

EFFECT OF REHABILITATION ON PELAGIC BIRD FECAL MICROBIAL COMMUNITIES AND SHEDDING OF *SALMONELLA* SPP.

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HYPOTHESIS AND OBJECTIVES:

Factors including diet, antimicrobial treatment, age, and exposure to infectious agents have been associated with *Salmonella* shedding and changes in gastrointestinal microbial diversity in animals and humans.^{2,4,5,9} Therefore, **we hypothesize that pelagic birds will have detectable changes in fecal bacterial community structure and increased shedding of *Salmonella* spp. during the rehabilitation process.**

Objectives:

1. To culture selected pelagic bird species for *Salmonella* spp. upon entrance to a rehabilitation center, during the rehabilitation process, and at departure from the rehabilitation center.
2. To characterize the microbial community diversity structure in selected fecal samples by comparing the banding patterns produced using Denaturing Gradient Gel Electrophoresis (DGGE).
3. To evaluate the association between changes in microbial community structure and *Salmonella* shedding with risk factors such as diet, antimicrobial treatment, bird species, age, oil exposure, or concurrent disease.

EXPERIMENTAL PLAN:

Study design and fecal sampling strategy:

Four common pelagic bird species involved in oil spills along the California coast will be evaluated in this study: common murre (*Uria aalge*), brown pelicans (*Pelicanus occidentalis*), western grebes (*Aechmophorus occidentalis*), and western gulls (*Larus occidentalis*). A total of 200 birds will be evaluated during their first weeks of rehabilitation at the San Francisco Bay Oiled Wildlife Care and Education Center (SFBOWCEC) in Cordelia, California. The sample size of 50 birds per species was selected based on the average 5% prevalence of *Salmonella* shedding detected in wildlife entering California rehabilitation centers in 1999-2000 and the expected caseload for these species under non-oil spill response conditions.⁸ This sample size will allow for 95% confidence of detecting *Salmonella* in each bird species with a power of 80%. If an oil spill occurs along the California coast during the study period, the study design may be modified to allow for increased sampling of birds affected by the spill in order to evaluate the effect of oil exposure and oil spill treatment protocols on fecal bacterial communities.

Upon each bird's arrival at SFBOWCEC, a fresh fecal sample will be collected from the bird transport carrier to represent the 'pre-admission' fecal bacterial community. Fecal samples will then be collected periodically during rehabilitation and again upon departure from the rehabilitation center. Screening approximately 50 birds of 4 species, with up to 4 fecal samples/bird, will allow for *Salmonella* screening of up to 800 fecal samples during the study. *Salmonella* screening of each sample will occur immediately, while feces will be archived frozen for bacterial community diversity analysis. A subset of 10 birds per species will be selected for microbial community structure analysis based on whether they are shedding *Salmonella*, with fecal community structure compared among birds as well as over time within the same bird. Data on risk factors such as dietary regimen, antimicrobial treatment, oil exposure, bird species, bird age, and concurrent disease will be recorded in the individual bird records.

Bacterial detection and characterization methodologies:

Fresh fecal samples will be processed in two ways. First, *Salmonella* will be detected by selenite enrichment and plating on selective media.⁸ Suspect *Salmonella* colonies will be further characterized with biochemical and serotype testing.³ Second, 1 gram fecal samples from each bird will be archived frozen at each collection time point, for later use in the microbial community structure analysis.

Fecal community structure analysis will be performed as described.⁶ Briefly, genomic DNA will be extracted from 200 mg fecal samples and a region of the 16S rRNA gene will be amplified using the Polymerase Chain Reaction (PCR). The PCR products will then be separated using DGGE, producing a complex banding pattern that represents the microbial community structure, with each band representing a unique bacterial species. The DGGE banding patterns can be compared qualitatively and quantitatively among samples from the same bird over time and among different birds receiving the same treatments. Additionally, bands of interest that are observed to change among samples will be excised and sequenced for bacterial identification. These methods have been used successfully by our laboratory for evaluation of equine microbial communities.

Data analysis:

Changes in microbial diversity and *Salmonella* shedding will be analyzed with univariate and multivariable methods. Differences in prevalence among groups will be compared using χ^2 or Fisher Exact statistical tests, while differences in the number of DGGE bands per sample will be compared using the student's t-test.¹ Logistic regression will be used to evaluate multiple risk factors simultaneously while adjusting for repeated sampling of birds using Stata software (Stata Co., College Station, TX). Risk factors of interest include dietary therapy, antimicrobial treatment, bird species, age, oil exposure, and concurrent disease.

SIGNIFICANCE TO OILED WILDLIFE HEALTH:

Studies in humans and animals have shown that the composition and diversity of fecal flora may change in response to diet, antimicrobial therapy, age, and exposure to infectious agents.^{1,4,5,9} Dietary and antimicrobial treatments are often utilized during wildlife rehabilitation, but their effects on pelagic bird fecal flora have not been evaluated. Laboratory studies have shown that antimicrobial treatment may decrease gastrointestinal colonization resistance, leading to increased susceptibility to *Salmonella* infection.⁷ If *Salmonella* infection or changes in bacterial composition in pelagic birds are associated with risk factors such as diet, antimicrobial treatment, bird species, age, oil exposure, or concurrent disease, then these factors should be considered to improve animal care at rehabilitation centers and in oil spill responses. For example, certain types of diets or antimicrobial regimens may prove to be more beneficial for preserving gastrointestinal community diversity and preventing *Salmonella* shedding. Furthermore, detection of *Salmonella* will help to assess wildlife health and evaluate the risk of exposure of rehabilitation personnel to this important fecal-oral zoonotic pathogen .

ESTIMATED BUDGET (funds are requested for one year):

PERSONNEL:	Dr. Miller (10%); Drs. Byrne and Kreuder (5% each) SRA II, 20% time, \$9205 salary + \$2762 benefits	\$0 \$11,967
EQUIPMENT:	Incubator for bacterial culture, to be housed at SFBOWCEC	\$1,289
SUPPLIES:	<i>Salmonella</i> detection supplies: 200 birds x 4 fecal samples/bird x \$12	\$9,600
	Bacterial community diversity analysis: 40 birds x 4 fecal samples = 160 samples	
	DNA extraction supplies	\$3,600
	DNA polymerase chain reaction supplies	\$3,900
	PCR product purification supplies	\$2,100
	Sequence analysis costs	\$6,400
TRAVEL:	Travel between Davis, Cordelia, and potential oil spill sites	\$1,000
Total funds requested:		\$39,856

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