresis on 1.5% agarose gels (Gibco–BRL, Milan,
93 Italy), stained with ethidium bromide and visualized under UV light. PCR amplified
94 without the DNA was used as negative control, whereas three reference *Campylobacter*
95 strains, *C. jejuni* ATCC 29428, *C. coli* ATCC 33559, and *C. lari* ATCC 43675,
96 obtained from LGC Promochem (LGC Promochem, Teddington, UK), were used as
97 positive controls. Furthermore, positive samples for *C. jejuni* and *C. coli* were also
98 examined for the presence of the cytolethal distending toxin genes (*cdtA*, *cdtB*, *cdtC*,

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

102 **3. Results**

103 Out of 70 common teals examined, 42 (60.0%; 95% confidence interval (CI) =
104 47.6-71.3%) were positive to *Campylobacter* spp. As proved by triplex PCR for the
105 presence of *C. jejuni*, *C. coli* and *C. lari* species, some of species isolated were
106 recovered from mixed infections. Specifically, *C. jejuni* were found in 40 out of 42
107 (95.2%; 95% CI = 82.6 - 99.2%) positive samples and *C. coli* were found in 13 out of
108 42 (30.9%; 95% CI = 18.1 – 47.2%) positive samples. In contrast, *C. lari* were
109 consistently not found.

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

115 **4. Discussion**

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

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

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

135 With respect to CDT, our results confirm the high prevalence of the *cdtA*, *cdtB*,
136 and *cdtC* genes in *C.jejuni* which is consistent with the results of previous studies
137 (Pickett et al., 1996; Martínez et al., 2006). Regarding *C. coli*, interestingly, not all
138 isolates carried *cdtA* and *cdtC* genes even though all *C. coli* isolates were positive to
139 PCR using *cdt* cluster primers. These findings are in agreement with Ripabelli et al.
140 (2010) who reported that several mismatches are present in the sequences of PCR
141 primers used in this study and *cdt* genes of *C. coli*. Furthermore, a recent study by Kabir
142 et al. (2011) suggested deletions within *cdtA*, *cdtB* and *cdtC* sequences used for several
143 multiplex PCR. However, as reported by the above cited authors a combination of *cdt*
144 cluster, *cdtA*, *cdtB*, and *cdtC* gene-based multiplex PCR provides more accurate and
145 reliable results for the specific identification of *C. jejuni* and *C. coli*. Several

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

151 Noteworthy is the detection of the *wlaN* gene carried by *C. jejuni* isolated from
152 common teals as it is presumably involved in the expression of ganglioside mimics in
153 Guillian-Barré syndrome (Linton et al., 2000). Interestingly, the prevalence (17.5%) of
154 this gene in common teal displayed in the present study was similar to that from poultry
155 meat (23.8 %) and to that from human clinical samples (25.0 %) reported by Datta et al.
156 (2003).

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

169 bird as a game bird. Finally, common teals, as migratory birds, may spread

170 *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 (*cdtA*, *cdtB*, *cdtC*, *cdt* cluster), and *wlaN* gene from 70 common teals (*Anas crecca*).

Species ^a	No. of isolates	No. of PCR positive with				
		<i>cdtA</i>	<i>cdtB</i>	<i>cdtC</i>	<i>cdt</i> cluster	<i>wlaN</i>
<i>C. jejuni</i>	40	40	40	40	40	7
<i>C. coli</i>	13	10	13	10	13	/

^a Some of species isolates were detected from mixed infections.