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Carriage of Bacteria and Protozoa in the Intestinal Tract of Common Tern Chicks

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Abstract.—Little is known about the intestinal microflora of Common Terns (Sterna hirundo) and their capacity to disseminate human gastrointestinal pathogens along migratory flyways. Common Tern chicks on Pettit Island in Barnegat Bay, New Jersey, USA, were evaluated for carriage of bacterial and protozoan pathogens. Oropharyngeal and cloacal swabs were taken for culture-based detection of bacterial gastrointestinal pathogens during the 2009 and 2010 breeding seasons. Bulk fecal samples were also taken during the 2010 nesting season to determine Cryptosporidium oocyst loads. Of 125 birds sampled in 2009, none carried Salmonella and only one carried Campylobacter. Moreover, the majority of birds sampled in the 2009 breeding season carried Escherichia coli and Klebsiella ozaenae, members of bacterial family Enterobacteriaceae. In 2010, one of the 54 birds sampled carried Salmonella and only one carried Campylobacter. Similarly, the birds sampled in 2009, the 2010 cohort showed relatively high carriage of E. coli and K. ozaenae. Microscopic examination of fecal smears obtained in 2010 revealed that 39 of 54 Common Tern chicks had microscopic structures consistent with Cryptosporidium oocysts in their intestines. These data indicate that Common Tern chicks in Barnegat Bay present low to no threat to public health through the spread of human gastrointestinal pathogens.

Key words.—Campylobacter, Common Tern, Cryptosporidium, gastrointestinal pathogens, Salmonella.

The Common Tern (Sterna hirundo) is the most widespread tern in North America and Europe (Nisbet 2002). Despite their relative abundance and distribution over a broad geographic range, little is known about the resident microflora inhabiting the digestive system of adult and juvenile members of this species. One study conducted by Hanson et al. (2009) demonstrated the presence of Escherichia coli in juvenile Common Terns on Interstate Island in the far western region of Lake Superior. E. coli populations in the intestinal tract of Common Tern juveniles were indistinguishable from those found in the intestinal tract of Ring-billed Gulls (Larus delawarensis) at the same sampling location (Hansen et al. 2009). Kapperud and Rosef (1983) swabbed the cloacae of 36 Common Terns in rural Norway and found that two carried Campylobacter jejuni, only one carried Yersinia enterocolitica, and none carried Salmonella.

During July and August 2004, epidemics of sick and dead fledgling Common Terns were reported at Seal Island National Wildlife Refuge in Maine and the Monomoy National Wildlife Refuge and Cape Cod National Seashore in Massachusetts (Hubalek 2004). Common Terns on Seal Island were unable to raise or extend one wing, while those in Massachusetts were unable to maintain their balance and either could not fly or were circling. No viruses were isolated, but many specimens from Massachusetts tested positive for Salmonella enteriditis Typhimurium. It was speculated that this was an opportunistic outbreak caused by immune system impairment (Hubalek 2004). In another study, researchers examined blood smears from 75 Common Terns on Bird Island, New Island and Plover Island off the coast of Massachusetts and found no hemoparasites (Fiorello et al. 2009).

There is some concern that migratory birds may play a role in the spread of zoonotic agents and impact water quality (Tsiodras et al. 2008; Krauss et al. 2004; Rappole et al. 2000). However, the authors of a recent review noted that evidence supporting direct pathogen transmission from wild birds to humans is rare (Tsiodras et al. 2008). Given
the feeding habits of Common Terns and their tendency to avoid human interaction, we hypothesize that this species does not play an important role in the carriage, spread or transmission of human pathogens. To evaluate this hypothesis, we sought to identify specific pathogenic bacteria isolated from cloacal and oropharyngeal samples of Common Tern chicks on Pettit Island in Barnegat Bay, New Jersey. Samples were evaluated for the presence of potential bacterial pathogens such as *E. coli*, *Salmonella* and *Campylobacter* and the protozoan parasite *Cryptosporidium*.

**METHODS**

**Cloacal and Oropharyngeal Sampling**

Pettit Island is a small saltmarsh island in Barnegat Bay, New Jersey, and the site of a Common Tern colony (see Palestis 2009 for site description). Field work was conducted under B.G.P.’s federal bird banding permit and state scientific collecting permits. Samples were collected on three different dates spanning two breeding seasons (17 June 2009, 8 July 2009, and 2 July 2010). There were approximately 250 breeding pairs present in 2009 and 300 in 2010. The range of dates allowed sampling from 179 chicks of different ages, ranging from newly hatched chicks to chicks near fledging, including twelve chicks that were swabbed at both time points in 2009. Tubes were labeled with the date, age (if known), hatching order and orifice sampled. Sterile cotton swabs were used to sample the oropharyngeal and/or the cloaca of Common Terns. The swabs were then placed in test tubes containing sterile Nutrient Broth (BD Difco™) for bacterial recovery (one sample per tube). Because the cloaca was often hidden by feathers in older chicks but protruding in young chicks, and because young chicks would not readily open their mouths for oral sampling, oropharyngeal swabs were taken mostly from older chicks while cloacal swabs were taken from younger chicks.

Oropharyngeal and cloacal samples in Nutrient Broth were then subjected to passage through selective media to identify bacteria present. Fifty soil samples from Pettit Island were also collected during the 2009 nesting season. These samples were tested for bacteria in a manner similar to the cloacal and oropharyngeal swabs.

In addition to the oropharyngeal and cloacal samples for bacterial detection, bulk fecal samples were collected in 2010 to check for carriage of *Cryptosporidium* oocysts. Chicks were oriented so their cloaca was over the opening of a 50-mL conical centrifuge tube containing 20 mL of sterile phosphate buffered saline. The chicks were then gently squeezed to induce defecation. Samples were transported to the lab and immediately fixed with formalin as indicated in the “Microscopic identification of *Cryptosporidium* oocysts” section below.

**Microbiological Assays**

We chose culture-based detection methods to assess bacterial carriage among Common Terns. Cultivation of the target organisms from turbid samples with diverse microecology is very reliable and does not suffer from false-positive or false-negative rates associated with nucleic acid- or surface antigen-based methods (Aburresh et al. 2006).

**Isolation of *Salmonella* Species**

After the recovery period, samples were inoculated into tetraionate broth (TTI) and selenite-cystine broth (BD Difco™) to select for *Salmonella* species (35 °C, 18-24 hours). Bacteria growing in the TTI and selenite-cystine broths were subjected to a second round of selection by streaking onto xylose lysine deoxycholate agar (XLD) (35°C, 18-24 hours) and bismuth sulfite agar plates (35°C, 48 hours) (BD Difco™).

Colonies displaying phenotypic characteristics consistent with *Salmonella* on XLD and bismuth sulfite agar were considered presumptive positive. Presumptive positive colonies were inoculated onto triple sugar iron (TSI) agar slants (BD Difco™) (37°C, 24-48 hours), and isolates that produced hydrogen sulfide and fermented glucose, but not lactose, were confirmed to be *Salmonella* spp. (United States Pharmacopeial Convention 2005).

**Isolation and Identification of Fecal Coliforms**

Non-salmonella coliform bacteria growing on bismuth sulfite and XLD agar were tested for oxidase activity using an oxidase DrySlide™ (BD BBL™). Fifty oxidase-negative, non-salmonella coliform isolates were selected at random (25 isolates from XLD and 25 from bismuth sulfite) for identification using the Enterotube II system (BD BBL™). Enterotubes were inoculated and incubated at 37°C for 48 hours and read according to the manufacturer’s instructions.

**Isolation of *Campylobacter* Species**

Samples from the recovery medium (Nutrient Broth inoculated in the field) were also streaked onto *Campylobacter* blood-free selective agar (Biomerieux) containing CCDA selective supplement (Oxoid) and incubated in air-tight jars with CampyPak™ Plus Microaerophilic System Envelopes with Palladium Catalyst (BD BBL™) (42°C for 48-72 hours). Grey, transparent, flat, mucoid colonies were Gram-stained and tested for oxidase activity. Gram-negative, spiral-shaped isolates that were oxidase positive were confirmed positive as *Campylobacter* spp.

**Microscopic Identification of *Cryptosporidium* Oocysts**

Avian fecal samples in phosphate-buffered saline were fixed by mixing with formalin (50:50). Microscope
slides were prepared by smearing formalin-fixed fecal samples on glass slides and placing on a slide warmer at 60°C until completely dry. Smears were stained using a modified acid-fast staining method as described by Vi-vesvara et al. (1997). Slides were observed through the microscope at 1,000x magnification and Cryptosporidium oocysts were identified as safranin stained structures that were 5-12 micrometers in diameter with furrowed edges.

Statistical Analysis

Inter-year comparison of bacterial carriage was evaluated with a Fisher’s Exact test.

RESULTS

Of 125 Common Tern chicks sampled during the 2009 breeding season, none tested positive for *Salmonella*, and one had phenotypic characteristics consistent with *Campylobacter* (Table 1). Approximately 52.8% of the samples were positive for *E. coli* when cultured on XLD and bismuth sulfite plates. Many (70.4%) of the Common Tern chicks sampled also carried *Klebsiella ozaenae* (Table 1). The non-salmonellae enteric species *Serratia liquefaciens* and *Klebsiella pneumoniae* were also isolated, but only rarely. Of 50 soil samples collected from the Pettit Island nesting area in 2009, only one contained any of the targeted organisms (confirmed positive for *Salmonella*). Birds from the 2009 cohort were not tested for fecal carriage of *Cryptosporidium* oocysts.

Of 54 Common Tern chicks sampled during the 2010 breeding season, one tested positive for *Salmonella* and none had *Campylobacter* (Table 1). Similar to the 2009 results, *K. ozaenae* and *E. coli* were frequently isolated, and *S. liquefaciens* and *K. pneumoniae* were occasionally found. There were no statistical differences between bacterial carriage rates for 2009 and 2010 (Fisher’s Exact Test, *P* > 0.3 for all species tested).

Almost three-quarters (72.2%) of the fecal samples collected in 2010 contained structures that were microscopically consistent with *Cryptosporidium* oocysts (Table 1). Fecal smears also contained microscopic structures with morphological similarity to nematodes. Approximately 19% of all birds sampled in
2010 contained nematode-like structures. We did not identify nematode species.

**DISCUSSION**

Little is known about the microflora of *Sterna hirundo*. One study demonstrated the presence of *E. coli* in juvenile terns in the Great lakes (Hansen *et al.* 2009), while another reported a 5% carriage rate for *Campylobacter jejuni* among a Common Tern population in rural regions of Norway (Kapperud and Rosef 1983). However, Common Terns tend to nest in isolated habitats that are free of human activity (Nisbet 2002), and their diet of freshly-caught fish likely results in relatively low exposure to human bacterial pathogens. Our results with Common Terns from the Pettit Island nesting colony in New Jersey confirm, in part, this supposition. We found very low carriage of important human gastrointestinal pathogens such as *Campylobacter* and *Salmonella*. The rate of carriage for *E. coli* was fairly high, agreeing with the previous findings of Hansen *et al.* (2009). However, our study did not identify *E. coli* at the strain level and we cannot rule out the possibility that these isolates were avirulent. *K. ozaenae*, an Enterobacteriaceae species associated with rare nasal infections in humans, was also commonly isolated (Botelho-Nevers *et al.* 2007). *K. ozaenae* is normally transmitted person-to-person via nasal discharge, and it is highly unlikely that carriage of this species among Common Terns poses a threat to human health.

*Cryptosporidium* is a protozoan parasite that forms hardy oocysts that cause diarrheal disease when ingested. To our knowledge, no previous studies have examined Common Terns for carriage of *Cryptosporidium* oocysts. One study found that non-migratory avian species at the Kuala Lumpur Zoo carried *Cryptosporidium*, but the authors did not assess species related to the Common Tern (Rohela *et al.* 2005). Using a modified acid-fast method developed by Visvesvara *et al.* (1997), we found that the majority of Common Terns had microscopic structures consistent with *Cryptosporidium* oocysts in their feces.

Overall, Common Terns appear not to pose a significant threat for the spread of bacterial human pathogens. While Common Terns may play a role in the dissemination of *Cryptosporidium* between marine environments, it is important to note that species determination is very difficult based on oocyst morphology alone, and many *Cryptosporidium* species have a narrow host range that does not include humans. Further work is needed to confirm the *Cryptosporidium* findings and determine the impact of Common Tern nesting colonies on local water quality.

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**LITERATURE CITED**


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