



Prevalence and characterization of *Salmonella* spp. among marine animals in the Channel Islands, California

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ABSTRACT: *Salmonella enterica* is a zoonotic pathogen that has been isolated from free-ranging marine mammals throughout the world, with animals in the Channel Islands of California (USA) showing the highest prevalence. The goal of this study was to determine prevalence, antimicrobial sensitivity and genetic similarity using pulsed-field gel electrophoresis (PFGE) of *Salmonella* in several non-domestic animal species on San Miguel and San Nicolas Islands. Fecal samples were collected from 90 California sea lion *Zalophus californianus* pups, 30 northern elephant seal *Mirounga angustirostris* pups and 87 western gulls *Larus occidentalis* in the Channel Islands and 59 adult male sea lions in Puget Sound, WA (USA). *Salmonella* were isolated, identified and serotyped, followed by antimicrobial susceptibility testing and PFGE. Of the California sea lion pups that were sampled on the islands, 21 % (n = 19) were positive for *Salmonella*, whereas no adults males in Puget Sound were positive. Of the northern elephant seal pups sampled, 87 % (n = 26) were harboring *Salmonella*. Only 9 % (n = 8) of western gulls were shedding *Salmonella*, with one of these gulls harboring the only antimicrobial resistant isolate. The serotypes found in these animals were Enteritidis, Montevideo, Newport, Reading, and Saint Paul. The only serotype that showed variation on PFGE was Newport. The pinnipeds of the Channel Islands harbor *Salmonella* at a higher prevalence than pinnipeds from other geographic areas observed in previous studies. Researchers and veterinarians should exercise increased caution when working with these animals due to the zoonotic potential of *Salmonella*.

KEY WORDS: *Salmonella enterica* · Channel Islands · California sea lions · Northern elephant seals · Western gulls · Marine animals

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INTRODUCTION

Salmonella enterica (hereafter '*Salmonella*') is a common bacterium isolated from many species of marine mammals, especially pinnipeds. *Salmonella* has been isolated from the intestinal tract of free-ranging juvenile California sea lions *Zalophus californianus* (Gilmartin et al. 1979), northern elephant seals *Mirounga angustirostris* (Stoddard et al. 2005) and northern fur seals *Callorhinus ursinus* (Vedros et al.

1982) in California, as well as from free-ranging harbor seals *Phoca vitulina* and grey seals *Halichoreus grypus* in the United Kingdom (Baker et al. 1995), free-ranging fur seals *Arctocephalus gazelle* in the sub-Antarctic (Palmgren et al. 2000) and free-ranging New Zealand sea lions *Phocarctos hookeri* (Fenwick et al. 2004). *Salmonella* have also been isolated from stranded marine mammal species that have been admitted for rehabilitation (Johnson et al. 1998, Smith et al. 2002, Stoddard et al. 2005).

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Based on the current published studies on prevalence of *Salmonella* in wild pinnipeds there may be a higher prevalence of salmonellosis in wild pinnipeds on the California Channel Islands compared to pinnipeds in other locations throughout the world. The reported prevalence of infection with *Salmonella* ranges from none in 35 northern fur seals in Alaska (Vedros et al. 1982) to 40% in free-ranging California sea lions on San Miguel Island in southern California (Gilmartin et al. 1979). The prevalence of *Salmonella* in pinnipeds in locations other than the Channel Islands has been reported to be lower than 25% (Vedros et al. 1982, Baker et al. 1995, Palmgren et al. 2000, Stoddard et al. 2005), while the prevalence for pinnipeds sampled on the Channel Islands has been greater than 25% (Gilmartin et al. 1979, Vedros et al. 1982). Vedros et al. (1982) sampled northern fur seals on both San Miguel Island in the Channel Islands and in Alaska's Pribilof Islands. They found no *Salmonella* in seals in Alaska but 25.8% of the seals sampled on the Channel Islands were positive (Vedros et al. 1982).

The Channel Islands are made up of 8 islands off the coast of southern California (Schoenherr et al. 2003), but San Miguel Island is the only location within this group of islands where studies on *Salmonella* prevalence in pinnipeds have been performed. Western gulls *Larus occidentalis* are the most common sea bird on the islands and they move between the mainland and other islands (Schoenherr et al. 2003). There are a limited number of mammals on the islands and many domestic species have been introduced by humans, including goats, cattle, pigs and sheep (Schoenherr et al. 2003). Six species of pinnipeds are found on the islands: harbor seals, northern elephant seals, California sea lions, northern fur seals, Guadalupe fur seals *Arctocephalus townsendi* and Steller sea lions *Eumetopias jubatus* (Schoenherr et al. 2003). Over 130 000 of these 6 species can be found on San Miguel Island, with northern elephant seals and California sea lions being the most abundant (Carretta et al. 2006). San Nicolas Island has similar-sized marine mammal populations, but has more extensive human impacts, as it houses a naval base whereas San Miguel Island is uninhabited apart from a ranger station and research station (Schoenherr et al. 2003). Adult male California sea lions do not feed while maintaining reproductive territories during the breeding season on the islands; they migrate from the Channel Islands north to areas from northern California through British Columbia, Canada (Peterson & Bartholomew 1967). They are essentially a coastal species, feeding on hake, herring, salmon, rockfish and squid (Antonelis et al. 1984). In contrast, although born on the same beaches on San Miguel Island as California sea lions, northern elephant seals migrate across wider areas of the north

Pacific basin to feed in pelagic waters mostly on cephalopods (LeBoeuf et al. 2000).

We undertook the current study to further define the prevalence of salmonellosis on the Channel Islands and to investigate potential reservoirs of infection. Previous studies on the islands focused exclusively on northern fur seals and California sea lions on San Miguel Island (Gilmartin et al. 1979, Vedros et al. 1982). Here we expand this knowledge by sampling additional species, including sea birds and seals with different migratory and feeding ecology, by sampling animals on an additional island with considerable human activity and by sampling sea lions during their migration away from the rookery islands to investigate potential sources of infection. The goal of this study was to determine the prevalence, antimicrobial sensitivity and pulsed-field gel electrophoresis (PFGE) patterns among *Salmonella* from California sea lions, northern elephant seals and western gulls on San Miguel and San Nicolas Islands, as well as in the adult California sea lions which had migrated from the Channel Islands to Puget Sound, Washington State. This information should further resolve the epidemiology of the bacterium.

MATERIALS AND METHODS

Animals sampled. Fecal samples were collected by rectal swab from 30 northern elephant seal pups on San Miguel Island in 2005. In 2005 and 2006 similar sampling was performed on 60 California sea lion pups on San Miguel Island, 30 sea lion pups on San Nicolas Island and 59 adult male California sea lions near Seattle, Washington State. Due to capture, restraint and sampling constraints, each animal was sampled only once. Fresh feces was collected from rocks in locations near shore where western gulls had been aggregated; a total of 58 gull samples from San Miguel Island and 29 gull samples from San Nicolas Island were collected in 2005 and 2006.

Bacterial isolation. Rectal swabs were placed in Cary-Blair transport medium (BD Diagnostics) and held at 4°C until processing within 48 h of collection. To isolate *Salmonella enterica*, selenite broth enrichment media (Hardy Diagnostics) was inoculated and incubated for 24 h at 37°C without carbon dioxide. Enriched selenite was then plated onto xylose-lysine-tergitol-4 (XLT4) agar (Hardy Diagnostics) and incubated at 37°C without carbon dioxide for 24 h. Up to 5 colonies that were positive for sulfur reduction on XLT4 were subcultured onto 5% defibrinated sheep blood agar and incubated at 37°C with carbon dioxide. Each isolate was spot tested for cytochrome oxidase and indole production. BBL™ *Salmonella* O Polyvalent

Antiserum (BD Diagnostics) was used to detect the isolates from serotypes A through I. If positive, isolates were further tested with BBL™ *Salmonella* O Antiserum Groups B, C1 and C2 (BD Diagnostics). Biochemical identification of isolates was performed using triple sugar iron (TSI) agar, urea agar, citrate agar and ortho-nitrophenyl-D-galactopyranoside (ONPG) media. *Salmonella* were identified as having an alkaline slant, acid butt, and H₂S production on TSI, urea negative, citrate positive, and ONPG negative. The first 20 isolates which were determined to be *Salmonella* were tested further on API 20E strips (Biomérieux). *Salmonella* isolates were sent to the National Veterinary Services Laboratory (Ames, Iowa) for further characterization by serotyping and phage typing of isolates that were found to be serotype Enteritidis. We stored 3 to 5 *Salmonella* colonies per animal in Microbank™ bead vials (Pro-Lab Diagnostics) and froze them at -80°C.

Antimicrobial sensitivity testing. Antimicrobial susceptibility testing was performed using the broth microdilution method (Sensititre®, Trek Diagnostic Systems). We used 4 to 5 well-isolated colonies grown from the frozen *Salmonella* stabulate to inoculate 2 ml BHI broth, incubated them at 37°C without carbon dioxide for 2 to 6 h, then diluted to a density equivalent to a 0.5 McFarland Standard. Inoculation of the minimum inhibitory concentration (MIC) plates was performed as described in the Sensititre® User Manual. The MIC plates were incubated at 37°C without carbon dioxide overnight and a MIC determined for each antimicrobial. Antimicrobials on the tray, which is for testing enteric isolates from domestic animals, are amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin, ceftiofur, ceftizoxime, chloramphenicol, enrofloxacin, gentamicin, tetracycline, ticarcillin-clavulanic acid and trimethoprim-sulphamethoxazole. Methods used for performing and interpreting antimicrobial sensitivity testing were based on Clinical and Laboratory Standards Institute (CLSI) standards (National Committee for Clinical Laboratory Standards 2002). Interpretation of resistance was based on human or food-animal MIC breakpoints depending on the drug tested.

PFGE. PFGE was performed on *Salmonella* following published PulseNet protocols (Centers for Disease Control and Prevention 2004). Agarose plugs containing *S. enterica* DNA were digested for 6 h with 60 U of *Xba*I (New England BioLabs) at 37°C. The resulting DNA fragments were separated by 1% (w/v) SeaKem Gold Agarose (Lonza) with the CHEF DR III system (Bio-Rad Laboratories) at 6 V cm⁻¹ with 0.5× Tris Borate Electrophoresis (TBE) buffer with the addition of 50 µM thiourea (Silbert et al. 2003) for 19 h. The initial pulse time was 2.2 s and the final pulse time was

63.8 s. The gels were stained with 1 µg ml⁻¹ of ethidium bromide in reagent grade water, photographed on a Typhoon 8600 (GE Healthcare) and visualized using ImageQuant version 5.1. Interpretation of chromosomal restriction patterns were based on published recommendations (Tenover et al. 1995).

Statistical analysis. Prevalence data was analyzed using Fisher's exact test (StatXact 4.0.1, Cytel Software). A p-value ≤0.05 was considered to be significant.

RESULTS

Prevalence and serotypes of *Salmonella*

The prevalence of *Salmonella* in juvenile California sea lions on the Channel Islands was 21.1%, with sea lions on San Miguel Island having a slightly higher prevalence (23.3%) than sea lions on San Nicolas Island (16.7%), but the difference was not significant (p = 0.588) (Table 1). Of the 59 adult sea lions sampled in Seattle, none were found to be shedding *Salmonella* (Table 1). There were 4 serotypes isolated from sea lions on San Miguel: Enteritidis phage type 8, Montevideo, Newport and Reading; only Montevideo and Newport were found on San Nicolas Island (Table 1). Montevideo (n = 10) and Newport (n = 7) were the most common serotypes found in sea lions (Table 1). There was one juvenile California sea lion on San Miguel Island that was harboring 2 serotypes, Reading and Montevideo.

Although there were only 30 juvenile northern elephant seals sampled on San Miguel, there was a very high prevalence of *Salmonella*, with 86.7% of the seals sampled being positive (Table 1). In addition to finding Enteritidis, Montevideo, Newport and Reading, as in the sea lions on this island, Enteritidis phage type 8 was isolated from the seals (Tables 1). Once again Montevideo and Newport were the most common serotypes found (Table 1). There were 2 seals that had both Enteritidis phage type 8 and Montevideo and one seal that had both Reading and Newport recovered from their rectal swabs.

Overall, the prevalence of *Salmonella* in western gulls was low, at 9.2%, however there was a much higher prevalence (that was statistically significant: p = 0.002) in the birds that were sampled on San Nicolas Island (24.1%) than in those sampled on San Miguel Island (1.7%) (Table 1). The sole *Salmonella* isolate from a western gull on San Miguel Island was found to be Newport (Table 1). All 5 of the serotypes identified in this study were found in western gull feces on San Nicolas Island (Table 1). One gull from San Nicolas Island harbored both Newport and Saint Paul.

Table 1. Number of animals sampled, location, prevalence and serotype of *Salmonella* isolated from California sea lions *Zalophus californianus* sampled in the Channel Islands, California, and Seattle, Washington State; from elephant seals *Mirounga angustirostris* sampled on San Miguel Island, California; and from western gulls *Larus occidentalis* sampled in the Channel Islands, California. Some animals harbored more than one serotype

Species/ location	No. of animals sampled	Prevalence (%)	Serotype				
			Enteritidis	Montevideo	Newport	Reading	Saint Paul
<i>Zalophus californianus</i>							
Channel Islands	90	19 (21.1)	1	10	7	2	0
San Miguel	60	14 (23.3)	1	8	4	2	0
San Nicolas	30	5 (16.7)	0	2	3	0	0
Seattle	59	0 (0)	0	0	0	0	0
<i>Mirounga angustirostris</i>							
San Miguel Island	30	26 (86.7)	3	12	7	6	1
<i>Larus occidentalis</i>							
Channel Islands	87	8 (9.2)	1	1	3	1	3
San Miguel	58	1 (1.7)	0	0	1	0	0
San Nicolas	29	7 (24.1)	1	1	2	1	3

Antimicrobial sensitivity of *Salmonella*

Of the 20 *Salmonella* isolated from juvenile California sea lions, 29 isolates from juvenile northern elephant seals and 9 isolates from western gulls on the Channel Islands, only 1 isolate was found to have any antimicrobial resistance to the drugs that were tested. A *Salmonella* Saint Paul isolated from a Western Gull on San Nicolas Island was resistant to ceftizoxime.

PFGE

Salmonella serotype-specific chromosomal PFGE fingerprinting patterns were produced for the 5 serotypes tested (Fig. 1). *Salmonella* Montevideo (Lanes 1 and 2) had 2 fingerprinting patterns, but only varied by 1 band (Fig. 1). The pattern seen in Lane 1 identified *Salmonella* Montevideo isolates from 9 California sea lions, 12 northern elephant seals and 1 western gull, whereas the pattern in Lane 2 came from a California sea lion on San Nicolas Island (Fig. 1). *Salmonella* Newport fingerprinting patterns (Lanes 3 and 4) were very different, varying by about 13 bands (Fig. 1). The Newport serotype fingerprinting pattern in Lane 3 identified a majority of the isolates consisting of 5 California sea lions, 7 northern elephant seals and 2 western gulls (Fig. 1). The second Newport fingerprinting pattern (Lane 4) identified 2 California sea lions and 1 western gull, all of which were sampled on San Miguel Island. The 2 banding patterns for serotype Reading were very similar and only varied by 2 bands (Fig. 1). The first *Salmonella* Reading fingerprinting pattern (Lane 5) identified isolates from 1 California sea lion, 5 northern elephant seals, and 1 western gull (Fig. 1). The second fingerprinting pattern for Reading (Lane 6)

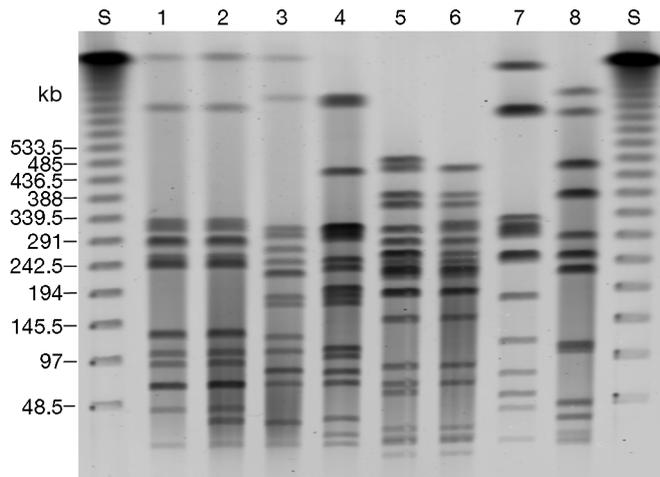


Fig. 1. PFGE of *Salmonella* spp. genomic DNA digested with *Xba*I. *Salmonella* Montevideo (Lanes 1 and 2), *Salmonella* Newport (Lanes 3 and 4), and *Salmonella* Reading (Lanes 5 and 6) all had 2 banding patterns. *Salmonella* Enteritidis (Lane 7) and *Salmonella* Saint Paul (Lane 8) each had only 1 banding pattern for all isolates. Bio-Rad Lambda Ladder was used as a standard (Lanes S)

was found for isolates from 1 California sea lion and 1 northern elephant seal from San Miguel Island. *Salmonella* serotypes Enteritidis (Lane 7) and Saint Paul (Lane 8) both had only 1 banding pattern (Fig. 1).

DISCUSSION

Salmonella enterica is a zoonotic pathogen that has been isolated from the intestinal tract of multiple species of free-ranging pinnipeds throughout the world (Gilmartin et al. 1979, Vedros et al. 1982, Baker et al.

1995, Palmgren et al. 2000, Stoddard et al. 2005). In this study conducted in 2005 and 2006, 5 serotypes of *Salmonella*—Enteritidis, Montevideo, Newport, Reading and Saint Paul—were isolated from juvenile California sea lions, juvenile northern elephant seals and western gulls, with some animals harboring more than 1 serotype. This study found different serotypes compared to previous studies on the Channel Islands, which reported Newport, Heidelberg and/or Oranienburg in California sea lions (Gilmartin et al. 1979), and Heidelberg, Newport and Adelaide in northern fur seals (Vedros et al. 1982). Studies on marine mammals and birds in California, but sampled outside of the Channel Islands, have identified the same serotypes as in this study (Thornton et al. 1998, Smith et al. 2002, Stoddard et al. 2005). Changing trends in serotypes have been observed over time in other species, including cattle (Sato et al. 2001) and humans (Olsen et al. 2001), but the reason for this is not known.

In previous studies, the highest reported prevalence of *Salmonella* in free-ranging animals was 40% in juvenile California sea lions on San Miguel Island (Gilmartin et al. 1979), which is higher than reported in this study. In the study by Gilmartin et al. (1979), California sea lion pups were sampled in October, while in this study we sampled sea lion pups in February or April. When looking at the differences in month sampled, sea lions had a prevalence of infection of 40% (n = 30) in February on San Miguel Island compared to 6.7% (n = 30) in April on San Miguel and 16.7% (n = 30) in April on San Nicolas. Both in humans (Cohen 1991) and animals (Ernst et al. 2004, Berge et al. 2006), a negative association between *Salmonella* infection and shedding and increasing host age exists. This may be one explanation for what is observed in these California sea lions, which is further supported by the fact that none of the adult sea lions sampled in Seattle were shedding *Salmonella* in their feces. The lack of *Salmonella* in sea lions sampled in Washington State after the coastal migration also suggests contact with mainland coastal waters is less important in determining a high prevalence of *Salmonella* in pinnipeds than exposure on the offshore Channel Islands.

In this study we were limited to sampling animals only once, but in the future it would be of value to repeatedly sample animals in order to determine if there is indeed a decrease in shedding bacteria in relation to age or if there is intermittent fecal shedding of bacteria. Other potential explanations for our observations would be test sensitivity, other host factors (such as nutritional status) that could vary among years and environmental factors, such as substrate suitability for *Salmonella* survival.

The high prevalence of shedding in northern elephant seals was significant, as *Salmonella* had only

been isolated previously from 3 out of 165 free-ranging seals sampled in northern California (Stoddard et al. 2005). Thus there appear to be important regional differences in *Salmonella* prevalence in elephant seals along the California coast. In addition to the high prevalence in northern elephant seal pups on San Miguel Island, there was also a significantly higher prevalence of *Salmonella* in western gulls sampled on San Nicolas (24.1%) than on San Miguel (1.7%). The reason for this higher prevalence in seals on offshore islands is not known, but Gilmartin et al. (1979) previously hypothesized that high prevalence of *Salmonella* in sea lions could be due to heavy fecal contamination. Spread through fecal contamination is possible; however, the northern elephant seal rookeries in central California are as heavily contaminated and a high prevalence of *Salmonella* is not reported there (Stoddard et al. 2005). The difference could be an original source or sources of *Salmonella* that contaminated the islands and spread through the marine mammal populations. Over the past 100 yr, feral animals such as black rats, pigs, sheep, deer, elk, and donkeys inhabited the Channel Islands, but with the exception of black rats, all feral mammals are now eradicated on San Miguel Island. Domestic cats are still present on San Nicolas Island. Gilmartin et al. (1979) hypothesized that western gulls could be responsible for spreading *Salmonella*, but the current study found a low level of *Salmonella* in these birds. Terrestrial mammal species on the island, such as the San Miguel Island deer mouse *Peromyscus maniculatus streator* and the black rat *Rattus rattus*, could potentially be a source of *Salmonella*, but there is little contact between these animals and the beach dwelling pinnipeds. Thus, the data in this study suggest northern elephant seals on San Miguel Island are currently the most important reservoirs of *Salmonella* on the Channel Islands, with gulls acting as vectors between land masses.

In this study there was little variation in the isolates based on PFGE and lack of antimicrobial resistance. The lack of antimicrobial resistance has been observed in *Salmonella* isolates from previous studies of free-ranging pinnipeds along the California coast (Stoddard et al. 2005). The lack of resistance in isolates from these animals may be due to the lack of selection pressure in wild animals, which are not exposed to antimicrobial drugs on these remote islands. The only serotype that showed variation on PFGE was Newport. The similar PFGE patterns suggest there may be transmission, direct or indirect, of *Salmonella* between species as has been demonstrated with New Zealand fur seals and feral pigs (Fenwick et al. 2004). However, when comparing *Salmonella* serotypes isolated from different wildlife species in different locations, they

were found to have the same PFGE restriction pattern (Smith et al. 2002). The same finding can be said for this study, since the patterns here were very similar to PFGE restriction patterns from *Salmonella* isolated in 2003 and 2004 from northern elephant seals in northern California (R. A. Stoddard unpubl. data). PFGE is thought to be a useful genetic tool for determining the source of infection in outbreak investigations; however, it does not always discriminate unrelated isolates (Barrett et al. 2006, Cardinale et al. 2006). Another technique, such as multilocus sequence typing (MLST), might prove useful in further discriminating genetic differences between *Salmonella* isolates from marine mammals.

The marine mammals of the Channel Islands appear to harbor *Salmonella* at a higher rate than marine mammals observed in other studies. Researchers and veterinarians should continue to exercise caution when working with these animals due to the zoonotic potential of *Salmonella*, although handlers should always be concerned about zoonotic diseases when handling wild animals. This study discovered more information about *Salmonella* in the marine mammals and birds of the Channel Islands, although further studies are needed. In the future it would be relevant to sample multiple terrestrial and marine species of different ages over several seasons to further resolve bacterial recruitment and epidemiology of *Salmonella* amongst this southern California island range.

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