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PREVALENCE OF *SALMONELLA* SPP., *CAMPYLOBACTER* SPP. AND *LISTERIA* SPP. IN RING-BILLED GULLS (*LARUS DELAWARENSIS*)

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ABSTRACT: Cloacal swabs collected from 264 ring-billed gulls (*Larus delawarensis*) at four sites near Montréal, Canada were cultured for the presence of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. All birds were apparently healthy when captured or killed. Of all birds examined, 8.7%, 15.9% and 9.5%, respectively, were infected with *Salmonella* spp., *Campylobacter* spp. and *Listeria monocytogenes*. Overall, 29.9% of gulls sampled harbored one or more of these bacteria. Gulls probably play only a minor role in the epizootiology of these bacteria.

Key words: *Salmonella* spp., *Campylobacter* spp., *Listeria monocytogenes*, gulls, Laridae.

INTRODUCTION

Intestinal carriage of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. by gulls is well recognized (Fenlon, 1985; Girdwood et al., 1985; Whelan et al., 1988). Most studies have been done in Europe and Japan on herring gulls (*Larus argentatus*), black-headed gulls (*Larus ridibundus*) and common gulls (*Larus canus*). In Québec and Ontario, Canada, populations of ring-billed gulls (*Larus delawarensis*) have increased considerably during recent years (Mousseau, 1984). An increase in the number of birds in public sites has been noted (Blokpoel and Tessier, 1986).

Girdwood et al. (1985) suggested that gulls were not an important factor in the epidemiology of human salmonellosis because of the low number of *Salmonella* recovered in gull feces. However, they suggested that a large number of gulls roosting in one site may represent a health hazard. Reilly et al. (1981) concluded that gulls were the source of environmental contamination in 3 of 26 occurrences of human and animal salmonellosis in Scotland. Gulls may play a significant role in bovine and ovine salmonellosis (Williams et al., 1977; Coulson et al., 1983; Sharp et al., 1983).

Whelan et al. (1988) found that 64% of the gulls sampled by cloacal swabbing carried *Campylobacter* spp. About 30% of

those were *C. jejuni*, an important human pathogen, but the most common serotypes present in gulls were not common in humans. They concluded that a health hazard to humans is possible only when large numbers of gulls roost in a common place. Fenlon et al. (1982) and Kaneuchi et al. (1987) found differences in intestinal carriage of *Campylobacter* spp. between different species of gull. Carter et al. (1987) studied the occurrence of *Campylobacter* spp. in surface waters and stated that the degree of surface water contamination with *Campylobacter* spp. depended in part on the prevalence of carrier animals in the environment.

Fenlon (1985) isolated *L. monocytogenes* from gulls' feces and suggested that gulls may be responsible for some pasture contamination with *Listeria* sp.; and if there are any defects in the silage process, the silage may remain contaminated.

To estimate fecal carriage rates and to evaluate the role of ring-billed gulls in the ecology of these bacteria, we determined the prevalence of these three genera of bacteria in feces of ring-billed gulls at four sites. We also determined whether prevalences of these bacteria varied with the type of land use.

MATERIALS AND METHODS

Fecal material from the cloaca of 264 apparently healthy gulls was collected with swabs

(Culturette, Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) near Montréal, Québec, Canada. Site 1 (Ile de la Couvée) was an embankment island, erected during construction of the St-Lawrence seaway, 1 km in length and 15 m in width in its northern extremity and 110 m in width in the south in the St-Lawrence River (45°28'N, 73°30'W); it was located 2 km south of Montréal. This island is near an urban setting and is covered by short-grass and a few shrubs. Site 2 (Ile St-Ours) also was an island in the St-Lawrence River (45°55'N, 73°15'W) 2 km in length and 800 m in width, located approximately 30 km east of Montréal in a rural area. This uninhabited island was covered with tall grass, shrubs and trees. Site 3 (45°43'N, 72°01'W) was a refuse dump surrounded by corn fields in a rural area about 80 km southeast of Montréal. Site 4 was a section of shoreline on the St-Lawrence River, 50 km east of Montréal close to a small town (46°02'N, 73°05'W). The shores were lined primarily with emergent vegetation.

One hundred and sixty-two birds were live-trapped with cages as described by Mills and Ryder (1979) at sites 1 and 2 and released following sampling; an additional 102 birds were shot by authorization of the Canadian Wildlife Service at sites 3 and 4. Sites 1 and 2 were nesting sites. Once collected, the swabs were kept in a styrofoam box with icepacks. Swabs for *Campylobacter* spp. isolation were stored in an anaerobic jar (Oxoid Canada Inc., Nepean, Ontario, Canada) and kept under microaerobic conditions by means of a gas generator envelope (CampyPack, Becton Dickinson Microbiology Systems). Samples were returned to the laboratory and put in their respective enrichment broths <24 hr after collection.

Swabs tested for *Salmonella* spp. were inoculated into 10 ml of brilliant green tetrathionate broth (Difco Laboratories, Detroit, Michigan, USA) with 0.2 ml of potassium iodine solution added (6 g iodine crystals and 5 g potassium iodide in 20 ml distilled water), and incubated for 18 hr at 43 C. Samples were streaked on a brilliant green sulfadiazine agar (Difco Laboratories) and incubated at 37 C for 24 hr. Suspect colonies were inoculated into a triple-sugar-iron (TSI) (Difco Laboratories) agar slant and streaked on an urea agar slant (Difco Laboratories). A slide-agglutination test (Tinghitella and Edberg, 1991) was done on each suspect isolate using a Salmonella polyvalent O antiserum (Bacto Salmonella O antiserum poly A to I and Vi, Difco Laboratories). Biochemical identification was done with an automated system (Vitek Systems, Hazelwood, Missouri, USA). Confirmed *Salmonella* were serotyped by slide-agglutination

with Salmonella O group D₁ antisera (Difco Laboratories) at the Microbiology section of the Health of Animals Laboratory of Agriculture Canada in Guelph, Ontario.

Swabs tested for *Campylobacter* spp. were inoculated into 10 ml of Rosef's enrichment broth (Rosef, 1981) supplemented with vancomycin (10 µg/ml) (Sigma Chemical Company, St. Louis, Missouri) trimetoprim (5 µg/ml) (Sigma Chemical Company), polymyxin B sulfate (2.5 IU/ml) (Sigma Chemical Company) and a 10% concentration each of ferrous sulfate, sodium metabisulfite and sodium pyruvate (Fisher Scientific Limited, Montréal, Québec, Canada). The tubes of Rosef's enrichment broth were incubated at 43 C under microaerophilic conditions (85% nitrogen, 10% carbon dioxide, 5% oxygen). Mueller-Hinton agar plates (Oxoid Canada, Inc.) supplemented with 10% citrated bovine blood and vancomycin (10 µg/ml), trimetoprim (5 µg/ml) and polymyxin B sulfate (2.5 IU/ml) were inoculated at 24 and 48 hr, respectively. Plates were incubated at 43 C under microaerobic conditions and examined after 24 and 48 hours for the presence of suspect colonies. Identification was carried out on suspect colonies by a Gram stain, catalase, oxidase and sensitivity to naladixic acid and cephalothin (30 µg SensiDiscs, Becton Dickinson Microbiology Systems) (Karmali et al., 1980). Biotyping was done on the basis of the rapid hippurate hydrolysis, rapid H₂S test and DNA hydrolysis (Lior, 1984). Classification of the isolates was done according to the scheme of Lior (1984).

Swabs tested for *L. monocytogenes* were inoculated into 10 ml of Listeria enrichment broth (LEB) (Lovett, 1988a) with 0.1 ml of a 0.12% (w/v) solution of acriflavin (Sigma Chemical Company) added. Following incubation for 24 hr at 30 C, 0.1 ml of this initial broth was transferred to a second LEB tube supplemented with 0.2 ml of 0.12% acriflavin. Tubes were incubated for 24 hr at 30 C. Oxford agar plates (Oxoid, Canada, Inc.) then were inoculated and incubated at 37 C for 24 hr. Suspect colonies were streaked onto trypticase soy agar plate (Becton Dickinson Microbiology System) with 0.6% yeast extract (Difco Laboratories) supplemented with 5% citrated bovine blood to detect the presence of hemolysis. All Gram-positive rod-shaped organisms were further tested. The identification scheme includes the CAMP test (Christie et al., 1944) using *Staphylococcus aureus* and *Rhodococcus equi* (Lovett, 1988b; McClain and Lee, 1988), catalase test, motility at 22 C, acid production from rhamnose, xylose, mannitol, maltose, glucose and methyl-alpha-D-mannopyranoside, and nitrate reduction (MacFaddin, 1980).

TABLE 1. Prevalence of enteric pathogens in ring-billed gull feces collected in Québec, Canada, April to July 1990.

Location	Bacteria isolated			
	<i>Salmonella</i> spp.	<i>Campylobacter</i> spp.	<i>Listeria monocytogenes</i>	One or more of these bacteria
Site 1 (Nesting site)	5/79 (6.3)*	9/79 (11.4)	7/79 (8.9)	20/79 (25.3)
Site 2 (Nesting site)	9/83 (10.8)	17/83 (20.5)	11/83 (13.3)	30/83 (36.1)
Site 3 (Refuse tip)	6/46 (13.0)	2/46 (4.3)	2/46 (4.4)	9/46 (19.6)
Site 4 (River shore)	3/56 (5.4)	14/56 (25.0)	5/56 (8.9)	19/56 (33.9)
Total	23/264 (8.7)	42/264 (15.9)	25/264 (9.5)	78/264 (29.6)

* Number of birds positive/number of birds sampled (% positive).

RESULTS

Salmonella spp. were recovered from the feces of 23 (8.7%) of 264 gulls (Table 1). Birds sampled at the refuse dump site had the highest isolation prevalence (13%). Ten serotypes were isolated: ten *S. hadar* were recovered, three *S. heidelberg*, two *S. berta*, two *S. thompson*, one *S. haardt*, one *S. typhimurium*, one *S. manilla*, one *S. kentucky*, one *S. infantis* and one *S. montevideo*. Furthermore, 15.9% of the gulls examined carried *Campylobacter*; 61.9% of all *Campylobacter* recovered were *C. jejuni*, an important human enteric pathogen (Table 2). Biotype I of this species was isolated most frequently. Isolation of *Campylobacter* was highest (25%) in birds on the river shore. One hundred (38%) of 264 birds sampled carried listeriae. *Listeria monocytogenes* was isolated from 25 (9.5%) of 264 gulls; it was most prevalent (13%) in birds from the rural nesting site. *Listeria innocua* was isolated 36 times (13.6%), *L. welshimeri* 37 times (14%), *L. grayi* and *L. ivanovii* one time each (0.4%). This is the first report of *L. ivanovii* isolation from gulls.

DISCUSSION

The prevalence of *Salmonella* spp. in this study (Table 1) is similar to that reported in other species of gulls (Fenlon, 1981; Butterfield et al., 1983; Girdwood et al., 1985; Monaghan et al., 1985). The serotypes found in gulls were among those most often isolated from humans (Fenlon,

1981; Butterfield et al., 1983; Girdwood et al., 1985). Further studies, especially in regard to the number of *Salmonella* in gull feces, are required before concluding on the role of ring-billed gulls in human and animal salmonellosis. We agree with Girdwood et al. (1985) that gulls probably represent a potential health hazard to humans and other animals only when a large number of birds roost at the same site. The presence of gulls near food processing plants (Berg and Anderson, 1972) has been identified as a possible source of contamination.

Our conclusions about *Campylobacter* spp. were different. Whelan et al. (1988) found that only 24% of *C. jejuni* and *C. coli* from gulls could be serotyped with Penner's antisera (Penner and Hennessy, 1980), in contrast with 76% of the human isolates. Whelan et al. (1988) concluded that gulls were not a likely source of infection for humans. Kaneuchi et al. (1989) also observed that only a low frequency of *C. jejuni* from gulls could be serogrouped within the scheme of Lior et al. (1982) and Itoh et al. (1982), but concluded that further investigations were necessary to clarify the significance of gulls as a potential source for human campylobacteriosis. While the *Campylobacter* spp. prevalence in our study is low (15.9%) compared with other findings (Fenlon et al., 1982; Kapperud and Rosef, 1983; Fricker et al., 1983), we concur with Kaneuchi et al. (1989). The number of *Campylobacter* spp. found in a single gull feces ($\leq 10^4$ /g) is sufficient to

TABLE 2. Biotypes of *Campylobacter* spp. isolated from ring-billed gulls, Québec, Canada, April to June 1990.

	<i>C. jejuni</i>				<i>C. coli</i>		<i>C. lari</i>	
	I*	II	III	IV	I	II	I	II
Number of isolates	19	4	1	2	2	—	12	2
Percent of all <i>Campylobacter</i> isolated	45.2	9.5	2.4	4.8	4.8	—	28.6	4.8

* Biotype of *Campylobacter* spp.

contaminate and cause illness in humans (Robinson, 1981; Fenlon et al., 1982). While only 30% of the *C. jejuni* or *C. coli* isolates were serologically related to human's isolates, this could still represent an appreciable number of microorganisms. There is increasing evidence that *C. lari* is associated with human gastrointestinal infections (Tauxe et al., 1985; Simor and Wilcox, 1987).

As ring-billed gulls populations increase, their usual feeding sites become saturated and gulls may turn to corn, tomatoes, strawberries and other fruits as food sources (Blokpoel and Tessier, 1986). The high prevalence of *L. monocytogenes* in gulls (Table 1) and the new feeding habits of ring-billed gulls would support the proposition of Fenlon (1985) that gulls may be a significant source for contamination of silage. Spoiled silage is a common source of *L. monocytogenes* in cattle (Blenden et al., 1987). Gulls also may contaminate vegetables or fruits and play a role in foodborne listeriosis (Blokpoel and Tessier, 1986).

When considering all three genera of bacteria, birds sampled at the refuse dump had the lowest prevalence (19.6%) (Table 1). *Campylobacter* spp. had its lowest recovery rate at the refuse dump. *Campylobacter* spp. are relatively sensitive to elevated temperature and dessication (Blaser et al., 1980a), and this site was fairly dry; hot weather prevailed during the sample collection period of summer 1990. In contrast, the highest prevalence of *Campylobacter* was found in birds from the river shore. *Listeria monocytogenes* was ob-

served most frequently at the Ile St-Ours nesting site, which is located in a rural area. This supports Welshimer and Donker-Voet's (1971) finding that *L. monocytogenes* is widespread in farm soil and vegetation. Differences noted in environmental contamination may explain the variation in prevalence noted in birds between the different sites (Table 1).

Salmonella spp., *Campylobacter* spp. and *L. monocytogenes* are widely distributed in the environment (Welshimer and Donker-Voet, 1971; Thomason et al., 1977; Penner and Hennessy, 1980; Watkins and Sleath, 1981; Prescott and Munroe, 1982; Carter et al., 1987; Pelzer, 1989). Gulls can become infected by feeding at a contaminated site (Reilly et al., 1981; Girdwood et al., 1985; Whelan et al., 1988). Thus the prevalence would reflect the degree of contamination of their environment (Butterfield et al., 1983; Fenlon, 1985; Monaghan et al., 1985).

Gulls could contribute to spreading these pathogens. However, gulls rarely have been implicated in human or animal illnesses from these bacteria (Fennel et al., 1974; Williams et al., 1977; Reilly et al., 1981; Benton et al., 1983; Coulson et al., 1983; Sharp et al., 1983). In our opinion, ring-billed gulls probably do not play a major role in the epidemiology of human or animal salmonellosis, campylobacteriosis or listeriosis. Other sources of infection (Blaser et al., 1980b; Manger and Dalziel, 1985; Blenden et al., 1987; Pelzer, 1989) should be considered first, but one can not neglect gulls as a possible source of infection when environmental contamination is suspected,

particularly when gulls are present in large numbers in the area.

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