

# BACTERIAL FLORA OF THE GIANT GARTER SNAKE (*THAMNOPHIS GIGAS*) AND VALLEY GARTER SNAKE (*THAMNOPHIS SIRTALIS FITCHI*) IN THE CENTRAL VALLEY OF CALIFORNIA

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**Abstract.**—This study investigates the normal bacterial flora found in two species of garter snakes. We obtained cultures by separately swabbing the mouth, skin and cloaca of 128 Giant Garter Snakes (*Thamnophis gigas*) and Valley Garter Snakes (*Thamnophis sirtalis fitchi*) distributed throughout four wildlife management areas in the Central Valley of California. We found 1,321 bacterial isolates including 14 anaerobic isolates from 384 swabs. The ten most commonly isolated gram negative aerobic bacteria were *Aeromonas* sp., *Citrobacter* sp., *Acinetobacter* sp., *Escherichia coli*, *Pseudomonas* sp., *Morganella morganii*, *Klebsiella* sp., *Shewanella* sp., *Proteus* sp., and *Providencia* sp. There were no significant differences in the number of isolates recovered from each species or geographic site. We obtained significantly more isolates from the skin compared to the mouth and cloaca. Many of the isolates recovered are potential human and reptile pathogens. The antibiotic sensitivity patterns of the aerobic gram negative isolates are similar to the sensitivity patterns of bacterial pathogens isolated from domestic animals and do not show significant resistance to appropriate antibiotics. These findings suggest that a wide variety of potentially pathogenic bacteria are present in the four habitats sampled and may impact the health of snakes and humans using these environments.

**Key Words.**—*Aeromonas*; bacteria; microbiology; *Vibrio*

## INTRODUCTION

In reptiles, bacterial associated morbidity and mortality can be the result of exposure to pathogenic strains or through opportunistic infection caused by normal flora during immunosuppression of the host (Tangredi 1997; Jacobson 2007), although outcomes in wild populations are unknown. Most bacterial infections in reptiles are opportunistic and caused by gram negative bacteria, which are commonly isolated from healthy reptiles (Draper 1981; Ross 1984). Less frequently, gram positive and anaerobic bacteria have caused disease in captive reptiles (Plowman 1987). The normal bacterial flora of snakes has been reported in a few species including free ranging Western Rat Snakes (*Pantherophis obsoletus*; Waugh 2017), Common Garter Snakes (*Thamnophis sirtalis*) that were in captivity for greater than a month (Goldstein 1981), and captive Reticulated Pythons (*Python reticulatus*; Yak 2015). Many reports of normal flora are in association with studies of snake venoms (Arroyo 1980; Shek 2009; Iqbal 2014).

Endemic to the wetlands and marshes of the Central Valley of California, the federally and California state-listed Giant Garter Snake (*Thamnophis gigas*; Fig. 1) depends on freshwater wetlands for the small fish and amphibians that are its primary food source (Fitch 1940). Unfortunately, conversion to agriculture and urban uses has led to an estimated 91% reduction in wetland habitat in California since the 1780s (Dahl 1990), with 43% of freshwater wetlands in the Central Valley lost

or converted since 1939 (Frayer 1989). This loss of historical habitat for the Giant Garter Snake has resulted in extirpations or serious declines throughout much of the former range of the species.

Although habitat loss remains the primary threat to extant Giant Garter Snake populations, other threats, such as insufficient or interrupted water supply during the active season of the snake (March through September), degraded water quality, environmental contamination, and parasite infestation have been identified as potential contributors to the ongoing decline of the species (U.S. Fish and Wildlife Service [USFWS] 1999, 2006). These factors may be particularly significant in the San Joaquin Valley, where recent surveys indicate a rapid decrease in Giant Garter Snake abundance in areas where habitat appears superficially suitable but where seasonal drying occurs (Hansen 2008; Wylie et al. 2010). Populations of Valley Garter Snakes (*T. sirtalis fitchi*), which co-occur with Giant Garter Snakes in the Central Valley, appear to remain stable, whereas the populations of Giant Garter Snakes are declining. The Valley Garter Snake (Fig. 2) is less aquatic and more versatile in its selection of prey, which, in addition to fish and amphibians, includes small mammals, small birds, and invertebrates, such as slugs and leaches (Rossman et al. 1996).

As part of a comprehensive study to assess the role of contaminants, water quality, and water management in the health and distribution of Giant Garter Snakes across their range, we examined Giant Garter Snakes and Valley Garter Snakes from four wildlife management



FIGURE 1. Giant Garter Snake (*Thamnophis gigas*) from the Central Valley of California, USA. (Photographed by Eric C. Hansen).

areas during the summer of 2008. The goal of this study was to use swabs collected from the skin, oral cavity, and cloaca of snakes to determine the bacterial flora present and if there was a difference in the flora between Giant Garter Snakes and Valley Garter Snakes. The four geographic sites provided the opportunity to compare snake bacterial flora among a broad cross section of the remaining habitats occupied by Giant Garter Snakes, including natural wetland habitats and rice agriculture in the Sacramento Valley and the waterfowl management areas dominating the San Joaquin Valley.

## METHODS

**Capture sites.**—We captured Giant Garter Snakes and Valley Garter Snakes during the peak of the 2008 active season (April through September) from four geographically independent study sites (Fig. 3) within the Central Valley of California, USA: Natomas Basin (Natomas; UTM 618789–633368 Easting, 4273215–4298568 Northing, NAD83, Zone 10S) in Sacramento and Sutter counties; Cosumnes River Preserve (Badger Creek; UTM644804–646012 Easting, 4242863–4244008 Northing, NAD83, Zone 10S) in southern Sacramento County; Grasslands Ecological Area (Los Banos; UTM 682764–686179 Easting, 4108849–4117672 Northing, NAD83, Zone 10S) in Merced County; and Mendota Wildlife Area (Mendota; UTM 735865–744699 Easting, 4060598–4068597 Northing, NAD83, Zone 10S) in Fresno County. Natomas and Badger Creek are located in the Sacramento Valley and represent two different major habitat profiles. Natomas is predominantly a rice growing region, with created wetlands specifically

managed for Giant Garter Snakes interspersed across the landscape, and Badger Creek is an unmodified, natural wetland. Los Banos and Mendota are located in the San Joaquin Valley and represent habitat profiles managed primarily for overwintering waterfowl.

**Sampling methods.**—We captured snakes in minnow traps (Cuba Specialty Manufacturing, Fillmore, New York, USA) modified to float (Casazza et al. 2000). We also captured snakes by hand when opportunities arose. We placed the traps along the open water-vegetation or open water-bankside interfaces of aquatic features (i.e., irrigation/drainage canals, sloughs, ponds, marshes) with sufficient water depth ( $\geq 6$  cm) and we checked the traps for captured snakes at least daily. We measured, determined the sex, photographed, and permanently identified each captured snake.

While wearing disposable exam gloves, we removed each snake from a trap and we sampled a 10 cm segment of the mid body skin by rolling a dry culturette swab (Fisher finest Transport Swab 14-907-12, Fisher Health Care, Houston, Texas, USA) cranial to caudal on all surfaces of the skin including the dorsal, both lateral, and ventral surfaces. The culturette was then sterilely replaced in the transport tube containing Amies Clear transport media gel. We obtained a sample of the pharyngeal flora by manually opening the mouth of the snake and rolling a culturette swab in the pharyngeal area caudal to the glottis. We then sterilely replaced the swab individually in the transport tube. We obtained a sample of the cloacal flora by gently inserting a third culturette swab 1 cm into the cloaca, slowly rotating it, and then removing the swab. We replaced the culturette





**FIGURE 2.** Valley Garter Snake (*Thamnophis siratalis fitchi*) from the Central Valley of California, USA. (Photographed by Eric C. Hansen).

individually in the transport tube. We placed all transport tubes containing the swabs in the transport media gel in a cooler that maintained the samples at below 27° C until delivery at the lab within 12 h of sampling.

As part of a concurrent study, we captured eight Giant Garter Snakes with grossly visible subcutaneous swellings. Each of these snakes was examined by a veterinarian and the swellings were diagnosed as abscesses. Using sterile technique, we cultured the necrotic tissue within each abscess as described above.

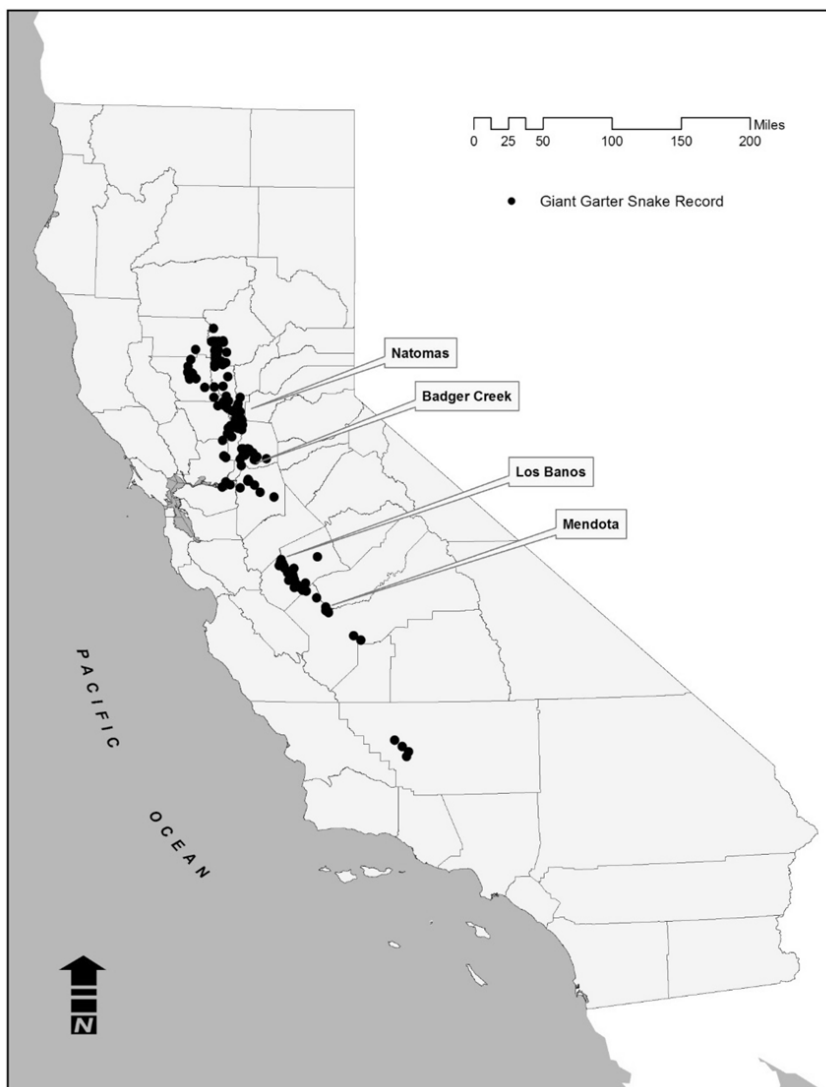
We sent our samples to a commercial veterinary clinical laboratory for analysis. All media were warmed to room temperature before plating. Swabs were removed from the transport media and used to streak plates using a standard four quadrate method (Sanders 2012). For recovery of aerobic and anaerobic bacteria, each sample was plated on Trypticase Soy Agar with 5% sheep blood (BA), MacConkey agar (MAC), CDC Anaerobic Blood Agar (ANA), Phenylethylalcohol agar (PEA), Colistin-Naladixic Acid agar (CNA), and Hektoen Enteric (HE) agar. Swabs were then placed in 4 ml tubes containing Thioglycolate broth (THIO). The BA, PEA and CNA plates were incubated at 33–37° C in 5–10% CO<sub>2</sub>. The MAC, HE and THIO plates were incubated at 33–37° C in room air atmosphere. ANA plates were placed in anaerobic jars and incubated at 33–37° C for 48 h. The BA, PEA, CNA, MAC, HE and THIO plates were examined at 18–24 h, 48 h, and 72 h for signs of growth and colony isolation. Organisms were identified using the Vitek™ identification system with confirmation using biochemistry tests. Bacterial organisms were identified by culture and biochemical characteristics only and not with DNA typing. The antibiotic sensitivity of the four most proliferative gram negative aerobic isolates from each swab was determined using standard Kirby Baur disk diffusion methods (Biemer 1973).

We compared the bacterial isolates from each collection source (skin, mouth, cloaca) to determine the number of isolates for each snake from all possible combinations of body sources (skin only, mouth only, cloaca only, all three sites, skin and mouth, skin and cloaca, mouth and cloaca). If the same organism was detected at multiple sources, we only counted it as one isolate in one body source. For example, if we isolated the same species of *Escherichia coli* from the mouth and cloaca, we counted it as one isolate from the body source of mouth/cloaca. In many snakes, we cultured multiple isolates of the same species of bacteria. These isolates differed in culture characteristics and biochemical testing.

Because data were not normally distributed and the sample sizes for many of the isolates were small, we used Kruskal-Wallis and rank sum tests to compare the number of bacterial isolates between species, geographic collection site, and body source. The significance level was set at  $\alpha = 0.05$ . For significant tests, we made pairwise post-hoc comparisons between samples using the Dunn's test. We conducted statistical analyses using STATA SE 11.0 (StataCorp, College Station, Texas USA).

## RESULTS

We obtained bacterial cultures from the skin, mouth, and cloaca of 128 snakes (Table 1). The average number of isolates ranged from eight to 13 (Table 1). The number of bacterial isolates did not differ significantly by species of snake ( $H = 1.14$ ,  $df = 1$ ,  $P = 0.286$ ) or geographic collection site ( $H = 3.12$ ,  $df = 3$ ,  $P = 0.374$ ). The number of isolates was significantly higher for skin swabs compared to mouth or cloaca swabs ( $H = 10.24$ ,  $df = 3$ ,  $P = 0.017$ ). *Clostridium* sp. was the most numerous of the 14 anaerobic bacterial isolates obtained from the 384 swabs submitted for anaerobic culture (Table 2).



**FIGURE 3.** Study sites in the Central Valley of California, USA, used to determine the bacterial flora of Valley Garter Snakes (*Thamnophis sirtalis fitchi*) and Giant Garter Snakes (*Thamnophis gigas*).

*Aeromonas* sp. was the most commonly isolated genus of gram-negative bacteria (Table 3). The antibiotic sensitivity pattern for the 10 most commonly isolated genera of gram-negative bacteria (1,269 aerobic isolates) showed expected resistance to antibiotics (Appendix 1). The four most common gram-positive isolates were non-hemolytic coagulase negative *Staphylococcus* species (153 isolates), Gamma hemolytic *Streptococcus* species (86 isolates), Alpha hemolytic *Streptococcus* species (66 isolates), and *Corynebacterium* species (56 isolates). Cultures of cutaneous abscesses in eight Giant Garter Snakes resulted in isolation of some of the same bacterial species as obtained from the skin, mouth, and cloaca (Table 4).

### DISCUSSION

We obtained 1,321 bacterial isolates (including 14 anaerobes) from 128 garter snakes collected at four sites in the Central Valley of California. This resulted in an average recovery rate of 10 aerobic isolates and

0.1 anaerobic isolates per snake. We cultured a bacterial isolate from two body sites from the same snake in 35% of the snakes and the same bacterial isolate was cultured from all three body sites in 24% of the snakes. There were no significant differences in the number of isolates obtained from Giant Garter Snakes compared to Valley Garter Snakes or in the number of isolates compared to the geographic collection site. There were significantly more isolates recovered from the skin than from other body sources (mouth or cloaca). This may be a result of cutaneous exposure to environmental bacteria. Both the Giant Garter Snakes and Valley Garter Snakes spend most of their time in very nutrient rich, slow moving bodies of water, which are expected to have abundant bacterial flora. Some of the bacteria isolated from the skin surface were likely secondary to surface contamination as the skin surface was not cleansed prior to swabbing. Therefore, the presence of bacteria on the surface of the skin does not demonstrate colonization of the skin or imply morbidity. The oral cavity and cloaca

**TABLE 1.** Number of bacterial isolates from each body source for two species of garter snakes collected at four different geographical sites in California USA. Abbreviations and acronyms are n = number of snakes cultured at each location, S = skin only, M = mouth only, C = Cloaca only, SMC = number of isolates found in three body sites (skin + mouth + cloaca), SM = number of isolates found in skin + mouth, SC = number of isolates found in skin + cloaca, MC = number of isolates found in Mouth + Cloaca, and AVG = average number isolates per snake.

Site	Species	n	S	M	C	SMC	SM	MC	SC	Total	AVG
Natomas	<i>sirtalis</i>	20	55	25	56	22	24	8	27	217	13
Natomas	<i>gigas</i>	21	58	29	48	30	14	11	32	222	11
Los Banos	<i>sirtalis</i>	21	43	27	56	24	15	9	24	198	9
Los Banos	<i>gigas</i>	17	41	15	44	17	9	5	17	148	8
Badger Creek	<i>sirtalis</i>	12	37	18	28	20	8	12	15	138	12
Badger Creek	<i>gigas</i>	20	58	27	52	18	18	13	26	212	11
Mendota	<i>sirtalis</i>	16	31	25	31	27	15	11	22	162	10
Mendota	<i>gigas</i>	1	2	0	1	1	0	1	5	10	10
Totals	<i>sirtalis</i>	69	166	95	171	93	62	40	88	715	10
	<i>gigas</i>	59	159	71	145	66	41	30	80	592	10
	Both species	128	325	166	316	159	103	70	168	1307	10.2

are much more isolated and protected sites. Therefore while positive cultures from these sites may also be the result of surface contamination, it is more likely that these isolates represent normal flora, especially if the same bacterial strain is identified in multiple locations.

We obtained the swabs cultured in this study immediately upon removal of the snake from the trap. We wore single use latex gloves while handling the snakes but these gloves were not cultured. It is possible that bacterial flora of the snakes changed during the less than 24 h that the snakes were in the traps. We used a standard culturette swab and transport media for obtaining the samples. This media/swab combination is marketed for transport of swabs for clinical use to isolate both aerobic and anaerobic bacteria. The swabs in transport media were delivered to the lab within 12 h of obtaining the sample, but it is possible that the number of anaerobic isolates in this study were reduced due to suboptimal conditions for transport and recovery of anaerobic organisms. However, given the aerobic environment on the skin of the snakes, the oral cavity, and the cloaca,

it may be that these anatomic regions do not have large anaerobic bacterial populations.

All swabs were plated for culture and identified by a commercial veterinary clinical laboratory using standard operating procedures in place in 2008. As these procedures are optimized for the recovery of bacteria from domestic animals, it is possible that the laboratory procedures were not optimal for recovery of bacterial isolates from reptiles. Varying temperatures at which isolates are incubated can make a difference in what isolates are found. Goldstein et al. (1981) found that duplicate cultures from garter snakes (*Thamnophis* spp.) incubated at room temperature and 37° C resulted in six isolates growing at room temperature but not at 37° C, 10 other isolates growing at 37° C but not room temperature, and 17 other isolates growing at both temperatures. Our results would likely have been different if a variety of incubation temperatures had been used.

The inability to identify many of the isolates in this study to the species level was likely due to differences in the bacterial strains from those normally encountered

**TABLE 2.** Anaerobic bacterial isolates from three body locations from Giant Garter Snakes (*Thamnophis gigas*) and Valley Garter Snakes (*T. sirtalis fitchi*) collected at four sites in the Central Valley of California, USA. The number of snakes cultured at each site is n.

Site	Species	n	Skin	Mouth	Cloaca	Organisms
Natomas	<i>sirtalis</i>	20	0	0	0	no anaerobic organisms
Natomas	<i>gigas</i>	21	3	0	0	three isolates of <i>Clostridium</i> species
Los Banos	<i>sirtalis</i>	21	2	0	1	two isolates of <i>Clostridium</i> species and one <i>Bacteroides</i> species
Los Banos	<i>gigas</i>	17	1	3	3	four <i>Clostridium</i> species one <i>Fusobacterium</i> species one unable to speciate
Badger Creek	<i>sirtalis</i>	12	0	0	0	no anaerobic organisms
Badger Creek	<i>gigas</i>	20	0	0	0	no anaerobic organisms
Mendota	<i>sirtalis</i>	16	0	0	1	one <i>Clostridium</i> species
Mendota	<i>gigas</i>	1	0	0	0	no anaerobic organisms
Totals	<i>sirtalis</i>	69	2	0	2	
	<i>gigas</i>	59	4	3	3	
	Combined	128	6	3	5	

**TABLE 3.** Ten most common genera of gram-negative bacteria isolated from Valley Garter Snakes (*T. sirtalis fitchi*) and Giant Garter Snakes (*Thamnophis gigas*) in the Central Valley of California including the body site cultured.

Organism	Total # of Isolates	Mouth	Cloaca	Skin	# Isolates for Genus
<i>Aeromonas</i> sp.	160	53	42	65	
<i>Aeromonas hydrophila</i>	13	3	2	8	
<i>Aeromonas veronii</i> biovar <i>veronii</i>	181	59	59	63	354
<i>Citrobacter</i> sp.	49	8	25	16	
<i>Citrobacter braakii</i>	83	11	48	24	
<i>Citrobacter freundii</i>	4	1	3	0	136
<i>Acinetobacter</i> sp.	73	33	7	33	
<i>Acinetobacter calcoaceticus-baumannii</i>	53	20	4	29	
<i>Acinetobacter junii</i>	3	2	0	1	
<i>Acinetobacter lowoffii</i>	1	0	0	1	130
<i>Escherichia</i> sp.	2	0	0	2	
<i>Escherichia coli</i>	95	16	52	27	97
<i>Pseudomonas</i> sp.	76	19	10	47	
<i>Pseudomonas aeruginosa</i>	5	0	2	3	
<i>Pseudomonas (flavimonas) oryzihabitans</i>	1	1	0	0	
<i>Pseudomonas mendocina</i>	2	1	1	0	84
<i>Morganella morganii</i>	68	7	49	12	68
<i>Klebsiella</i> sp.	1	0	0	1	
<i>Klebsiella oxytoca</i>	35	0	22	13	
<i>Klebsiella ozaenae</i>	1	1	0	0	
<i>Klebsiella pneumoniae</i>	23	5	8	10	60
<i>Shewanella</i> sp.	59	12	10	37	59
<i>Proteus mirabilis</i>	12	3	5	4	
<i>Proteus penneri</i>	1	1	0	0	
<i>Proteus vulgaris</i>	45	6	33	6	58
<i>Providencia</i> sp.	1	0	0	1	
<i>Providencia rettgeria</i>	55	4	32	19	56

in domestic animals for which the Vitek™ identification system is designed. The limitations of this study are consistent with the use of a commercial veterinary laboratory for processing samples from non-domestic animals. In future studies, use of molecular methods (such as DNA sequencing) may result in fewer unidentified isolates. Current best practices rely on the use matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) and 16S sequencing rather than growth characterization and biochemistry testing used in this study (Clark 2013). MALDI-TOF has been recently shown to be effective in the identification of aerobic bacterial flora of boid snakes (Plenz 2015). Optimization of growth media and incubation techniques for reptile-associated bacteria would also enhance accuracy.

The findings in this study are consistent with the literature where aerobic bacteria predominate over anaerobic bacteria in snakes (Jacobson 2007). Similar to other studies of the oral bacterial flora of snakes, the most commonly identified genus of anaerobic bacteria was *Clostridium* followed by *Bacteroides* (Draper 1981;

Yak 2015). Some studies have found that gram-positive bacteria predominate in the flora of snakes (Draper 1981; Goldstein 1981) while in this study, gram-negative bacteria predominated the aerobic flora. The bacterial species isolated in this study were also isolated in garter snakes by others but in different frequency (Mergenhausen 1956; Goldstein 1981). The most common aerobic bacteria identified in this study are commonly considered opportunistic pathogens of reptiles (Rosenthal 2006). The antibiotic sensitivities of these isolates are typical of the antibiotic sensitivities encountered in the same bacterial taxa from domestic animals (Aucoin 2007). This similarity in sensitivity patterns suggests that these bacterial isolates have not been subjected to selection pressures exerted by exposure to subtherapeutic doses of antibiotics in the environment.

Cultures of cutaneous abscesses in eight Giant Garter Snakes resulted in isolation of some the same bacterial species as we found from other parts of the body. This finding suggests that while the snakes in this study appeared normal, under some circumstances, these bacterial organisms may become pathogenic. Not



**TABLE 4.** Bacterial isolates from eight clinical cases of abscesses in Giant Garter Snakes (*Thamnophis gigas*) in the Central Valley of California, USA.

Isolate	Case							
	1	2	3	4	5	6	7	8
<i>Aeromonas</i> sp.					X			
<i>Alcaligenes faecalis</i>			X					
<i>Bacteroides</i> sp.	X							
<i>Citrobacter braakii</i>					X		X	
<i>Citrobacter</i> sp.			X			X		
<i>Clostridium</i> sp.							X	
<i>Corynebacterium</i> sp.		X						
<i>Dermatophilus chelonae</i>								X
<i>Enterobacter cloacae</i>	X							
<i>Escherichia coli</i>				X				
<i>Fusobacterium</i> sp.	X		X					
Gram negative rod	X						X	
<i>Klebsiella oxytoca</i>					X			
<i>Morganella morganii</i>							X	
<i>Providencia rettgeri</i>			X	X		X	X	
<i>Pseudomonas aeruginosa</i>				X				
<i>Pseudomonas</i> sp.					X			
<i>Shewanella</i> sp.							X	
<i>Streptococcus</i> (alpha hemolytic)		X						
<i>Streptomyces</i> sp.	X							

surprisingly because necrotic tissue in abscesses has low oxygen tension, the isolation of anaerobic bacteria was more common in the abscesses than from the body surfaces sampled. One of the snake abscesses grew a pure culture of a gram-positive bacteria, *Dermatophilus chelonae*. This snake was also severely emaciated.

In addition to being pathogenic for reptiles, most of the bacteria isolated in this study have been identified as possible pathogens in humans (Philips 2015). A significant finding of this study is the isolation of *Vibrio cholera*, *Salmonella choleraesuis*, *Plesiomonas shigelloides*, coagulase negative hemolytic *Staphylococcus*, *Hafnia (enterobacter) alvei*, *E. coli*, and *Edwardsiella tarda*, all of which have been associated with significant infections in humans (Philips 2015). We obtained both of the *Vibrio cholera* isolates from the San Joaquin Valley region (Los Banos and Mendota) where the Giant Garter Snakes are in most rapid decline and the habitat is more disturbed. *Plesiomonas* was isolated from both the Los Banos and Natomas collection sites. We recovered the hemolytic *Staphylococcus* isolates from the northern (Natomas and Badger Creek) collection sites. *Hafnia (enterobacter) alvei*, *E. coli*, and *E. tarda* were distributed throughout all four geographic collection sites. It is commonly assumed that reptiles in captivity are carriers of species *Salmonella* species. In this study, we obtained 15 *Salmonella* isolates from 12 snakes (approximately 10% of snakes sampled) distributed throughout all four geographic collection sites and represented approximately 1% of the

bacterial isolates. As a result of these findings, people who are engaged in activities in areas with these snakes should take precautions to reduce their exposure to these potential pathogens.

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**ERIC HANSEN** is a Consulting Environmental Biologist living in Sacramento, California. Specializing in threatened and endangered reptiles and amphibians of the Central Valley, Mr. Hansen has a lifetime of experience with the Giant Garter Snake and has focused on this and other protected species as an independent consultant and researcher since 1998. With primary interests centering on conservation ecology, his independent and collaborative projects have covered a range of interests, including population genetics, contaminants and toxicology, demography, and spatial ecology. (Photographed by Eric C. Hansen).



**CHRISTINE KREUDER JOHNSON** is Professor of Epidemiology and Ecosystem Health in the School of Veterinary Medicine and Director of the EpiCenter for Disease Dynamics at the One Health Institute at University of California, Davis. Her research focuses on wildlife population health and the impact of ecological processes on species at risk and patterns of disease transmission. Recent activities investigate zoonotic disease spillover dynamics, viral host shifts, and epidemiologic patterns facilitating zoonotic disease transmission and spread. She provides epidemiologic support to federal and state agencies during unusual outbreak events and directs global surveillance activities for the Emerging Pandemic Threats PREDICT program. Christine graduated with distinction from Duke University in 1990, obtained a veterinary degree (V.M.D.) from the University of Pennsylvania in 1994, and a Ph.D. in Epidemiology from University of California, Davis in 2003. (Photograph courtesy of University of California, Davis School of Veterinary Medicine).



**ROBERT POPPENGA** has over 24 y of post-graduate experience conducting or collaborating on wildlife toxicology studies and investigating the effects of a variety of chemical contaminants on wildlife and domestic animal species. Most recently he has been involved in projects assessing the impact of anticoagulant rodenticides on a broad range of non-target wildlife species, the impact of the banning of lead ammunition on lead exposure in the California Condor (*Gymnopyps californianus*) using surrogate avian species, and assessment of contaminant exposure as a factor in the health and population stability of the Giant Garter Snake in the Central Valley of California. He is a veterinarian with specialty board certification in toxicology by the American Board of Veterinary Toxicology. Currently, he is Head of the Toxicology Section of the California Animal Health and Food Safety Laboratory System. (Photograph courtesy of UC Davis School of Veterinary Medicine).

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**APPENDIX 1.** Kirby Baur disk diffusion antibiotic sensitivities of the 10 most common genera of gram negative aerobic bacteria from 1,269 isolates obtained from Valley Garter Snakes (*Thamnophis sirtalis fitchi*) and Giant Garter Snakes (*Thamnophis gigas*) in the Central Valley of California. Abbreviations are S = percentage of isolates sensitive, R = percentage of isolates resistant, I = percentage of isolates intermediate in sensitivity, n = total number of isolates tested, AM = amikacin, CL = amoxicillin & clavulanic acid, AX = amoxicillin, AP = ampicillin, AU = amoxicillin & clavulanate potassium, C = carbenicillin, CD = ceftazidime, CF = ceftiofur, CA = cephalixin, CT = cephalothin, CH = chloramphenicol, CP = ciprofloxacin, EN = enrofloxacin, GN = gentamicin, PI = piperacillin, TE = tetracycline, TI = ticarcillin, TO = tobramycin, TR = trimethoprim & sulfadiazine, and NT = not tested.

	AM	CL	AX	AP	AU	C	CD	CF	CA	CT	CH	CP	EN	GN	PI	TE	TI	TO	TR
<i>Aeromonas</i> species																			
S	100	0	0	1	2	0	100	100	15	51	100	100	100	100	100	99	13	100	97
R	0	70	100	99	90	100	0	0	85	40	0	0	0	0	0	1	71	0	0
I	0	30	0	0	8	0	0	0	0	9	0	0	0	0	0	0	16	0	3
n	354	20	20	336	336	55	354	318	20	336	354	354	354	354	345	354	354	354	354
<i>Citrobacter</i> Species																			
S	100	75	0	6	1	100	98	97	38	9	99	99	96	100	100	100	100	100	100
R	0	25	100	91	95	0	1	2	62	80	1	1	0	0	0	0	0	0	0
I	0	0	0	3	4	0	1	1	0	11	0	0	4	0	0	0	0	0	0
n	135	8	3	31	115	19	135	135	8	127	135	135	135	135	9	51	50	51	51
<i>Acinetobacter</i> species																			
S	99	100	40	66	81	100	94	17	0	0	51	100	100	99	87	59	99	99	100
R	1	0	20	27	13	0	1	61	100	100	43	0	0	1	7	36	0	0	0
I	0	0	40	7	6	0	5	22	0	0	6	0	0	0	6	5	1	1	0
n	129	8	5	82	116	5	126	126	9	120	129	129	129	129	68	129	77	129	77
<i>Escherichia coli</i> species																			
S	100	75	100	44	43	100	98	96	100	65	99	100	97	100	75	99	92	100	100
R	0	0	0	50	48	0	2	3	0	33	1	0	0	0	25	1	8	0	0
I	0	25	0	6	9	0	0	1	0	2	0	0	3	0	0	0	0	0	0
n	97	4	4	96	96	2	97	97	4	96	97	97	97	97	4	97	72	97	97
<i>Pseudomonas</i> species																			
S	100	17	17	7	14	20	100	42	0	0	21	100	79	100	99	67	48	100	51
R	0	50	67	4	83	60	0	26	86	100	11	0	0	0	0	10	43	0	48
I	0	33	16	89	3	20	0	32	14	0	68	0	21	0	1	23	9	0	1
n	80	7	7	71	71	5	79	74	7	72	79	79	79	79	79	77	77	77	67
<i>Morganella</i> species																			
S	100	0	0	2	2	NT	96	91	0	2	74	100	100	100	100	69	97	100	100
R	0	100	100	98	98	NT	3	7	100	98	19	0	0	0	0	31	2	0	0
I	0	0	0	0	0	NT	1	2	0	0	7	0	0	0	0	0	1	0	0
n	68	3	2	65	65		68	68	3	65	68	68	68	68	5	68	67	68	68
<i>Klebsiella</i> species																			
S	100	100	0	15	78	NT	98	93	100	93	100	100	100	100	100	100	54	100	100
R	0	0	100	85	15	NT	0	3	0	0	0	0	0	0	0	0	44	0	0
I	0	0	0	0	7	NT	2	4	0	7	0	0	0	0	0	0	2	0	0
n	59	5	5	54	54		59	59	5	54	59	59	59	59	2	59	57	58	58
<i>Shewanella</i> species																			
S	100	66	66	88	97	8	100	98	0	20	98	100	100	100	100	97	85	100	100
R	0	33	33	9	2	15	0	2	0	21	0	0	0	0	0	2	5	0	0
I	0	0	0	3	1	77	0	0	100	59	2	0	0	0	0	1	10	0	0
n	59	3	3	56	56	13	59	51	3	56	59	59	59	59	58	59	59	59	59

**APPENDIX 1 (CONTINUED).** Kirby Baur disk diffusion antibiotic sensitivities of the 10 most common genera of gram negative aerobic bacteria from 1,269 isolates obtained from Valley Garter Snakes (*Thamnophis sirtalis fitchi*) and Giant Garter Snakes (*Thamnophis gigas*) in the Central Valley of California. Abbreviations are S = percentage of isolates sensitive, R = percentage of isolates resistant, I = percentage of isolates intermediate in sensitivity, n = total number of isolates tested, AM = amikacin, CL = amoxicillin & clavulanic acid, AX = amoxicillin, AP = ampicillin, AU = amoxicillin & clavulanate potassium, C = carbenicillin, CD = ceftazidime, CF = ceftiofur, CA = cephalexin, CT = cephalothin, CH = chloramphenicol, CP = ciprofloxacin, EN = enrofloxacin, GN = gentamicin, PI = piperacillin, TE = tetracycline, TI = ticarcillin, TO = tobramycin, TR = trimethoprim & sulfadiazine, and NT = not tested.

	AM	CL	AX	AP	AU	C	CD	CF	CA	CT	CH	CP	EN	GN	PI	TE	TI	TO	TR
<i>Proteus</i> species																			
S	100	66	33	11	29	NT	97	97	0	7	88	100	100	100	75	49	81	100	100
R	0	0	34	84	58	NT	2	3	100	93	7	0	0	0	25	51	17	0	0
I	0	34	33	5	13	NT	1	0	0	0	5	0	0	0	0	0	2	0	0
n	57	3	3	55	55		57	57	3	54	57	57	57	57	4	51	57	57	57
<i>Providencia</i> species																			
S	100	NT	NT	4	4	66	96	100	NT	4	57	100	100	100	100	0	96	100	100
R	0	NT	NT	7	4	34	0	0	NT	0	21	0	0	0	0	98	2	0	0
I	0	NT	NT	88	92	0	4	0	NT	96	22	0	0	0	0	2	2	0	0
n	56			56	56	6	56	50		56	56	55	56	56	8	56	48	54	54