Zero prevalence of *Clostridium difficile* in wild passerine birds in Europe

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Introduction

*Clostridium difficile* is a Gram-positive, anaerobic, spore-forming bacillus (Hall & O’Toole, 1935). It is an important pathogen of humans and a variety of animal species, including companion and farm animals (Borriello et al., 1983; O’Neill et al., 1993; Songer et al., 2000; Weese et al., 2001a, b; Kiss & Bilkei, 2005; Rodriguez-Palacios et al., 2006; Keel et al., 2007), where it is recognized as an important cause of antimicrobial-associated diarrhea and enteritis/colitis syndrome (Poutanen & Simor, 2004).

A major public health concern is the possibility of inapparent animal reservoirs of *C. difficile* and shedding of bacteria to noninfected individuals or populations, as well as being a source of food contamination. Migrating birds can be a key epizootiological factor for transmission and distribution of pathogens over a wide geographic range. Therefore, the purpose of this study was to investigate whether migrating passerine birds can be a source of spread of *C. difficile* along their migration routes. Cloacal samples were taken from 465 passerine birds during their migration south over the Alps. Selective enrichment was used for detection of *C. difficile*. *Clostridium difficile* was not isolated from any of the samples, which indicates that migrating passerine birds are unlikely to serve as a reservoir and a carrier of *C. difficile*.

Materials and methods

Ringing and sampling of wild living passerine birds was conducted in August 2009 and 2010 at the bird ringing station near Vrhnika town (45°46′N, 14°18′E) in the central part of Slovenia. All sampled birds were captured with mist nets. They were placed in net bags/sacks in groups of 1–10 according to species. They were ringed, weighed, measured, and their age was determined. Captured birds were...
migrating passerines breeding in north and temperate regions of Europe and overwintering in Mediterranean and Africa. All birds \((n = 465)\) were sampled with special micro-applicators (Hygroplastic Corp.) to avoid cloacal damage.

A total of 98 cloacal swabs were cultured individually; the remaining \((n = 367)\) samples were pooled according to the species and cultured in pools of up to 10 samples (Table 1).

Cloacal swabs were stored in an anaerobic environment no more than 3 h after collection and transported to the laboratory within 24 h. The samples were then inoculated into clysoerine-cefoxitin fructose enrichment broth (Oxoid, UK) supplemented with 0.1% sodium taurocholate (Sigma-Aldrich) for 7 days. Subsequently, 1 mL of inoculated broth from each sample/pool was mixed with an equal amount of ethanol and left at room temperature for 30 min. After the alcohol shock, the samples were inoculated onto clysoerine-cellosolve C agar base and \(C. difficile\) selective supplement; Oxoid and incubated anaerobically at 37 °C for 2 days (Arroyo et al., 2005; Avbersek et al., 2009). Identification of isolates was based on morphological criteria and typical odor.

**Results and discussion**

A total of 465 samples was taken from six different species of wild living migrating passerine birds (Table 1). None of the samples was positive for \(C. difficile\). Most samples were taken from young birds \((n = 440, 94.6\%)\) on their first migration (Table 1). The change from individual to pooled culture was performed to accommodate a larger population sample in this study after negative initial culture results on individual samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>Individual samples</th>
<th>Pooled samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birds total</td>
<td>Adults</td>
<td>Birds total</td>
</tr>
<tr>
<td>Sedge Warbler</td>
<td>Acrocephalus schoenobaenus</td>
<td>6</td>
</tr>
<tr>
<td>Reed Warbler</td>
<td>Acrocephalus scirpaceus</td>
<td>1</td>
</tr>
<tr>
<td>Robin</td>
<td>Erithacus rubecula</td>
<td>1</td>
</tr>
<tr>
<td>Icterine Warbler</td>
<td>Hippolais icterina</td>
<td>6</td>
</tr>
<tr>
<td>Blackcap</td>
<td>Sylvia atricapilla</td>
<td>27</td>
</tr>
<tr>
<td>Garden Warbler</td>
<td>Sylvia borin</td>
<td>57</td>
</tr>
<tr>
<td>Total ((n = 465))</td>
<td>6 species</td>
<td>98</td>
</tr>
</tbody>
</table>

Bird terminology according to Jancar et al. (1999).

To the authors’ knowledge, this is the first report on assessment of the level of colonization of migrating passerine birds with \(C. difficile\), and the first report of complete lack of detection of \(C. difficile\) in an animal population. The incidence of \(C. difficile\) colonization in samples from this study was expected to be similar to or smaller than those in other animal species epidemiological studies. However, most animals studied to date were subject to intensive breeding where the incidence of \(C. difficile\) colonization is traditionally high (Borriello et al., 1983; Simango, 2006; Rodriguez-Palacios et al., 2007b; Pirs et al., 2008; Simango & Mwakurudza, 2008; Avbersek et al., 2009; Weese et al., 2010).

More than 80% of passerine birds are juvenile on an autumn migration to south (Jakubas & Wojczulanis-Jakubas, 2010). Accordingly, most samples taken in this study were from juvenile birds (94.6%). \(Clostridium difficile\) colonization among different age groups can decrease substantially over time, which is documented in calves, piglets, and chickens (Rodriguez-Palacios et al., 2007b; Zidaric et al., 2008; Alvarez-Perez et al., 2009; Norman et al., 2009). In a single poultry farm in Slovenia, 100% of fecal samples from 2-week-old birds were culture positive. The colonization rate decreased to 71.4% in 14 weeks old birds, and to 40.9% in 18-week-old birds, which indicated a significant age-related variation (Zidaric et al., 2008). Similar findings were evident in a report of an outbreak of a fatal \(C. difficile\) necrotizing enteritis, which selectively affected only juvenile captive ostriches (Struthio camelus) on a single farm (Frazier et al., 1993). In the present study, most samples were taken from birds that were young and on their first migration, which would be just after the peak of their \(C. difficile\) colonization (Zidaric et al., 2008; Weese, 2010). Therefore, negative cultures for \(C. difficile\) were a surprising discovery, especially because \(C. difficile\) in humans and animals is reported from the migration destinations of both the north and south hemisphere (Simango, 2006; Simango & Mwakurudza, 2008; Weese, 2010).

The results of this study indicate that migrating passerine birds in Europe and their southern migratory locations are unlikely to serve as a reservoir or a carrier of \(C. difficile\). Similar results would not be expected in birds that come in closer contact with humans or dwell in habitats intensively cultivated by humans. \(Clostridium difficile\) has been found in > 60% of rivers and water samples (Zidaric et al., 2010); therefore, animals that are in active contact with such habitats would also more likely to be positive for \(C. difficile\) (Levett, 1986).

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References


