Risk Factors for Infection with Pathogenic and Antimicrobial-Resistant Fecal Bacteria in Northern Elephant Seals in California

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SYNOPSIS

Objectives. The goal of this study was to identify potential environmental and demographic factors associated with *Campylobacter jejuni* (*C. jejuni*), *Salmonella enterica* (*Salmonella* spp.), and antimicrobial-resistant *Escherichia coli* (*E. coli*) infection in northern elephant seals stranded along the California coastline.

Methods. *E. coli*, *Salmonella* spp., and *C. jejuni* were isolated from rectal swabs from 196 juvenile northern elephant seals, which were found stranded and alive along the California coast and brought to The Marine Mammal Center in Sausalito, California, for rehabilitation. Gender, weight, county where the animal stranded, month stranded, coastal human population density, exposure to sewage outfall or freshwater outflow (river or stream), and cumulative precipitation in the previous 24 hours, seven days, 30 days, 90 days, and 180 days were analyzed as potential risk factors for infection.

Results. The odds of *C. jejuni* and antimicrobial-resistant *E. coli* were higher in feces of seals stranded at sites with higher levels of freshwater outflow compared with lower levels of freshwater outflow. The odds of *Salmonella* spp. in feces were 5.4 times greater in seals stranded in locations with lower levels of 30-day cumulative precipitation, along with substantially lower odds of *Salmonella* shedding for seals stranded in Monterey or Santa Cruz county compared with seals stranded in regions further north or south of this central California location.

Conclusions. Juvenile northern elephant seals that have entered the water are being colonized by antimicrobial-resistant and pathogenic fecal bacteria that may be acquired from terrestrial sources transmitted via river and surface waters.

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California is the most populous state in the U.S., with nearly 34 million inhabitants, 72% of whom live in coastal counties.[1] Municipal, industrial, and agricultural effluents generated from coastal or inland of California can eventually drain into the Pacific Ocean through point- and non-point source discharges, storm runoff, and sewage outfalls, many of which utilize rivers to be conveyed to the ocean.[2–5] The ocean is relied upon to sufficiently dilute contaminated water to make coastal waters safe for human recreation, fishing, and shellfish harvesting.

Even after dilution in the ocean, humans can be exposed to pathogens through water contact during recreation and from seafood consumption.[6–11] In California, people surfing near urban environments are more likely to become ill than those surfing in rural areas.[12] According to the Centers for Disease Control and Prevention (CDC), waterborne bacterial infections account for half of the three to five billion cases of diarrhea each year in the U.S., with *Campylobacter jejuni* (*C. jejuni*) and *Salmonella enterica* (*Salmonella* spp.) commonly implicated.[13]

Direct pathogen exposure is not the only concern associated with fecal pollution of the marine environment. Nearly one-third of fecal coliforms isolated from sewage, surface waters, and seawater were found to be resistant to one or more antimicrobial drugs.[14] The aquatic environment could not only serve as a reservoir for antimicrobial-resistant bacteria, but also as a location for the spread and evolution of antimicrobial resistance.[15] Antimicrobial-resistant bacteria are a concern for both humans and animals because they decrease efficacy of commonly used antimicrobials, limit the range of treatment options, increase the cost and duration of treatment, and lead to higher morbidity and mortality.[16–18]

An increasing flow of infectious pathogens from land to sea is not just a concern for humans, but also for marine mammals.[6] *Toxoplasma gondii*, a fecal protozoal pathogen that is shed by cats, causes a fatal encephalitis in southern sea otters (*Enhydra lutris nereis*), and freshwater runoff is a risk factor for infection.[19] Marine mammals are also being infected with zoonotic bacteria, with some of these bacteria having antimicrobial resistance.[20–22] Juvenile northern elephant seals (*Mirounga angustirostris*) in California that have entered the Pacific Ocean are more likely to be infected with *C. jejuni* and *Salmonella* spp. compared with seals that have not yet entered the ocean.[23]

The goals of this study were to identify risk factors associated with pathogenic and antimicrobial-resistant fecal bacteria in northern elephant seals stranded along the California coastline and compare the prevalence of antimicrobial-resistant *Escherichia coli* (*E. coli*) in stranded seals with seals that had not left their natal beach (free-ranging), thereby having minimal exposure to open ocean conditions.

METHODS

Study animals

From February through July in 2003 and 2004, 165 free-ranging juvenile northern elephant seals on three natal beaches that had recently weaned and had not yet entered the water, and 196 juvenile seals that stranded along the California coast were swabbed rectally for bacterial culture (Figure 1). Stranded seals were sampled within 24 to 48 hours of being admitted to The Marine Mammal Center (TMMC) in Sausalito, California, for rehabilitation.

Bacterial isolation

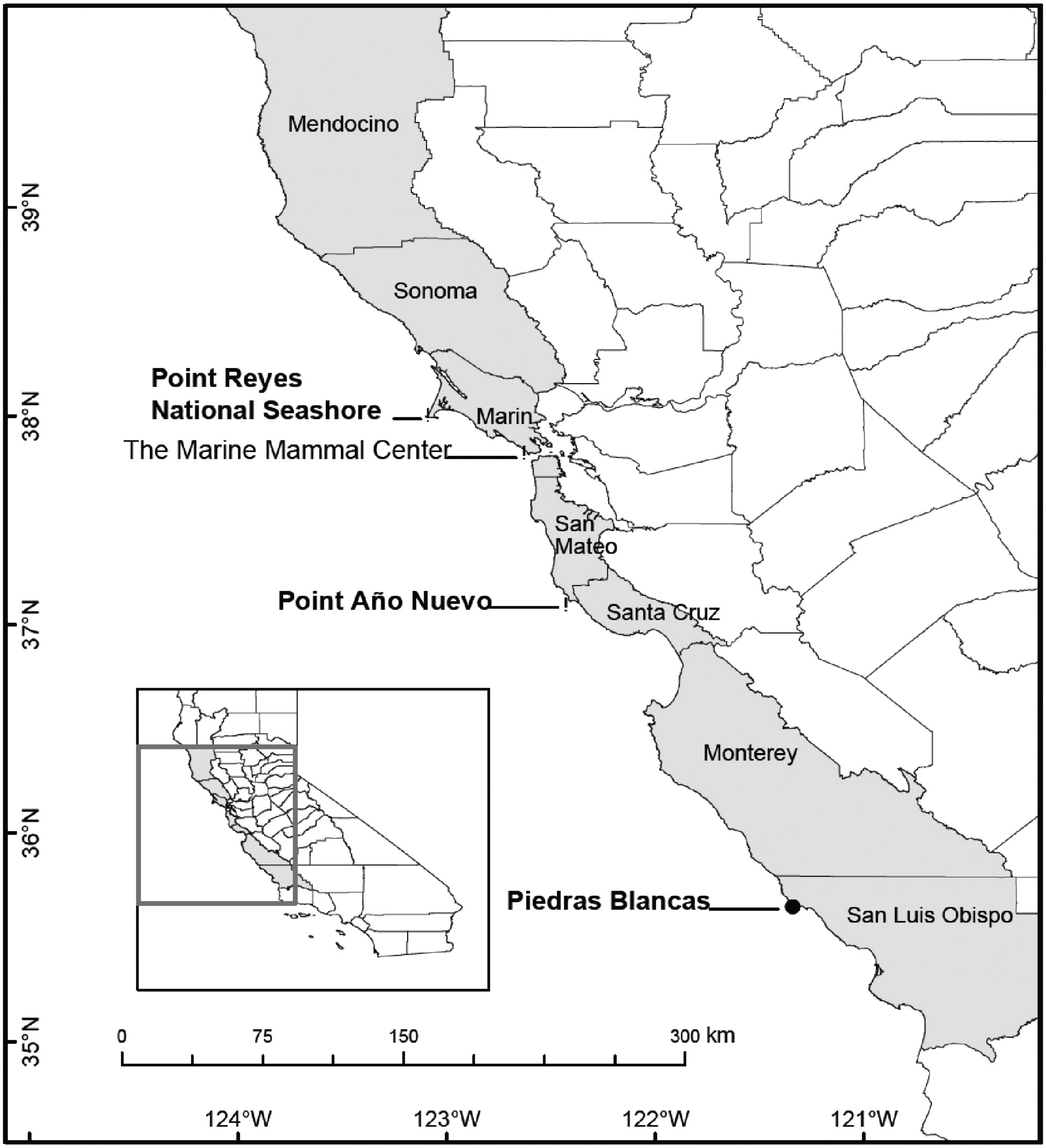
All *Salmonella* spp. and *C. jejuni* isolated from 195 seals used in this study were previously reported,[23] with one additional seal in this study that was not culture positive for either bacterium. A total of 73 seals harbored *Salmonella* spp. and 54 seals harbored *C. jejuni*. Rectal swabs from the same 196 stranded and 165 free-ranging seals were also used to isolate *E. coli*.

All culture and antimicrobial sensitivity testing was performed at the Microbiology Laboratory, Veterinary Medical Teaching Hospital, School of Veterinary Medicine at the University of California in Davis. Rectal swabs placed in Cary-Blair transport medium (BD Diagnostics, Franklin Lakes, New Jersey) were used to isolate *E. coli* using standard procedures.[24] Isolates were stored in Microbank® bead vials (Pro-Lab Diagnostics, Austin, Texas) at −80°C. *Salmonella* spp. and *C. jejuni* were isolated as previously described,[23] using standard techniques.[24,25]

Antimicrobial sensitivity testing

Antimicrobial susceptibility was performed on up to three *E. coli* isolates from each fecal sample using the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI) methods[26] using Sensititre® Automated Microbiology Systems.[27] Antimicrobial drugs on the minimum inhibitory concentration (MIC) plates were amikacin, amoxicillin-clavulanic acid, ampicillin, cefazolin, ceftiofur, ceftizoxime, chloramphenicol, enrofloxacin, gentamicin, tetracycline, ticarcillin-clavulanic acid, and trimethoprim-sulphamethoxazole. Methods used to perform and interpret antimicrobial sensitivity testing were based on CLSI standards.[26] A seal was considered to have resistant *E. coli* if one or more isolates were

Figure 1. Location of The Marine Mammal Center (TMMC), rescue range of TMMC (shaded), and northern elephant seal rookeries (Point Reyes National Seashore, Point Año Nuevo, Piedras Blancas) where seals were sampled along the California coastline



either intermediate or fully resistant to ≥1 of the 12 antimicrobial drugs tested.

Definition of risk factors

Risk factors potentially associated with infection of stranded northern elephant seals with *Salmonella* spp., *C. jejuni*, and antimicrobial-resistant *E. coli* were investigated similarly to those described by Miller et al.[19] These included gender, weight, county where the animal stranded, month stranded, coastal human population density, exposure to sewage outfall or freshwater outflow, and cumulative precipitation in the previous 24 hours, seven, 30, 90, and 180 days prior to stranding. Risk factors specifically associated with antimicrobial-resistant *Salmonella* spp. and *C. jejuni* were not determined due to small sample size of these bacteria. Although serovar information was available for Salmonella isolates, data were analyzed for the species as a whole because four of the five serovar samples size groups were less than 20.[23]

Animals were weighed upon admission to TMMC and assigned to the following weight classes: 26.0–32.0 kg, 32.1–38.0 kg, 38.1–44.0 kg, and >44.0 kg. Stranded animals along the central California coast ranged from Mendocino to San Luis Obispo County (Figure 1). Due to the small numbers of animals stranding in areas north of San Francisco County, animals from San Francisco northward were pooled as a single group. Animal stranding dates were grouped by two-month intervals spanning February through July (February and March, April and May, June and July) in 2004 and 2005.

Each animal was assigned a numerical value for its stranding location using previously described methods.[19] Briefly, the California coastline, which included stranded seal locations, was divided into 0.5 km increments and each increment was assigned a numerical value, with point 0 being the mouth of the San Francisco Bay; locations north had a negative value and locations south had a positive value. Locations north of the San Francisco Bay ranged from −1 to −607 and those ranging south of the bay ranged from 1 to 955. Each animal's relative exposure to coastal human population density, municipal sewage outfall, freshwater outflow, and previous precipitation were determined for each of the geographic numeric values using previously established methods.[19]

Categorical values for coastal human population density were determined using U.S. 2000 Census data[1] that were condensed into four groups: <100, 100–1,000, 1,001–3,000, and >3,000 humans per square mile. Freshwater outflow was quantified as previously described[19] and was categorized as: 0–10,000, 10,001–100,000, and >100,000 acre-feet per year. Relative exposure to precipitation was determined using the closest rain gauge to each stranding location, using a previously described method with data collected by the California Department of Water Resource Data Exchange Center, the University of California Department of Agriculture and Natural Resources Integrated Pest Management Program, and California Irrigation Management System.[28] Precipitation for the 24 hours prior to each seal's stranding was categorized as either none or >0 inches. Cumulative precipitation over the seven days prior to stranding was categorized as either ≤1 inch or >1 inch. Cumulative precipitation over the 30 days prior to stranding was classified as 0, 0.01–2.00, 2.01–4.00, or >4.00 inches. Cumulative precipitation 90 days prior to stranding was categorized as <4.00, 4.01–8.00, 8.01–12.00, 12.01–16.00, and >16.00 inches. Finally, precipitation 180 days prior to stranding was classified as <10.00, 10.01–18.00, 18.01–26.00, and <26.01 inches.

Statistical analysis

Significant differences among the prevalence of antimicrobial resistance of fecal *E. coli* isolated from free-ranging and stranded seals was determined using Fisher's exact test using StatXact 4.0.1.[29] Minitab™ Release 14.2 was used to perform univariate risk analysis.[30] LogXact Version 4.1 was used for univariate analysis of the stranding month as a risk factor for *Salmonella* spp., as no seals stranding in June and July tested positive.[31] Risk factors were considered significant if the *p*-value was ≤0.05 in univariate analyses. Relationships between risk factors were further analyzed using multivariable logistic regression using LogXact with a forward-stepping algorithm and a *p*-value of ≤0.05 for inclusion in the model based on the likelihood ratio test. Freshwater outflow and precipitation were examined for significance, both by leaving the data as continuous and by categorizing the relative runoff and sewage exposure as low, medium, or high to identify the best-fitting model.

RESULTS

Prevalence of antimicrobial-resistant *E. coli*

E. coli were isolated from all but four stranded seals. The majority of free-ranging and stranded seals (98.8% and 88.6%, respectively) harbored *E. coli* without resistance or with resistance to only one antimicrobial drug (Table 1). Multidrug-resistant *E. coli* were more common in stranded seals than in seals that had not entered the water, and no free-ranging seals were resistant to >1 antimicrobial drug (Table 1). The most common antimicrobial drugs to which *E. coli* were resistant were ampicillin and tetracycline.

Table 1. Prevalence of *Escherichia coli* (*E. coli*) resistance in 165 free-ranging and 192 stranded northern elephant seals stranding in California

Number of antimicrobials resistant to[a]	*Free-ranging seals (percent)*	*Stranded seals (percent)*	*P-value*
0	152 (92.1)	119 (62.0)	≤0.001[b]
1	11 (6.7)	51 (26.6)	≤0.001[b]
≥2	2 (1.2)	22 (11.4)	≤0.001[b]

[a]Determined by the most resistant *E. coli* from each seal

[b]Statistically significant

Univariate categorical analyses

Risk factors associated with infection of stranded northern elephant seals with *Salmonella* spp. (72/196 seals), *C. jejuni* (54/195 seals), and antimicrobial-resistant *E. coli* (73/192 seals) were investigated. The odds of shedding antimicrobial-resistant *E. coli* were 50% less (odds ratio [OR] = 0.46, p=0.04) for seals stranding near locations with a population density between 100 and 1,000 people/mile^2 compared with seals stranding near locations with <100 people/mile^2 (Table 2). All other potential risk factors were not statistically significant for detection of *E. coli* in elephant seal feces.

Precipitation within the 90 days preceding stranding was positively associated with presence of fecal *Salmonella* spp. (Table 3). Seals stranding in locations with low (4.01–8.00 inches) or moderate (8.01–12.00 inches) rainfall in the immediate pre-stranding period were approximately three to four times more likely to harbor *Salmonella* spp., when compared with seals stranding in locations exposed to minimal (<4.00 inches) rainfall in the immediate pre-stranding period (OR=2.96, 95% confidence interval [CI] 1.02, 8.66, p=0.05, and OR=3.98, 95% CI 1.24, 12.81, p=0.02, respectively) (Table 3). All other potential risk factors were not statistically significant for detection of *Salmonella* spp. in elephant seal feces. Based on the univariate analysis for *C. jejuni*, there were no significant risk factors for the categories tested (Table 4).

Logistic regression analysis

The odds of a juvenile seal having antimicrobial-resistant *E. coli* in its feces increased 1.24 times for each additional log increase (tenfold increase) in the freshwater outflow (acre-feet per year) at the stranding location (95% CI 1.04, 1.49, p=0.02) (Figure 2). This same trend was identified for *C. jejuni*: for each additional log increase in exposure to freshwater outflow (in acre-feet per year), the odds of a seal shedding *C. jejuni* at stranding increased by 1.24 (95% CI 1.02, 1.51, p=0.03) (Figure 2).

In the final logistic regression model, an increased risk for *Salmonella* spp. infection was associated with the county in which each seal stranded, as well as exposure to lower levels of coastal precipitation over the 30 days prior to stranding (Table 5). Seals stranding in Santa Cruz County (OR=0.44, 95% CI 0.22, 0.99, p=0.05) and Monterey County (OR=0.26, 95% CI 0.10, 0.68, p<0.01) were less likely to have fecal *Salmonella* spp. when compared with seals stranding in San Luis Obispo County (Table 5). The odds of shedding *Salmonella* was 5.4 times greater in seals that stranded in coastal locations with low precipitation (between 0.1 and 2.0 inches of rain) compared with seals stranding in areas with higher precipitation (95% CI 1.64, 17.66, p<0.01) (Table 5).

DISCUSSION

The goal of this study was to identify potential environmental and demographic factors associated with *C. jejuni*, *Salmonella* spp., and antimicrobial-resistant *E. coli* infection in seals stranded along the California coastline. Juvenile northern elephant seals that had left their natal beach and later stranded had a higher prevalence of pathogenic and antimicrobial-resistant bacteria in their feces, with some of those bacteria being multidrug resistant, when compared with juvenile seals that had not yet left their natal beach. Increased exposure to freshwater runoff was associated with a higher probability that a seal was infected with *C. jejuni* and antimicrobial-resistant *E. coli*. Exposure to lower rather than higher levels of precipitation in the 30 days prior to stranding was associated with a higher odds or probability that a seal was shedding *Salmonella* spp., while stranding in Santa Cruz and Monterey Counties was associated with a lower chance of *Salmonella* spp. infection compared with stranding in a more southerly county.

We initially hypothesized—based on the importance freshwater outflow had on the presence of fecal protozoal parasites in both sea otters[19] and shellfish[28] in California—that high exposure to freshwater runoff would be a significant risk factor for infection of northern elephant seals with pathogenic and

Table 2. Categorical risk factors, using univariate analysis, for northern elephant seals that are harboring antimicrobial-resistant fecal *Escherichia coli* at stranding

Risk factor	*Group*	*Percentage culture positive*	*Odds ratio*	*95% CI*	P-*value*
Gender	Male	37 (*n*=119)	1.00	—	—
	Female	40 (*n*=73)	1.12	0.62, 2.04	0.70
Weight range (kilograms)	26.0–32.0	32 (*n*=31)	1.00	—	—
	32.1–38.0	40 (*n*=80)	1.40	0.58, 3.36	0.45
	38.1–44.0	40 (*n*=57)	1.42	0.57, 3.57	0.46
	>44.0	33 (*n*=24)	1.05	0.34, 3.27	0.93
Stranding county	San Francisco and north	36 (*n*=22)	1.00	—	—
	San Mateo	45 (*n*=20)	1.43	0.42, 4.93	0.57
	Santa Cruz	24 (*n*=49)	0.57	0.19, 1.68	0.31
	Monterey	39 (*n*=36)	1.11	0.37, 3.34	0.85
	San Luis Obispo	46 (*n*=65)	1.50	0.55, 4.06	0.43
Month stranded	February to March	40 (*n*=96)	1.00	—	—
	April to May	35 (*n*=89)	0.78	0.43, 1.42	0.42
	June to July	42 (*n*=7)	1.10	0.23, 5.17	0.91
Human population (number of humans per square mile)	<100	46 (*n*=69)	1.00	—	—
	100–1,000	28 (*n*=53)	0.46	0.21, 0.98	0.04[a]
	1,001–3,000	35 (*n*=34)	0.63	0.27, 1.47	0.29
	>3,000	39 (*n*=36)	0.74	0.32, 1.67	0.46
Sewage outfall (acre-ft/year)	<1	38 (*n*=154)	1.00	—	—
	1–16,000	39 (*n*=38)	1.08	0.52, 2.23	0.84
Freshwater outflow exposure (acre-ft/year)	0–10,000	32 (*n*=94)	1.00	—	—
	10,001–100,000	41 (*n*=64)	1.56	0.81, 3.01	0.19
	>100,000	47 (*n*=34)	1.90	0.85, 4.22	0.12
Precipitation in past 24 hours (inches)	0	36 (*n*=164)	1.00	—	—
	>0	45 (*n*=31)	1.42	0.65, 3.10	0.37
Precipitation in past 7 days (inches)	0–1.00	36 (*n*=176)	1.00	—	—
	>1.00	56 (*n*=16)	2.25	0.80, 6.33	0.12
Precipitation in past 30 days (inches)	0	22 (*n*=9)	1.00	—	—
	0.01–2.00	38 (*n*=102)	2.52	0.51, 12.47	0.26
	2.01–4.00	38 (*n*=56)	2.40	0.46, 12.39	0.30
	>4.00	44 (*n*=25)	3.14	0.55, 17.89	0.20
Precipitation in past 90 days (inches)	<4.00	36 (*n*=22)	1.00	—	—
	4.00–8.00	44 (*n*=90)	1.27	0.48, 3.37	0.63
	8.01–12.00	30 (*n*=40)	0.70	0.23, 2.11	0.52
	12.01–16.00	20 (*n*=20)	0.41	0.10, 1.66	0.21
	>16.00	45 (*n*=20)	1.33	0.38, 4.62	0.65
Precipitation in past 180 days (inches)	<10.00	41 (*n*=27)	1.00	—	—
	10.00–18.00	38 (*n*=91)	0.91	0.38, 2.18	0.83
	18.01–26.00	38 (*n*=58)	0.89	0.35, 2.26	0.81
	>26.00	31 (*n*=16)	0.66	0.18, 2.44	0.54

[a]Statistically significant

CI = confidence interval

Table 3. Categorical risk factors, using univariate analysis, for northern elephant seals that were culture positive for *Salmonella* spp. at stranding

Risk factor	*Group*	*Percentage culture positive*	*Odds ratio*	*95% CI*	*P-value*
Gender	Male	38 (*n*=120)	1.00	—	—
	Female	34 (*n*=76)	0.84	0.46, 1.52	0.56
Weight (kilograms)	26.0–32.0	39 (*n*=31)	1.00	—	—
	32.1–38.0	39 (*n*=80)	1.00	0.43, 2.35	0.93
	38.1–44.0	40 (*n*=58)	1.04	0.43, 2.54	1.00
	>44.0	23 (*n*=26)	0.48	0.15, 1.52	0.21
Stranding county	San Francisco and north	43 (*n*=23)	1.00	—	—
	San Mateo	35 (*n*=20)	0.70	0.20, 2.41	0.57
	Santa Cruz	30 (*n*=50)	0.56	0.20, 1.55	0.26
	Monterey	36 (*n*=36)	0.37	0.12, 1.16	0.09
	San Luis Obispo	48 (*n*=67)	1.19	0.46, 3.08	0.72
Month stranded[a]	February to March	32 (*n*=96)	1.00	—	—
	April to May	45 (*n*=91)	1.71	0.91, 3.26	0.10
	June to July	0 (*n*=9)	0.17	-inf, 1.15	0.07
Human population (number of humans per square mile)	<100	41 (*n*=70)	1.00	—	—
	100–1,000	31 (*n*=54)	0.68	0.29, 1.60	0.37
	1,001–3,000	32 (*n*=34)	0.65	0.31, 1.37	0.26
	>3,000	39 (*n*=38)	0.92	0.41, 2.06	0.84
Sewage outfall (acre-ft/year)	<1	36 (*n*=157)	1.00	—	—
	1–16,000	38 (*n*=39)	1.10	0.53, 2.26	0.80
Freshwater outflow exposure (acre-ft/year)	0–10,000	37 (*n*=95)	1.00	—	—
	10,001–100,000	32 (*n*=65)	0.82	0.42, 1.59	0.56
	>100,00	44 (*n*=36)	1.37	0.63, 2.99	0.43
Precipitation in past 24 hours (inches)	0	37 (*n*=165)	1.00	—	—
	>0	35 (*n*=31)	0.94	0.42, 2.09	0.88
Precipitation in past 7 days (inches)	0–1.00	37 (*n*=180)	1.00	—	—
	>1.00	31 (*n*=16)	0.77	0.26, 2.30	0.64
Precipitation in past 30 days (inches)	0	27 (*n*=11)	1.00	—	—
	0.01–2.00	44 (*n*=104)	2.11	0.53, 8.43	0.29
	2.01–4.00	34 (*n*=56)	1.37	0.33, 5.77	0.67
	>4.00	16 (*n*=25)	0.51	0.09, 2.79	0.44
Precipitation in past 90 days (inches)	<4.00	22 (*n*=23)	1.00	—	—
	4.00–8.00	45 (*n*=93)	2.96	1.02, 8.66	0.05[b]
	8.01–12.00	53 (*n*=40)	3.98	1.24, 12.81	0.02[b]
	12.01–16.00	5 (*n*=20)	0.19	0.02, 1.78	0.15
	>16.00	15 (*n*=20)	0.64	0.13, 3.08	0.57
Precipitation in past 180 days (inches)	<10.00	38 (*n*=29)	1.00	—	—
	10.00–18.00	39 (*n*=92)	1.05	0.45, 2.48	0.91
	18.01–26.00	29 (*n*=58)	0.68	0.27, 1.74	0.42
	>26.00	47 (*n*=17)	1.45	0.43, 4.89	0.55

[a]Analyzed using LogXact Version 4.1 due to negative results

[b]Statistically significant

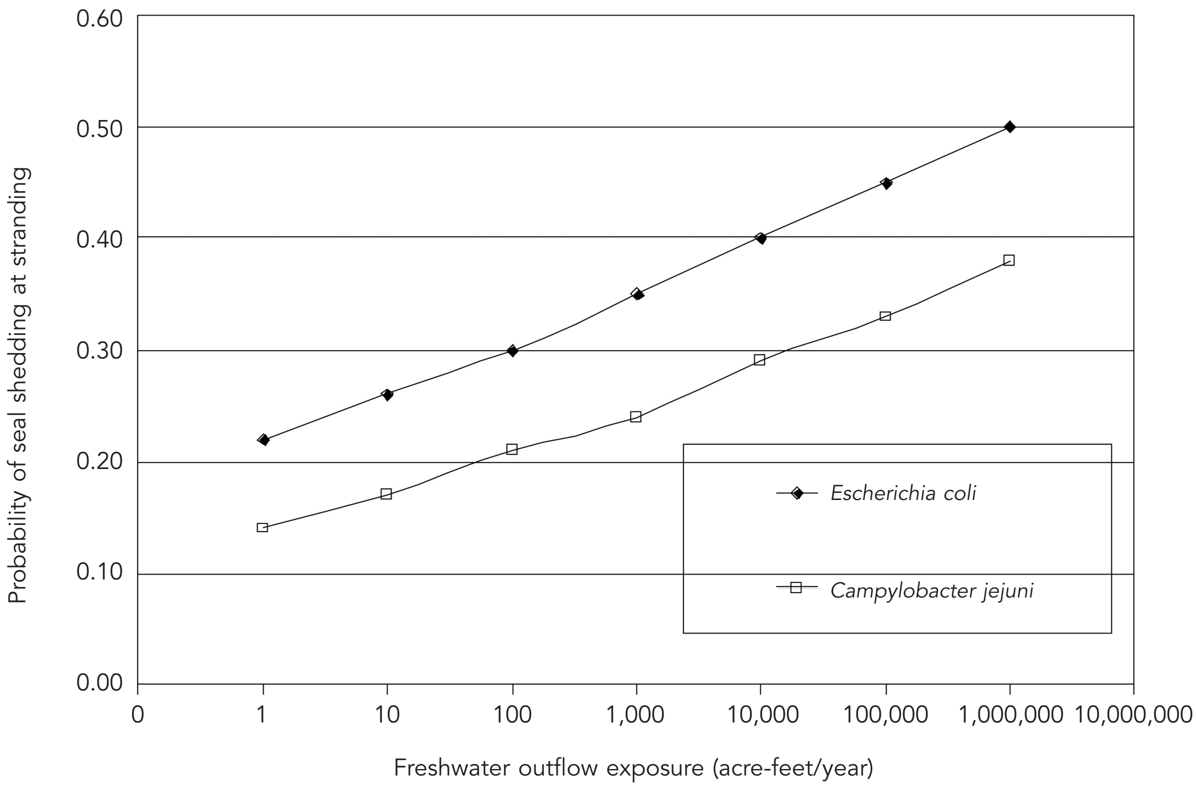
CI = confidence interval

Table 4. Categorical risk factors, using univariate analysis, for northern elephant seals that were culture positive for *Campylobacter jejuni* at stranding

Risk factor	*Group*	*Percentage culture positive*	*Odds ratio*	*95% CI*	P-*value*
Gender	Male	28 (*n*=119)	1.00	—	—
	Female	28 (*n*=76)	1.01	0.53, 1.91	0.98
Weight (kilograms)	26.0–32.0	39 (*n*=31)	1.00	—	—
	32.1–38.0	25 (*n*=79)	0.54	0.22, 1.30	0.17
	38.1–44.0	28 (*n*=58)	0.60	0.24, 1.52	0.28
	>44.0	23 (*n*=26)	0.48	0.15, 1.52	0.21
Stranding county	San Francisco and north	39 (*n*=23)	1.00	—	—
	San Mateo	25 (*n*=20)	0.52	0.14, 1.93	0.33
	Santa Cruz	28 (*n*=50)	0.60	0.21, 1.71	0.34
	Monterey	19 (*n*=36)	0.38	0.12, 1.22	0.10
	San Luis Obispo	29 (*n*=66)	0.36	0.23, 1.70	0.36
Month stranded	February to March	33 (*n*=96)	1.00	—	—
	April to May	22 (*n*=90)	0.56	0.29, 1.08	0.09
	June to July	22 (*n*=9)	0.57	0.11, 2.91	0.50
Human population (number of humans per square mile)	<100	28 (*n*=69)	1.00	—	—
	100–1,000	26 (*n*=54)	1.10	0.44, 2.72	0.84
	1,001–3,000	29 (*n*=34)	0.92	0.41, 2.06	0.84
	>3,000	29 (*n*=38)	1.07	0.45, 2.58	0.88
Sewage outfall (acre-ft/year)	<1	29 (*n*=156)	1.00	—	—
	1–16,000	21 (*n*=38)	0.62	0.26, 1.44	0.27
Freshwater outflow exposure (acre-ft/year)	0–10,000	24 (*n*=94)	1.00	—	—
	10,001–100,000	28 (*n*=65)	1.20	0.58, 2.46	0.62
	>100,00	39 (*n*=36)	1.77	0.77, 4.04	0.18
Precipitation in past 24 hours (inches)	0	26 (*n*=164)	1.00	—	—
	>0	35 (*n*=31)	1.56	0.69, 3.52	0.28
Precipitation in past 7 days (inches)	0–1.00	27 (*n*=179)	1.00	—	—
	>1.00	31 (*n*=16)	1.21	0.40, 3.65	0.74
Precipitation in past 30 days (inches)	0	18 (*n*=11)	1.00	—	—
	0.01–2.00	22 (*n*=103)	1.29	0.26, 6.41	0.75
	2.01–4.00	39 (*n*=56)	2.91	0.57, 14.76	0.20
	>4.00	28 (*n*=25)	1.75	0.03, 10.21	0.53
Precipitation in past 90 days (inches)	<4.00	32 (*n*=22)	1.00	—	—
	4.00–8.00	23 (*n*=93)	1.15	0.32, 4.17	0.83
	8.01–12.00	25 (*n*=40)	1.75	0.50, 6.16	0.38
	12.01–16.00	35 (*n*=20)	0.62	0.23, 1.73	0.37
	>16.00	42 (*n*=20)	0.71	0.23, 2.25	0.57
Precipitation in past 180 days (inches)	<10.00	17 (*n*=29)	1.00	—	—
	10.00–18.00	32 (*n*=91)	2.25	0.78, 6.48	0.14
	18.01–26.00	22 (*n*=58)	1.39	0.44, 4.35	0.58
	>26.00	41 (*n*=17)	3.36	0.86, 13.15	0.08

CI = confidence interval

Figure 2. Probability of a seal shedding *Campylobacter jejuni* and antimicrobial-resistant *Escherichia coli* based on freshwater outflow exposure (acre-feet/year) at location of stranding



antimicrobial-resistant fecal bacteria. This hypothesis is further supported by studies that found antimicrobial-resistant bacteria in multiple rivers in the U.S.[32] and high bacterial counts at beaches near river mouths.[33] In addition, swimming in natural sources of water is a novel risk factor for *C. jejuni* and *E. coli* gastrointestinal infections in humans.[34] This hypothesis may be true based on our final model, in which increasing levels of freshwater outflow were associated with increased odds that a stranded juvenile elephant seal was shedding *C. jejuni* and resistant species of *E. coli*.

Using the coefficients from the logistic regression model to calculate the association between freshwater outflow and the probability of shedding of *C. jejuni* and resistant species of *E. coli* among stranded seals, one can see that although this association is significant, large increases of outflow were needed for just moderate increases in the probability of shedding these bacteria. For example, for each base-10 logarithm increase in outflow, the probability of shedding *C. jejuni* and resistant species of *E. coli* increased only 3% to 5% (Figure 2). Based on this association, we speculate that seals could be exposed to bacteria from local coastal streams and rivers draining directly into the ocean or through major rivers like the San Joaquin and Sacramento Rivers that discharge into the ocean through the San Francisco Bay along with the Salinas River.

Exposure to increased freshwater outflow was not a significant risk factor for seals with *Salmonella* spp. in their feces; in fact, seals stranding in locations of lower compared with higher precipitation in the 30 days prior to stranding were at increased risk for infection. *Salmonella* spp. may require steady precipitation over a longer time period to enter the ocean, due to lower concentrations of *Salmonella* in terrestrial animal and human feces.[24,35] Depending on stranding location, this higher-risk category of 30-day cumulative precipitation of 0.1–2.0 inches occurred from February through May, similar to the range of months for the referent category of >4.0 inches of precipitation. Hence, month of occurrence does not appear to be the underlying causal factor, but instead may be the result of fecal accumulation during bouts of longer inter-storm intervals, leading to higher concentrations of fecal-borne pathogens once a precipitation event does occur.[36]

The county of stranding for each juvenile northern elephant seal was found to be a significant risk factor for the isolation of *Salmonella* spp. from the seals' gastrointestinal tract. Seals stranding in Santa Cruz and Monterey Counties were more likely to have *Salmonella* spp., compared with seals stranding in San Luis Obispo County. The increased risk associated with seal stranding in these counties could be related to environmental and anthropogenic risk factors that were not considered in the present analysis. Increased human population density could play a role in increasing risk for bacterial exposure in the different counties; however, it was not determined to be a risk factor in this study.

Previous studies have demonstrated that increased water contact recreation in coastal locations near dense human populations increased the risk of illness.[12,37] High human population density and increased exposure to municipal sewage outfalls did not appear to increase the isolation frequency of any of the fecal bacteria included in this study. This finding was surprising, considering that antimicrobial-resistant *E. coli* are known to be present in sewage outfall.[38,39] One explanation for not finding an association between sewage outfall and presence of the bacteria in this study is that very few seals (n=39) stranded in locations with significant exposure to municipal sewage, resulting in insufficient power to detect this potential risk factor.

Limitations

There are complications and potential sources of error for risk factor analysis in this study. One source of error is related to precipitation data. These data were gathered through the use of rain gauges along the California coastline. However, the rain gauges are sometimes taken offline and the data are no longer available. In the few cases in which this occurred, a gauge from the next-closest location was used. Therefore, measurement error may exist for the precipitation data obtained for some stranding locations.

There is also the potential bias due to an animal's stranding location, as it does not precisely account for all areas in which a seal may have traveled and foraged throughout its lifetime. These travels could greatly influence the seals' range of exposure to enteric bacterial flora. When juvenile northern elephants begin to forage, they spend approximately four months at sea and can travel long distances during that period.[40] Because of the unknown distances that the elephant seals in this study could have traveled before stranding, it is difficult to determine the precise locations where pathogen exposure may have occurred.

CONCLUSIONS

The cause of the higher prevalence of pathogenic and antimicrobial-resistant bacteria in stranded juvenile northern elephant seals compared with those that have not left their natal beaches is important to determine, especially if animals are becoming infected by fecal pollution in the marine environment. This study supports previous findings that increased freshwater outflow is an important risk factor for fecal-associated pathogens isolated from marine mammals and shellfish harvested from the ocean.[19,28] Fecal pollution from terrestrial locations could impact the ecology of the marine environment, as well as the health of humans and animals that rely on it, demonstrating a critical need to better understand the complex ecology at the terrestrial-marine interface.

Table 5. Logistic regression of significant catagorical risk factors for northern elephant seals that were culture positive for *Salmonella* spp. at stranding

Risk factor	*Group*	*Adjusted odds ratio*	*95% CI*	*P-value*
Stranding county	San Francisco and north	1.03	0.37, 2.88	0.96
	San Mateo	0.77	0.26, 2.28	0.64
	Santa Cruz	0.44	0.20, 0.99	0.05[a]
	Monterey	0.26	0.10, 0.68	<0.01[a]
	San Luis Obispo	1.00	—	—
Precipitation in past 30 days (inches)	0	2.09	0.35, 12.44	0.42
	0.01–2.00	5.37	1.64, 17.66	<0.01[a]
	2.01–4.00	3.01	0.86, 10.44	0.67
	>4.00	1.00	—	—

[a]Statistically significant

CI = confidence interval

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