Zoonotic pathogens isolated from wild animals and environmental samples at two California wildlife hospitals

Jennifer L. Siembieda, DVM, MPVM, PhD; Woutrina A. Miller, DVM, MPVM, PhD; Barbara A. Byrne, DVM, PhD, DACVIM; Michael H. Ziccardi, DVM, MPVM, PhD; Nancy Anderson, DMV, PhD; Nadira Chouicha, PhD; Christian E. Sandrock, MD, MPH; Christine K. Johnson, VMD, MPVM, PhD

Objective—To determine types and estimate prevalence of potentially zoonotic enteric pathogens shed by wild animals admitted to either of 2 wildlife hospitals and to characterize distribution of these pathogens and of aerobic bacteria in a hospital environment.

Design—Cross-sectional study.

Sample—Fecal samples from 338 animals in 2 wildlife hospitals and environmental samples from 1 wildlife hospital.

Procedures—Fecal samples were collected within 24 hours of hospital admission. Environmental samples were collected from air and surfaces. Samples were tested for zoonotic pathogens via culture techniques and biochemical analyses. Prevalence of pathogen shedding was compared among species groups, ages, sexes, and seasons. Bacterial counts were determined for environmental samples.

Results—Campylobacter spp, Vibrio spp, Salmonella spp, Giardia spp, and Cryptosporidium spp (alone or in combination) were detected in 105 of 338 (31%) fecal samples. Campylobacter spp were isolated only from birds. Juvenile passerines were more likely to shed Campylobacter spp than were adults; prevalence increased among juvenile passerines during summer. Non-O1 serotypes of Vibrio cholerae were isolated from birds; during an oil-spill response, 9 of 10 seabirds screened were shedding this pathogen, which was also detected in environmental samples. Salmonella spp and Giardia spp were isolated from birds and mammals; Cryptosporidium spp were isolated from mammals only. Floors of animal rooms had higher bacterial counts than did floors with only human traffic.

Conclusions and Clinical Relevance—Potentially zoonotic enteric pathogens were identified in samples from several species admitted to wildlife hospitals, indicating potential for transmission if prevention is not practiced. (J Am Vet Med Assoc 2011;238:773–783)
In the United States, there are more than 2,100 wildlife hospitals in which sick and injured wild animals receive medical treatment. Because most wild animals brought to wildlife hospitals are found in peri-urban areas, these facilities are important, yet mostly underutilized, critical points for monitoring zoonotic pathogens shed by wildlife. Wildlife hospitals are also particularly high-risk interfaces for transmission of pathogens among wildlife species or among humans and wild animals because a wide range of wildlife species are often housed in close proximity. Stressed animals are more likely to shed pathogens, and humans working at these centers are in direct and frequent contact with the animals in their care. The most important zoonoses at a wildlife hospital may be enteric pathogens because these are often environmentally resistant and easily transmitted via the fecal-oral route, and direct contact with pathogens can occur while staff are treating patientst and performing regular husbandry duties. The prevalence of enteric bacteria among seabirds and raptors has been reported in some studies conducted in wildlife rehabilitation centers in California, Washington, and Spain. However, to the authors’ knowledge, no studies have been performed at wildlife hospitals to evaluate enteric zoonotic pathogens in the wide range of species treated at these centers or to identify critical control points for management of fecal contamination and associated health risks by means of aerobic bacterial counts.

The study reported here focused on common species of wild birds and mammals admitted to wildlife rehabilitation centers along the west coast of the United States. To evaluate the potential occupational risk of zoonotic pathogen exposure for hospital staff and volunteers at centers that treat various wildlife species, we conducted a broad surveillance to detect enteric zoonotic pathogens in fecal samples from newly hospitalized animals and to determine aerobic bacterial counts in air and surface samples obtained from the hospital environment. The objectives of the study were to estimate the prevalence of Campylobacter spp, Vibrio spp, Salmonella spp, Escherichia coli O157:H7, Yersinia spp, Cryptosporidium spp, and Giardia spp shed by birds and mammals newly admitted to 2 wildlife hospitals in Northern California; to characterize these pathogens and assess patterns of shedding among wildlife species and age groups; and to evaluate distribution of these pathogens and of aerobic bacteria in a wildlife hospital environment.

Materials and Methods

Sample collection periods and sites—Fecal samples were collected from wild birds and mammals from January 14, 2007, through November 15, 2007, at 2 San Francisco Bay wildlife hospitals (facilities A and B) that receive various peri-urban wildlife species native to the western United States (Appendix). To assess environmental contamination, samples were obtained at facility A from multiple surfaces and from ambient air throughout a 2-day period in November 2007 during a moderate oil spill response when the facility was almost filled to patient capacity.

Fecal sample collection—Fecal samples were obtained opportunistically from 338 birds and mammals within 24 hours of admission to each facility. Up to 5 g of fecal matter/sample were collected from the examination table or cage floor. A sterile swab was inserted to the approximate center of each fecal sample and placed into a Cary-Blair agar and transport container. Samples were maintained at approximately 4°C during storage and transported on ice packs in a cooler to maximize recovery of bacteria. Species, oil exposure status (oiled or nonoiled), age group (juvenile or adult subjectively assessed on the basis of features such as plumage, beak characteristics, and weight), and sex (for species with visually distinct sexual dimorphism) of each animal were recorded during admission.

Environmental sample collection—Cleaning of floors and cages was performed via removal of grossly visible debris followed by application of an all-purpose cleaning solution diluted 1:10 in tap water. Medical supplies and equipment were cleaned with a chlorhexidine diacetate solution diluted 1:10 in tap water. Enclosure surfaces and mats were tested after cleaning, unless indicated otherwise. Samples for culture and heterologous aerobic bacterial counts were obtained from a standard area (5 replicates of 81 cm²) of each of the following surfaces by use of sterile cotton-tip applicators moistened with sterile water: floors (before and after cleaning), floor mats in the main bird room, disinfecting mat at the entrance of the main bird room, and bird enclosures (pools designated A and B), wall cages, net-bottomed cage (before and after cleaning), and wooden aviary with plastic-lined pool. Samples for culture (5 replicate swabs) were also collected from the distal 5 cm of 16F (large, 5.3-mm-diameter) and 8F (small, 2.7-mm-diameter) red rubber catheters, from inner aspects of I-ML syringes used to administer food and medications (after cleaning and disinfection as described), and from medical equipment (inner aspects of the rebreathing bag, breathing circuit tubes, and scavenging system of the anesthesia machine; inner aspects of endotracheal tubes; and surface of the stainless steel surgery table). Sample swabs were placed into cryovials with 1.75 mL of sterile saline (0.9% NaCl) solution refrigerated at approximately 2°C, and then kept on ice during transport to the laboratory.

Air samples were collected on 5% SBA and MacConkey agar plates by use of a 401-hole, impact air sampler outside the facility (temperature, 12°C [54°F]) and inside the facility in the main animal room (26°C [79°F]) and drying room (24°C [75°F]) when birds were present. Before each set of air samples was obtained, the air sampler was cleaned with a cotton cloth and 70% isopropyl alcohol. Air samples were collected for 2 continuous minutes at each location in triplicates.

Bacterial and protozoal screening—Fecal samples collected at facilities A and B and environmental samples collected at facility A were tested for the presence of Campylobacter spp, Salmonella spp, E.coli O157:H7, Vibrio spp, Yersinia spp, Giardia spp, and Cryptosporidium spp. Microbial cultures were performed at the Veterinary Medical Teaching Hospital Laboratory (School of Veterinary Medicine, University of California-Davis).
within 48 hours of sample collection because of transport time constraints.

Feces were screened for Campylobacter spp, Salmonella spp, E coli O157:H7, Vibrio spp (including Vibrio cholerae, Vibrio parahaemolyticus, and Vibrio alginolyticus), and Yersinia spp by use of standard media and enrichment techniques as described elsewhere; because only a small amount of feces could be collected from some animals, 10 samples were tested only for Campylobacter spp and Salmonella spp. Suspect colonies were subcultured onto fresh media and characterized by use of Gram stain, selective media, and biochemical tests or identification strips. Briefly, Campylobacter spp were isolated by use of 5% SBA supplemented with cefoperazone, vancomycin, and amphotericin B. Isolates were analyzed by standard biochemical tests, including sensitivity to cephalothin (30-µg disk) and nalidixic acid (30-µg disk), presence of catalase, and hippurate hydrolase. Cultures were enriched for Salmonella spp by use of selenite broth incubated for 24 hours before subculturing onto xylose-lysine tergitol 4 agar. Black colonies consistent with Salmonella spp were confirmed via biochemical testing and serogroup identification by agglutination with Poly O and group-specific antisera. Fecal samples were screened for E coli O157:H7 via selective culture on cefixime-tellurite sorbitol MacConkey agar. Vibrio spp were detected via enrichment with alkaline peptone water followed by subculture on thiosulfate-citrate-bile salts sucrose agar after 18 to 24 hours. Vibrio spp were identified according to reactions on triple sugar iron and urea agars, ability to split indole, and presence of cytochrome oxidase; final identification was confirmed by use of API 20E strips. Screening for Yersinia enterocolitica was performed by use of cefulodin-irgasan novobiocin agar.

Feces were examined for the presence of Giardia spp and Cryptosporidium spp by use of a direct immunofluorescence assay detection kit, and cyst- or oocyst-positive fecal samples were concentrated and purified via immunomagnetic separation for genotyping, as described in another study.

Additional characterization was performed for Salmonella spp, Campylobacter spp, V cholerae (non-O1 serotypes), Cryptosporidium spp, and Giardia spp. Serotype analysis of Salmonella spp was performed at the National Veterinary Services Laboratory in Ames, Iowa.

Campylobacter spp isolates were cultured on 5% SBA in a microaerophilic environment at 37°C for 48 hours. To prepare isolates for DNA extraction, 4 to 6 colonies were collected from each plate by use of a sterile loop and mixed with 200 µL of sterile distilled water. Each bacterial solution was vortexed for 15 seconds and then heated to 100°C for 10 minutes. Samples were centrifuged for 5 minutes at 12,000 × g, and the supernatant was removed and frozen at –80°C until PCR amplification was performed. Amplification of the 16S rRNA gene was performed by use of universal primers with a thermal cycler, as previously described. Product size was determined via gel electrophoresis on a 1% agarose gel that contained 0.005% ethidium bromide. The amplicon was cleaned with a reagent and submitted to a laboratory for sequencing. A commercially available software package was used to optimize the DNA sequences and compare these to known DNA sequences via interfaces with a local alignment search tool and multiple sequence alignment software. Vibrio cholerae (non-O1) isolates were cultured on 5% SBA at 37°C for 24 hours, and DNA extraction and gel electrophoresis were performed as described for Campylobacter spp. A PCR assay was used to determine the presence of the cholera toxin gene (ctxAB) in isolates of V cholerae (non-O1), as described elsewhere.

To genotype protozoa isolated from fecal samples, an 18S rRNA PCR protocol in conjunction with DNA sequence analysis that targets all Cryptosporidium spp was used. Giardia spp were analyzed via amplification of the glutamate dehydrogenase (gdh) and β-giardin genes. For DNA extraction, the 50-µL purified immunomagnetic separation product was mixed with 180 µL of lysis buffer solution in a cryotube, placed in liquid nitrogen for 4 minutes, and then placed in boiling water for 4 minutes to break open oocysts and cysts. A DNA extraction kit was used to digest each sample and extract DNA according to the manufacturer’s instructions, and DNA was eluted with 50 µL of 95°C DNAase-free PCR water. The targeted DNA region was 300 bp in size for Cryptosporidium spp; for Giardia spp, sizes were 432 and 753 bp for the gdh and β-giardin genes, respectively. Gel electrophoresis was used to separate the PCR products on a 2% agarose gel that contained 0.005% ethidium bromide. The PCR products were purified from the gel by use of a kit according to the manufacturer’s instructions, and sequences were obtained and analyzed as previously described. Sequences were deposited in the GenBank database under accession Nos. GQ426097 through GQ426099.

For heterologous aerobic bacterial counts of environmental samples, each cryovial that contained a surface sample was vortexed for 15 seconds and 5 serial, 10-fold dilutions were made by the addition of sterile saline; a 100-µL aliquot of the original sample and of each dilution was added to individual 5% SBA culture plates. Plates (including those used to collect air samples) were incubated at 37°C for 24 hours without additional CO2, and CFUs were counted. If colonies on the plate were not individually discernable, data were recorded as too numerous to count. The first plate with distinct countable colonies was used to estimate the total number of bacteria. Counts of surface samples were adjusted for the volume and dilution of that sample, and the estimated total CFUs per square centimeter of the surface from which the sample was obtained was used for statistical analysis. The estimated total CFUs for each cultured air sample was adjusted to CFUs per cubic meter by use of a table supplied by the manufacturer of the air sampler.

Statistical analysis—The prevalence of pathogen shedding was calculated as the proportion of fecal samples that tested positive for Campylobacter spp, Vibrio spp, Salmonella spp, Campylobacter spp, or Giardia spp among all animals from which samples were obtained. To evaluate potential occupational risk to staff at wildlife hospitals and to compare pathogen shedding among disparate wildlife taxa, species were categorized into groups considered most reflective of the typical segregation of animals at wildlife hospitals (Appendix). A Fisher exact test was used to compare the prevalence...
of pathogen shedding according to species group (mammals, passerines, raptors and turkey vultures, nonoiled seabirds, oiled seabirds, wading birds, and waterfowl), time of year (winter, January 14, 2007 to February 27, 2007; spring, March 1, 2007 to May 31, 2007; summer, June 3, 2,007 to September 10, 2,007; and fall, October 1, 2,007 to November 15, 2007), age (juvenile vs adult), and sex, if known. To account for variability in pathogen shedding among different species groups, tests used to compare prevalence by age and sex were stratified according to species group. Species groups were further stratified according to age group when testing for seasonal effects. A nonparametric Kruskal-Wallis rank test was used to detect differences in the aerobic bacterial counts for each surface and air sample site because data were not normally distributed. Mean values and 95% CIs were calculated via Poisson distribution and used to determine significance of bacterial values and 95% CIs were calculated via Poisson distribution and used to determine significance of bacterial counts. The median, range, outliers, and 25% and 75% quartiles for each surface and air sample site were determined, and box-and-whiskers plots were created. Values of $P < 0.05$ were accepted as significant.

**Results**

**Sample collection**—Seasonal trends in species composition and in numbers of patients admitted to the facilities resulted in varying numbers of samples obtained during January 14 to February 27 ($n = 64$ samples), March 1 to May 31 (149), June 3 to September 10 (103), and October 1 to November 15 (22). Environmental samples were collected at facility A in November 2007 during a moderate oil-spill response effort when an additional 1,084 seabirds (ie, seabirds from which samples were not obtained) were treated in 2 large rooms and several outside pools and aviaries.

**Enteric zoonotic pathogens in fecal samples from wild animals**—Campylobacter spp, Vibrio spp, Salmonella spp, Giardia spp, and Cryptosporidium spp (alone or in combination) were detected in fecal samples from 105 birds and mammals out of 338 wild animals from which samples were obtained (Table 1). Three Campylobacter spp (Campylobacter jejuni [n = 23 samples], Campylobacter coli [10], and Campylobacter lari [5]) were isolated from a variety of terrestrial and aquatic animals.

Table 1—Prevalence of enteric zoonotic pathogens shed in fecal samples obtained from 338 wild birds and mammals within 24 hours of admission to either of 2 wildlife hospitals in California from January 14, 2007, through November 15, 2007.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Positive tests (No./total)</th>
<th>Prevalence (%)</th>
<th>Wildlife species with positive tests (No. of animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter spp</td>
<td>46/338</td>
<td>13.6</td>
<td>— American coot (2), grebe (3), green heron (1), Pacific loon (1), surf scoter (1), and scrub jay (2)</td>
</tr>
<tr>
<td>Campylobacter coli</td>
<td>10/338</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>Campylobacter lari</td>
<td>5/338</td>
<td>1.5</td>
<td>Brown pelican (1), common murre (1), ruddy duck (1), surf scoter (1), and western grebe (1)</td>
</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>23/338</td>
<td>6.8</td>
<td>American crow (7), American robin (1), bufflehead (1), common murre (4), common raven (1), green heron (1), rhinoceros auklet (1), ruddy duck (2), snowy egret (1), surf scoter (2), western gull (1), and western screech owl (1)</td>
</tr>
<tr>
<td>Unknown spp</td>
<td>8/338</td>
<td>2.4</td>
<td>American white pelican (1), horned grebe (2), Pacific loon (1), ruddy duck (1), snowy egret (1), common murre (1), and willet (1)</td>
</tr>
<tr>
<td>Vibrio spp</td>
<td>33/328</td>
<td>10.1</td>
<td>—</td>
</tr>
<tr>
<td>Vibrio cholerae (non-O1 serotypes)</td>
<td>31/328</td>
<td>9.5</td>
<td>American coot (1), black-crowned night heron (4), brown pelican (1), common goldeneye (1), common murre (3), eared grebe (1), green heron (2), greater scaup (2), mallard (2), rhinoceros auklet (1), ruddy duck (2), surf scoter (6), western grebe (2), western gull (1), and unknown wading bird (2)</td>
</tr>
<tr>
<td>Vibrio alginolyticus</td>
<td>2/328</td>
<td>0.6</td>
<td>America crow (1) and common murre (1)</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>8/338</td>
<td>2.4</td>
<td>—</td>
</tr>
<tr>
<td>Arizonae III, serogroup B, strain III SS26-223, and Typhimurium; serogroup C1, 6,7,1.5, Thompson, and Ohio; serogroup D, Enteritidis; and serogroup E, Liverpool</td>
<td>8/338</td>
<td>2.4</td>
<td>Turkey vulture (2), mallard (2), long-tailed weasel (1), black-crowned night heron (2), and Canada goose (1)</td>
</tr>
<tr>
<td>Cryptosporidium spp</td>
<td>3/328</td>
<td>0.9</td>
<td>—</td>
</tr>
<tr>
<td>Skunk isolate genotype</td>
<td>1/328</td>
<td>0.3</td>
<td>Raccoon (1)</td>
</tr>
<tr>
<td>Genotype unknown</td>
<td>2/328</td>
<td>0.6</td>
<td>Bobcat (1) and gopher (1)</td>
</tr>
<tr>
<td>Giardia spp</td>
<td>15/328</td>
<td>4.6</td>
<td>—</td>
</tr>
<tr>
<td>Assemblage A genotype</td>
<td>1/328</td>
<td>0.3</td>
<td>Bobcat (1)</td>
</tr>
<tr>
<td>Genotype unknown</td>
<td>14/328</td>
<td>4.3</td>
<td>Barn owl (1), great horned owl (1), Canada goose (4), greater scaup (1), mallard (1), pocket gopher (1), ruddy duck (3), and gull (2)</td>
</tr>
</tbody>
</table>

Prevalence was calculated as the proportion of fecal samples that tested positive for each pathogen among all animals from which samples were obtained. Because only a small amount of feces could be collected from some animals, 10 samples were tested only for *Campylobacter* spp and *Salmonella* spp. Some samples tested positive for >1 pathogen.

— = Numbers are reported according to subcategories (species, genotype, or serotype).
ic birds for an overall Campylobacter spp shedding prevalence (calculated among birds and mammals) of 13.6%. Campylobacter spp were not isolated from mammals, and prevalence of Campylobacter spp was 14.9% (46/309) among all samples from avian species. Isolates for which species could not be identified were classified as Campylobacter spp; these were either catalase positive (n = 6 samples) or catalase negative (2). Vibrio spp were shed by several avian species, mostly aquatic birds, with an overall prevalence of 10.1% and a prevalence of 10.8% (33/305) among avian species. Most Vibrio spp isolates were V cholerae (non-O1), and eXAB genes were not detected. Eight Salmonella spp serotypes were identified in samples from a variety of birds and mammals, with an overall prevalence of 2.4%. The 5 enterica serotypes detected included Arizonae III; serogroup B, strain III 53:z4,z23 and Typhimurium; serogroup C1, 6,7:1,3, Thompson, and Ohio; serogroup D, Enteritidis; and serogroup E, Liverpool. Escherichia coli O157:H7 and Y enterocolitica were not detected in samples from any wild animals evaluated.

Giardia spp were detected in fecal samples from 4.6% of all animals tested. Animals that had Giardia-positive samples represented a variety of species that included aquatic birds, raptors, and a gopher. Comparisons with genetic sequence data and genetic groups (assemblages) contained in the GenBank database revealed that the Giardia isolate from a bobcat was within assemblage A at the gdh (accession No. GQ426099) and β-giardin (GQ426098) loci. The overall prevalence of Cryptosporidium spp shedding was 10.7% (3/28) in mammals, and Cryptosporidium spp were detected in samples from only 3 juvenile mammals (a raccoon, bobcat, and gopher). High-quality sequence data were produced for 1 Cryptosporidium-positive sample and 1 Giardia-positive sample (assessed via microscopy). Low numbers of oocysts and cysts (<10/slide well) prevented this analysis in other samples. Characterization of the raccoon Cryptosporidium isolate (GQ426097) produced an 185 rDNA sequence identical to the genotype of the skunk isolate (EU437415) and different from other reference genotypes.

Patterns in enteric zoonotic pathogen shedding—Prevalence of enteric pathogen shedding differed significantly among the 7 species groups (mammals, passerines, raptors and turkey vultures, seabirds [oiled and nonoiled], wading birds, and waterfowl) examined (Appendix; Table 1). The prevalence of Campylobacter spp shedding differed significantly (P = 0.022) among groups; the highest prevalence (21/98 [21.4%]) was detected in seabirds. Vibrio cholerae (non-O1) was isolated from nearly all oiled seabirds (9/10 [90%]), and the prevalence of this pathogen differed significantly (P < 0.001 for all comparisons) among species groups as well; prevalence was much lower in samples from nonoiled seabirds (9/98 [9.2%]), wading birds (8/44 [18.2%]), and waterfowl (5/37 [13.5%]) than in those of other species groups. Salmonella enterica prevalence was significantly (P = 0.024) increased among samples from waterfowl (3/37 [8.1%]), raptors (2/39 [5%]), wading birds (2/44 [4.5%]), and mammals (1/29 [3.4%]) than among other species groups evaluated. Giardia spp prevalence similarly differed significantly (P < 0.001) for all comparisons among species groups; more waterfowl shed cysts (8/37 [21.6%]) than did oiled seabirds (1/10 [10%]), mammals (2/29 [6.9%]), raptors and turkey vultures (2/38 [5.3%]), or nonoiled seabirds (2/99 [2%]).

After prevalence estimates were stratified according to species group, the prevalence of Campylobacter spp shedding was determined to differ significantly (P = 0.021) between juvenile and adult passerines (10/46 [21.7%] and 1/33 [3%], respectively). Juvenile passerines were also more likely to shed Campylobacter spp in summer months (P = 0.001) than they were during other seasons. Shedding of Campylobacter spp among juvenile passerines increased from 11% in the spring to 47% in the summer. Shedding of Campylobacter spp was detected at similar prevalences among juvenile (3/16 [18.8%]) and adult (18/82 [22%]) seabirds. However, shedding among adult nonoiled seabirds was significantly (P = 0.004) more likely to occur in fall months, even though adult seabirds were admitted to the facility throughout the year.

Similar to findings for Campylobacter spp, V cholerae (non-O1) was detected in a greater proportion of samples from adult seabirds in the fall months, compared with samples obtained at other times of the year (P = 0.022). Oiled seabirds, among which 9 of 10 shed V cholerae (non-O1), were all admitted during the fall, but nonoiled seabirds, which were admitted to the 2 facilities throughout the year, were also more likely to shed V cholerae (non-O1) during summer and fall months than during other times of the year. Shedding of V cholerae (non-O1) was detected in wading birds during summer months only.

Environmental distribution of pathogens and aerobic bacteria—Culture results of samples obtained from the main bird room floor and from ambient air at facility A were positive for V cholerae (non-O1). No other pathogens (E coli O157:H7, Campylobacter spp, Salmonella spp, or Yersinia spp) were detected in environmental samples. Aerobic bacterial counts were significantly (P = 0.001) different among all environmental sample locations. Relatively small amounts of bacterial contamination were detected for most environmental surfaces (median counts, < 25,000 CFUs/cm²; Figure 1), whereas some surfaces (drying room floor, floor mats, and outdoor aviary cage and pools) had relatively large amounts of bacterial contamination (median counts, > 800,000 CFUs/cm²; Figure 2); concentrations for ambient air were considered low (median counts, < 2,000 CFUs/m³; Figure 3).

Bacterial counts of samples obtained from large red rubber catheters (mean, 1.2 CFUs/cm² [95% CI, 0.4 to 2.6 CFUs/cm²]) and syringes (mean, 0.8 CFUs/cm² [95% CI, 0.2 to 2.1 CFUs/cm²]) were significantly lower than those obtained from small red rubber catheters (mean, 9.399 CFUs/cm² [95% CI, 9.314 to 9.484 CFUs/cm²]). Floors throughout facility A varied greatly in bacterial counts. The floor of the employees’ room (where no animals are allowed) had the least amount of bacterial contamination (mean, 12 CFUs/cm² [95% CI, 9 to 15 CFUs/cm²]). The least contaminated floor in a room that housed birds was that of the intensive care unit (mean bacterial count, 269 CFUs/cm² [95% CI, 255 to 284 CFUs/cm²]), followed by those of the main bird room.
Figure 1—Box-and-whiskers plot indicating various environmental surfaces at a wildlife hospital (facility A) on which low concentrations (median counts, < 25,000 CFUs/cm²) of aerobic bacteria were detected. Bacterial counts were determined via culture of samples obtained throughout a 2-day period during an oil-spill response. Boxes indicate the 25th to 75th percentiles; horizontal lines within each box indicate the median value. Whiskers represent the range of values, and outliers (> 2 SDs from the mean) are represented by filled circles. *Samples obtained after cleaning.

Figure 2—Box-and-whiskers plot indicating environmental surfaces at facility A for which high concentrations (median counts, > 800,000 CFUs/cm²) of aerobic bacteria were determined by means of culture. See Figure 1 for key.

Figure 3—Box plot of concentrations of bacteria in air samples obtained inside and outside of facility A when birds were present. Bacterial counts were determined via culture of samples collected directly onto culture plates by use of an impact air sampler. See Figure 1 for key.

(mean, 894 CFUs/cm² [95% CI, 867 to 921 CFUs/cm²]), food preparation room (mean, 2,725 CFUs/cm² [95% CI, 2,679 to 2,771 CFUs/cm²]), oiled seabird wash room (mean, 11,798 CFUs/cm² [95% CI, 11,731 to 11,866 CFUs/cm²]), and oiled seabird drying room (mean, 845,499 CFUs/cm² [95% CI, 844,694 to 846,306 CFUs/cm²]). The floor mats in the main bird room (mean, 3.241 X 10⁶ CFUs/cm² [95% CI, 3.239 X 10⁶ CFUs/cm² to 3.242 X 10⁶ CFUs/cm²]) and the disinfecting foot mat at the entrance of the main bird room (mean, 14,563 CFUs/cm² [95% CI, 14,458 to 14,670 CFUs/cm²]) had significantly higher bacterial counts than did the actual flooring (mean, 894 CFUs/cm² [95% CI, 868 to 921 CFUs/cm²]). The main bird room floor had significantly lower bacterial counts after cleaning (mean, 4 CFUs/cm² [95% CI, 1 to 10 CFUs/cm²]) than before cleaning.

The anesthesia machine had significantly higher bacterial counts (mean, 41 CFUs/cm² [95% CI, 35.8 to 47.2 CFUs/cm²]) than did endotracheal tubes (mean, 4.2 CFUs/cm² [95% CI, 2.6 to 6.4 CFUs/cm²]) and the surgery table (mean, 0.3 CFUs/cm² [95% CI, 0.01 to 1.4 CFUs/cm²]), but all had minimal bacterial contamination. Of anesthesia machine parts, the rebreathing bag was more contaminated (mean, 205 CFUs/cm² [95% CI, 178 to 233 CFUs/cm²]) than was the breathing circuit (mean, 0 CFUs/cm²) and the scavenging system (mean, 0 CFUs/cm²).

Bacterial counts of samples obtained from ambient air differed significantly among all locations tested. Outside air had the lowest mean bacterial count (223 CFUs/m³ [95% CI, 207 to 241 CFUs/m³]), compared with bacterial counts of air inside rooms of the facility (oiled-seabird drying room mean, 420 CFUs/m³ [95% CI, 397 to 444 CFUs/m³]; main bird room mean, 1,710 CFUs/m³ [95% CI, 1,664 to 1,757 CFUs/m³]).

Discussion

The study reported here describes the estimated prevalence of potentially zoonotic bacteria and protozoa shed by a diverse group of terrestrial birds and mammals at an important wildlife-human interface in North America. The assessments were performed at 2 wildlife hospitals in the coastal region of California. Facility A receives approximately 2,000 aquatic birds (ie, gulls,
shorebirds, wading birds, and waterfowl) annually, and staff members routinely respond to oil-spill events. Facility B receives approximately 6,000 passerines, raptors, and terrestrial mammals (eg, raccoons, opossum, rodents, rabbits, squirrels, and sometimes wild carnivores) each year. *Campylobacter jejuni* was the most prevalent pathogen detected in the present study. Although this pathogen has been previously described in a variety of birds, it is not thought to be an important cause of illness or mortality in avian species. However, in humans, this bacterium accounts for the majority of bacterial diarrheal disease in the United States, causing an estimated 2.4 million cases annually. In most cases, infections are self-limiting, although treatment can hasten recovery. Albeit rare, there can be complications from *Campylobacter* infections in humans (most often from *C. jejuni*) such as meningitis, arthritis, colitis, pancreatitis, and Guillain-Barre syndrome. Of the approximately 18 species of *Campylobacter*, we detected *C. lari, C. coli*, and most commonly *C. jejuni*, all of which have been identified in humans with diarrhea. The zoonotic potential of the *B. campylobacter* spp isolates that could only be identified as catalase negative or positive is uncertain; however, potential candidates for species that are catalase positive are *C. lari, C. coli*, and *C. upsaliensis*, all of which have been implicated as causes of diarrhea in humans. Catalase-negative *Campylobacter* spp such as *Campylobacter concisus* have been implicated in gastroenteritis in children and immunocompromised individuals. In the present study, *Campylobacter* spp were detected in samples from avian species only, and these bacteria are the zoonotic pathogens most likely to be encountered when working at wildlife hospitals that receive these animal species. Further investigation is warranted to assess the pathogenicity of *Campylobacter* spp shed by wild animals and their potential transmissibility to humans.

*Vibrio cholerae* (non-O1) was detected in fecal samples from birds and in environmental samples collected during an oil-spill response. *Vibrio cholerae* (non-O1) can cause cholera-like illness as well as bacteremia, wound infections, and ear infections in humans. In California, *V. cholerae* (non-O1) has been detected in coastal waters along central California and caused gastrointestinal illness in a surfer in this region. We did not detect the cholera toxin–producing gene ctxAB in samples from birds or environmental samples in the study reported here. *Vibrio alginolyticus* was detected in fecal samples from 2 birds in the present study. This opportunistic bacterium is the most widespread of *Vibrio* spp found in the environment and can cause gastroenteritis or wound infections in humans in some circumstances. When *V. cholerae* (non-O1) was detected in environmental samples (ie, samples from the air and floor of the main bird room), fecal samples from 9 of 10 the oiled seabirds housed in the main bird room tested positive for the pathogen as well. These samples were obtained from birds within 24 hours of arrival at the wildlife facility; thus, the test results indicated that birds were infected prior to admission. *Vibrio* spp are ubiquitous in the marine environment, and their concentrations in marine waters increase when hydrocarbons from pollution or an oil spill cause eutrophication. Oiled seabirds may be exposed to *V. cholerae* (non-O1) by means of preening or foraging on prey infected with the bacterium. To minimize the threat of *V. cholerae* (non-O1) spread among animals, good ventilation and frequent sanitization of areas occupied by these birds is critical, particularly during oil spill response efforts involving seabirds. Personal protective equipment typically used during oil spill responses should minimize exposure risk to staff at the response facility; and similar precautions should be taken by members of the public who rescue sick and oiled birds.

Interestingly, we found that a greater percentage of adult, nonoiled seabirds shed *Campylobacter* spp and *V. cholerae* (non-O1) during fall months than at other times of the year. In the marine environment, temperature-dependent *Vibrio* spp are more abundant in late summer and early fall when the water is warmer and more nutrient-rich, which is consistent with the finding in the present study. Similar seasonal differences have been reported among juvenile seabirds shedding *C. jejuni*. Clearly, repeated collection of samples over multiple years is necessary to determine the validity of seasonal patterns in pathogen shedding prevalence detected in the present study.

Eight serotypes of *Salmonella* were detected in samples from aquatic birds (wading birds and waterfowl), turkey vultures, and a mammal (Table 1). Salmonellosis is an important cause of mortality in birds and has been reported as an important cause of die-offs in wild passerines in recent years. All of the *Salmonella* serotypes detected in the study reported here have been associated with clinical disease in humans, and most are in *Salmonella* serogroups B, C1, D, and E, which account for 99% of *Salmonella* infections in humans and warm-blooded animals. Two of the isolates we detected, *S. enterica* serotype Typhimurium and *S. enterica* serotype Enteritidis, have been associated with invasive disease in humans. Although overall prevalence of *Salmonella* spp shedding was comparatively low in the wild animals screened in the present study, this pathogen should be considered an important zoonotic threat at wildlife hospitals, especially for humans handling terrestrial and freshwater aquatic avian species such as waterfowl, wading birds, turkey vultures, and passerines.

*Giardia duodenalis* (also called *Giardia intestinalis* and *Giardia lamblia*) causes diarrheal disease in humans and animals, and within the *G. duodenalis* species, there are multiple assemblages that have various host specificities. Assemblages A and B are considered potentially zoonotic and may infect a broad range of mammals. At the gdh locus, the DNA sequence of this isolate was identical to the A1 genotype of *Giardia* isolates from a ferret in Japan (GenBank accession No. AB469364) and from cattle in Brazil (EF507642). At the β-giardin locus, the bobcat *Giardia* isolate genotype was identical to the A2 genotype of isolates from a moose in Norway (DQ648778) and a human in Israel (AY072723). Detection of an assemblage A genotype of *Giardia* isolated from a bobcat suggests zoonotic potential, but further studies are needed to characterize the host specificity and life cycle of *Giardia* isolates of this genotype. It is interesting that the gdh locus of the bobcat isolate suggests the A1 genotype while the β-giardin
locus matches the A2 genotype, as has been reported for isolates in 2 other studies. It has been hypothesized that Giardia isolates of the A1 genotype have both animal and human hosts and could be zoonotic, while those of the A2 genotype have only human hosts and are not zoonotic. The results of genetic sequencing of isolates from a bobcat in California as well as a moose in Norway do not support this hypothesis because they genotype as A2 on the basis of β-giardin analysis.

Similarly for Cryptosporidium spp, there are a number of recognized species and genotypes, some that are thought to be primarily host specific while others are zoonotic. The raccoon isolate matched a genotype originally described in striped skunks in California in 2002 and was subsequently detected in raccoons in the eastern United States. This genotype has also been reported in a human patient with diarrhea in the United Kingdom. Unfortunately, we were unable to obtain genotype information from other immunofluorescence assay-positive samples. Protozoal cysts or oocysts were present in very low numbers in the samples obtained, which is consistent with a chronic phase of shedding low numbers of parasites in the feces, as compared to the acute infection phase in which high numbers of parasites are shed. Animals that shed low numbers of parasites are still of interest because the median infective doses (ID₅₀) of some parasite strains have been shown to be in the 10⁻³ to 100-cyst or -oocyst ranges.

All of the pathogens targeted in the study reported here can be shed in feces and can contaminate surface waters; C jejuni, Salmonella spp, and Cryptosporidium spp are considered emerging infectious waterborne pathogens. This is consistent with the detection of C jejuni and Salmonella spp in samples from aquatic host species in the present study. Interestingly, Cryptosporidium spp were only isolated from mammals in our study, even though this pathogen has been detected in a wide variety of birds and mammals and has been responsible for large waterborne outbreaks in humans. In the study reported here, low-volume fecal samples from birds that were dehydrated and malnourished at the time of admission to the facility and poor sensitivity of an assay with immunofluorescent antibodies originally raised against the C parvum antigen in cattle may have been factors that limited detection in samples from aquatic avian species.

Bacterial contamination among surfaces, equipment, and air was assessed via counts of aerobic bacterial during an oil-spill response when the hospital had nearly reached full patient capacity. The large number of hospitalized animals and associated fecal load present at the facility may have enhanced potential survival and transmission of pathogens among animals and humans via fecal-oral and aerosol routes. Treatment supplies such as small red rubber catheters were found to be contaminated with oil and bacteria during each sample collection. During an oil-spill response, a more effective cleaning regimen should be used to clean these supplies or they should be discarded after use. Our comparisons of aerobic bacterial counts in areas of the hospital not used during the oil-spill response may be generalized to other facilities operating at near full patient capacity. Medical equipment was found to have minimal bacteria present with the exception of the anesthetics machine rebreathing bag (205 CFUs/cm²). Care should be taken to disinfect the rebreathing bag and allow it to dry completely before each use. Air inside the facility, especially in rooms with high temperatures (>23.9°C [75°F]) that contained large numbers of birds, was more contaminated than air outside the facility, an indication that better ventilation may help prevent disease transmission within and among species. Numerous beneficial management practices measured in the wildlife hospital in this study likely minimized the spread of fecal contamination, and similar management should be practiced in other wildlife hospitals. Floor mats appeared to reduce contamination on the main bird room floor by collecting debris and feces. The ICU floor was cleaner likely because of the enhanced sanitation practices to prevent nosocomial infections. The employees' room where workers ate lunch and held meetings was significantly less contaminated than were other parts of the hospital that were evaluated.

Because of the constraints of opportunistic sampling and the time needed for sample transport, our samples were not analyzed until 48 hours after collection, which may have reduced the sensitivity of microbial culture methods and caused us to underestimate the potential for wild animals to serve as a source of pathogen exposure in this setting. Workers at wildlife hospitals in which most patients are seabirds may be at risk of exposure to Giardia spp, Campylobacter spp and V cholerae (non-O1). Risk of exposure to animals shedding V cholerae (non-O1) is 9 times that for individuals handling oiled seabirds, compared with the risk for those handling other wild animal species. An increased risk of zoonotic pathogen exposure also exists at facilities that house other aquatic birds, such as waterfowl and wading birds, compared with facilities that primarily house terrestrial birds and mammals. Individuals handling these aquatic species are likely to be additionally exposed to Salmonella spp. For facilities in which diverse terrestrial species are treated, workers may be exposed to Campylobacter spp when treating passerines and to Salmonella spp and Giardia spp when caring for turkey vultures and raptorial species. Hospital staff working with mammals should be aware that patients may be shedding Salmonella spp, Cryptosporidium spp, and Giardia spp with unknown zoonotic potential, and appropriate measures should be used to minimize cross-species transmission.

We identified potentially zoonotic pathogens in birds and mammals that could be transmitted to workers at wildlife hospitals and even to the households of these workers if good hygiene and preventive measures are not practiced. It is possible that animals stressed by illness may be more likely to shed pathogenic bacteria than are healthy free-ranging animals, and the risk of zoonotic pathogen exposure may increase further when animals are retained at facilities for long periods of time. Extra precautions should be taken during summer and early fall months when wildlife hospitals are busiest and pathogen prevalence may be highest. To reduce the risk of exposure to these pathogens, fecal-oral transmission can be prevented by eliminating feces in the environment and by use of simple hygienic.
measures such as hand washing. Also, staff and volunteers should be encouraged to wear personal protective equipment such as face masks and gloves on a routine basis. \(^2\) A diverse range of wild animal species is often housed in sometimes crowded conditions at wildlife hospitals, and this important interface among species may be particularly a high-risk location for emergence of pathogens that can cross species barriers.

a. A detailed list of species is available from the corresponding author upon request.
b. Simple Green, Sunshine Makers Inc, Huntington Harbour, Calif.
e. SAS Super 100/180, blow rate: 100L/min, PBI International, Milan, Italy.
g. BioMerieux, Hazelwood, Mo.
h. Meridian Diagnostics Inc, Cincinnati, Ohio.
i. Eppendorf Centrifuge 5417C, Westbury, NY.
j. Mastercycler gradient thermal cycler, Eppendorf North America, Westbury, NY.
k. Exo Sap-IT, USB Corp, Cleveland, Ohio.
l. Chromas software, version 2.33, Technelysium Pty Ltd, Tewantin, QLD, Australia.
m. ATL buffer, QIAGEN Inc, Valencia, Calif.
n. QIAGEN DNA mini kit, QIAGEN Inc, Valencia, Calif.
o. QIAQUICK gel purification kit, QIAGEN Inc, Valencia, Calif.
p. Stata SE, version 9.2, StataCorp, College Station, Tex.
q. Hardy Diagnostics, Santa Maria, Calif.
r. Biological Media Services, University of California-Davis, Davis, Calif.
s. SFPPL, Integrated DNA Technologies, Coralville, Iowa.
t. T492RPL, Integrated DNA Technologies, Coralville, Iowa.
u. Davis Sequencing Inc, Davis, Calif.

References


### Appendix

Wild animals (total, 338) from which fecal samples were collected and cultured for enteric zoonotic pathogens in either of 2 wildlife hospitals in California from January 14, 2007, through November 15, 2007.

<table>
<thead>
<tr>
<th>Species group</th>
<th>Order</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammal (29)</td>
<td>Carnivora (12)</td>
<td>Raccoon (10) and other mammals (3)</td>
</tr>
<tr>
<td></td>
<td>Didelphimorphia (12)</td>
<td>Opossum (12)</td>
</tr>
<tr>
<td></td>
<td>Lagomorpha (1)</td>
<td>Cottontail rabbit (1)</td>
</tr>
<tr>
<td></td>
<td>Rodentia (2)</td>
<td>Pocket gopher and fox squirrel (2)</td>
</tr>
<tr>
<td></td>
<td>Soricomorpha (1)</td>
<td>Flat footed mole (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Passerine (79)</td>
<td>Columbiformes (16)</td>
<td>Mourning dove (14) and band-tailed pigeon (2)</td>
</tr>
<tr>
<td></td>
<td>Passeriformes (61)</td>
<td>American crow (12), American robin (13), western scrub jay (12), and other songbirds (24)</td>
</tr>
<tr>
<td></td>
<td>Piciformes (2)</td>
<td>Northern flicker and mockingbird (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raptor and turkey vulture (39)</td>
<td>Ciconiiformes (3)</td>
<td>Turkey vulture (3)</td>
</tr>
<tr>
<td></td>
<td>Falconiformes (22)</td>
<td>American kestrel (6), red-tailed hawk (5), and other raptors (11)</td>
</tr>
<tr>
<td></td>
<td>Strigiformes (14)</td>
<td>Barn owl (5), great horned owl (6), and western screech owl (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonoiled seabird (100)</td>
<td>Anseriformes (8)</td>
<td>Surf scoter (8)</td>
</tr>
<tr>
<td></td>
<td>Charadriiformes (44)</td>
<td>Common murre (31), western gull (7), and other seabirds (6)</td>
</tr>
<tr>
<td></td>
<td>Gaviiformes (9)</td>
<td>Common, Pacific, and red-throated loons (9)</td>
</tr>
<tr>
<td></td>
<td>Pelecaniformes (13)</td>
<td>Brown pelican (6) and other seabirds (7)</td>
</tr>
<tr>
<td></td>
<td>Podicipediformes (25)</td>
<td>Western grebe (11), Clark’s grebe (6), and other grebes (8)</td>
</tr>
<tr>
<td></td>
<td>Procellariidae (1)</td>
<td>Sooty shearwater (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oiled seabird (10)</td>
<td>Anseriformes (6)</td>
<td>Scaup and scoter (6)</td>
</tr>
<tr>
<td></td>
<td>Charadriiformes (1)</td>
<td>Glaucous-winged gull (1)</td>
</tr>
<tr>
<td></td>
<td>Podicipediformes (3)</td>
<td>Eared and western grebe (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wading bird (44)</td>
<td>Anseriformes (2)</td>
<td>American coot (2)</td>
</tr>
<tr>
<td></td>
<td>Charadriiformes (2)</td>
<td>Killdeer and willet (2)</td>
</tr>
<tr>
<td></td>
<td>Gaviiformes (39)</td>
<td>Black-crowned night heron (16), green heron (8), snowy egret (7), and other wading birds (8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waterfowl (37)</td>
<td>Anseriformes (37)</td>
<td>Virginia rail (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Canada goose (13), mallard (12), ruddy duck (5), and other ducks (7)</td>
</tr>
</tbody>
</table>

Number of animals is shown in parentheses.