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Influence of Refuse Sites on the Prevalence of *Campylobacter* spp. and *Salmonella* Serovars in Seagulls[▽]

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Wild animals are well-known reservoirs of *Campylobacter* and *Salmonella*. We investigated the influence of insalubrious diets on the prevalence of both enterobacteria in seagulls. *Campylobacter* occurrence in gull chicks sampled along the northeastern Iberian coast was directly related to the degree of refuse consumption. High *Salmonella* values from the sampling sites did not reflect any dietary relationship.

Campylobacter and *Salmonella* spp. are the leading causes of zoonotic enteric infections in developed and developing countries, and their incidence is increasing even in countries with adequate public health surveillance (14, 27, 44). Despite the health impact of these enteropathogenic bacteria, their epidemiology remains poorly understood, and the full epidemiological pathways leading to infection in humans have not yet been elucidated.

Well-known modes of transmission to humans include physical contact with domestic animals, person-to-person spread, and consumption of contaminated food and water (44). In addition, wild animals might play a significant role in the epidemiology of enterobacteria (3, 34). For instance, the role of wild birds in the bacteriological deterioration of drinking and recreational water reservoirs by fecal contamination is well documented (2, 19). Furthermore, due to their ability to fly freely and to cover long distances during annual movements, wild-living birds are suspected of functioning as effective dispersers of disease via the aforementioned fecal contamination of pastures and surface waters throughout the world (33).

Seagulls in particular, due to their scavenging feeding habits, are one of the most documented carriers of *Campylobacter* and *Salmonella* (8, 11, 18, 23). The increasing number of studies concerning seagulls and environmental public health are also partially due to the fact that populations of several species of gulls (*Larus* spp.) have increased dramatically throughout Australia, North America, and Europe during the past several decades (36, 41). Although feeding habits related to garbage and sewage have been largely assumed to increase the risk of microbiological infection on wildlife (8, 19), to our knowledge no studies prove this assumption by combining dietary analysis and microbiological carriage determination.

During the late chick-rearing period of 2005, 182 yellow-legged gull chicks were sampled in three colonies along the northeastern Iberian coast (Medes Islands, $n = 75$; Ebro Delta, $n = 36$; and Columbretes Islands, $n = 71$) (see Table 1 for colony details). A single fledgling from each brood was captured, sampled, measured, weighed, and marked. Avian health status was evaluated through a body condition index computed from residuals from a regression of body size (principal-component analysis based on head-bill, tarsus, and wing lengths) against mass (39, 43). We collected 6 to 8 growing scapular feathers from each bird as well as some food samples spontaneously regurgitated (kept frozen at −20°C) for stable isotope analysis of carbon (C), nitrogen (N), and sulfur (S). Once regurgitated items were identified and classified according to their origin (marine, brackish, and freshwaters; crops and terrestrial environments; or refuse sites), we followed standard procedures (7, 13, 32), weighing and placing into tin capsules subsamples of powdered feathers and powdered food for final combustion in a stable isotope mass spectrometer (ThermoFinnigan, Bremen, Germany). Stable isotope ratios were expressed in the standard δ notation relative to Pee Dee Belemnite ($\delta^{13}C$), atmospheric nitrogen ($\delta^{15}N$), and troilite of the Canyon Diablo meteorite ($\delta^{34}S$) (32). Relative indexes of the different food sources were estimated for every individual, using a triple-isotope, four-endpoint (marine and freshwaters, terrestrial environments, and refuse sites) mixing model (30). Finally, for bacterial isolation, duplicate cloacal swabs from each chick were taken and placed in Amies charcoal medium (Deltalab, Barcelona, Spain), stored under refrigeration, and cultured within 2 to 4 days after sampling. *Salmonella* isolation was performed according to ISO 6579:2002 (1), and presumptive colonies on MacConkey agar (Oxoid, United Kingdom) were confirmed with the Mucap test kit (Biolife, Italy) and API 20E system (bioMérieux, France). *Salmonella* species isolates were serotyped at the National Reference Centre for Animal Salmonellosis (Algete, Madrid, Spain). *Campylobacter* isolation was performed as described previously (42). Identification of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter lari* were carried out with a species-specific PCR (10, 37).

Salmonella spp. were isolated from 31 birds (17.0%; 95% confidence interval [CI], 11.9 to 23.1%), which generated an overall incidence similar to those described from other studies within the same area (5) as well as from the Atlantic Iberian

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TABLE 1. Main informative parameters of studied breeding sites

Location (coordinates)	Breeding site	Distance from human settlements (km)	No. of breeding pairs	Fishing vessel activity around each area (2007)[a]			Reference
				Relative estimation	No. of vessels	Gross tonnage	
Medes Islands (42°0′N, 3°13′E)	Protected islands off the coast of a tourist resort	0.9	6,500[b]	Moderate–high	392	8,956	6
Ebro Delta (40°40′N, 0°45′E)	Isolated peninsula in a natural park	7.5	6,000	High	358	11,195	28
Columbretes Islands (39°54′N, 0°41′E)	Isolated archipelago in a marine reserve	55	450	High	245	9,248	28

[a] Fishing vessel information for each area was taken from http://ec.europa.eu/fisheries.
[b] After several cullings.

Peninsula (12), the British Islands (23), Fennoscandia (34), and Central Europe (11, 16). However, *Campylobacter* spp. were recovered only from 19 (10.4%; 95% CI, 6.6 to 15.7%) of all the samples collected (Table 2), which, compared to the results of other studies, represented a relatively low carrier rate (8, 16, 24). The highest values of *Salmonella* species prevalence among fledging gulls were observed in the Medes Islands (Table 2), although differences among localities were not statistically significant ($\chi^2 = 1.99$, $P = 0.39$). Similarly, the prevalence of *Salmonella enterica* serovar Typhimurium did not show any significant geographical variation ($\chi^2 = 2.53$, Monte Carlo $P = 0.36$). A conditional logistic model using locality as the stratum did not find any reliable model to fit *Salmonella* species and *S.* Typhimurium prevalences using the consumption of refuse, the body condition index of gulls, and their possible interaction as dependent variables (likelihood ratio [LR] test = 3.29, Monte Carlo $P = 0.19$, and LR test = 0.8, Monte Carlo $P = 0.71$, respectively). On the other hand, *Campylobacter* prevalence was significantly greater in gulls from the Medes Islands

TABLE 2. *Campylobacter* species and *Salmonella* serovar carriage rates of fecal samples from yellow-legged gull chicks sampled throughout three western Mediterranean colonies

Species or serovar	No. (%) of infected gull chicks from[a]:		
	Medes Islands ($n = 75$)	Ebro Delta ($n = 36$)	Columbretes Islands ($n = 71$)
Campylobacter species			
C. jejuni	8	0	1
C. coli	0	0	0
C. lari	0	0	0
Not determined	6	2	2
Total carriers	14 (18.67)	2 (5.56)	3 (4.23)
S. enterica subsp. *salamae* serovar Sofia (4,12:b:-)	1 (1.33)	0 (0)	0 (0)
S. enterica subsp. *enterica* serovar			
Azteca	1	0	0
Bardo	1	0	0
Brandenburg	0	0	1
Bredeney	2	2	1
Corvallis	0	0	2
Derby	1	0	2
Enteritidis	0	0	1
Hadar	0	2	2
Ituri	1	0	0
Lexington	1	0	0
Newport	2	0	0
Paratyphi B	1	0	0
Rissen	1	0	0
Typhimurium	6	1	2
Virchow	0	0	1
1,4,5,12:i:-	1	0	0
1,4,12:i:-	1	0	0
4,12:i:-	1	0	0
4,5,12:i:-	1	0	0
Total carriers	16 (21.33)	4 (11.11)	11 (15.49)

[a] Bacterial prevalences are in parentheses.

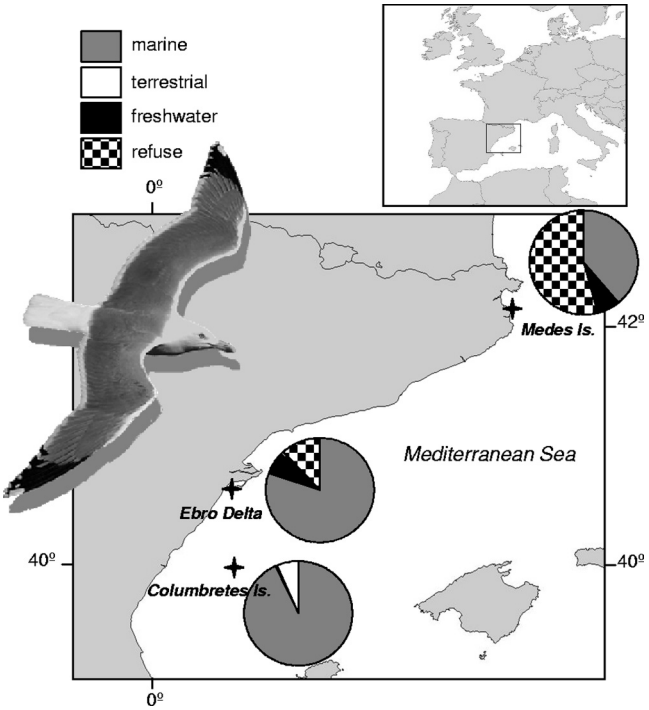

FIG. 1. Map locations and foraging habitat exploitation of the yellow-legged gull colonies sampled in the study along the western Mediterranean coast. Foraging habitat exploitation percentages estimated by individual isotopic mixing models are represented as colony means in the circle diagrams.

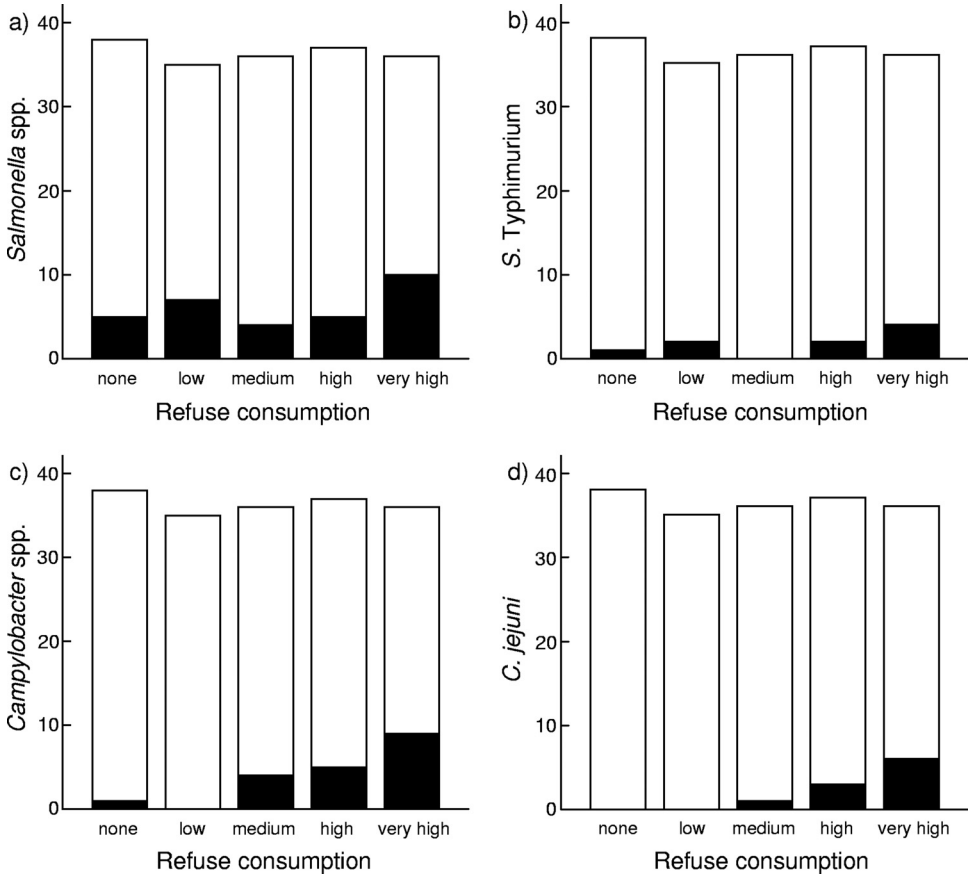

FIG. 2. Enterobacterial prevalence on yellow-legged gull chicks according to refuse consumption on the Iberian Mediterranean coast. The numbers of birds positive for *Salmonella* spp. (a), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (b), *Campylobacter* spp. (c), and *Campylobacter jejuni* (d) are shown in black according to refuse consumption. The numbers of gulls negative for enterobacteria are shown in white. *x* axis categories represent the quintiles ($n = 182$; none = 38, low = 35, medium = 36, high = 37, very high = 36) according to the individual refuse consumption percentages estimated from isotopic mixing models.

(18.7%) than in gulls from the Columbretes Islands, where only three birds (4.2%) were found positive for *Campylobacter* ($\chi^2 = 9.28$, $P = 0.0007$) (Table 2). A conditional logistic model using locality as the stratum showed that *Campylobacter* prevalence was positively related to refuse consumption (parameter ± standard error, 7.19 ± 3.31; $P = 0.029$) (see Fig. 2), whereas the body condition index did not show a significant relationship. Similarly, *C. jejuni* carriage was more probable in gulls from the Medes Islands than in gulls from the other localities ($\chi^2 = 10.5$, Monte Carlo $P = 0.001$), although we failed to find any relationship with refuse consumption or body condition using conditional logistic regression (LR test = 2.41, Monte Carlo $P = 0.29$).

Campylobacter and *Salmonella* have been isolated from a variety of ecological sources, although in sealife both pathogens are thought to cause little or no disease (22). In addition, several extensive studies on Antarctic seabirds found them all to be *Salmonella* and *Campylobacter* negative (4, 29), suggesting that seabirds, in general, acquire both bacteria after exposure to human-altered environments, particularly to those related to garbage and sewage (40). In agreement with this, birds from the Medes Islands feeding abundantly on refuse waste showed greater *Campylobacter* bacterial prevalence than those from the Columbretes Islands, where chicks fed almost exclusively on fish (Fig. 1). However, chicks from the Columbretes Islands showed a relatively high prevalence of *Salmonella* but a low prevalence of *Campylobacter*, which might be due to differential ecological behaviors between these bacteria. *Salmonella* can persist in the environment for long periods (20) and probably survives in the soil of the breeding colony between reproductive periods. On the other hand, *Campylobacter* infection may be restricted to direct transmission, since some abiotic variables, such as temperature and aerobic atmosphere (26, 35), particularly dehydration, negatively affect the survival of *Campylobacter* in the environment (25). In support of these different ecological behaviors in the infection pathways of *Salmonella* and *Campylobacter*, we found no associative relationship between both bacteria within each individual (Fisher's exact test, $P = 0.25$; only one bird from the Medes Islands was positive for both bacteria). In addition, constant values of *Salmonella* incidence in gulls throughout Europe (from 10% to 20%) may represent a stable level of carriage compared to the variable *Campylobacter* prevalence (ranging from 1% to 62%), further corroborating the difference in persistence between these bacteria in the environment.

Two of the most threatening enterobacteria for human

health, *S.* Typhimurium and *C. jejuni* (38), were the most frequently isolated bacteria in the studied area. However, other bacteria more related to wild avifauna, such as *C. lari*, were surprisingly not detected. *S*. Typhimurium and *C. jejuni* were more abundant in the Medes Islands (Table 2), where yellow-legged gulls extensively exploited refuse sites. Although we failed in detecting a specific significant relationship of *S.* Typhimurium and *C. jejuni* with gulls' feeding habits, the results presented here provide additional insights into this issue (Fig. 2). An unusual serovar, *S. enterica* subsp. *salamae* serovar Sofia (4,12:b:-), was also isolated; its habitat is believed to be cold-blooded animals and the environment (31), although it has also been isolated from other sources (9, 12, 17). On the other hand, *S. enterica* serotype Paratyphi B (isolated from one chick from the Medes Islands) is able to cause both enteric fever and gastroenteritis and is recovered mainly from humans (21). The presence of this virulent human pathogen in seagulls is also of notable public health concern because of the potential risk that these birds may pose for the transmission of enteric fevers.

The dispersal ranges of infectious pathogens are linked to the movement capacity of their infected hosts as well as of their animal reservoirs (15). In spite of that fact, there are few published epidemiological studies focusing on the potential effect of bacterial carriage on the health status of wildlife. Presumably, birds with enterobacterial infections or with food limitations are in poorer body condition than noninfected birds and they might be negatively affected, especially during the sensitive chick development stage (39). However, our results suggested that *Campylobacter* and *Salmonella* did not affect the body condition of chick gulls, providing some evidence that gulls may merely act as nonaffected carriers of these enterobacteria rather than show clinical signs of disease. Therefore, as subclinical carriers, there would be no health limitations imposed on yellow-legged gulls by infection. In turn, the fledgling and adult movement capacity is not hampered, and therefore there is potential for the dispersal of pathogenic *Campylobacter* and *Salmonella* over large geographical areas. This could, in part, contribute to the nearly worldwide distribution of both enteropathogens (44).

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