

OSTEOMYELITIS ASSOCIATED WITH *SALMONELLA ENTERICA* SS *ARIZONAE* IN A COLONY OF RIDGENOSE RATTLESNAKES (*CROTALUS WILLARDI*)

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Abstract: The identification of three Arizona ridgenose rattlesnakes (*Crotalus willardi*) with *Salmonella arizonae*-associated osteomyelitis led to a 5-yr prospective study of radiographic signs and *Salmonella* intestinal carriage rates in a 19-member colony of this rattlesnake species. Ventrodorsal radiographs were performed and cloacal swabs were cultured for *Salmonella* spp. annually. Ten snakes survived the 5-yr period, with six of them remaining free of bony lesions. Three snakes that had no bony lesions in 1995 developed radiographic signs of osteomyelitis during the study. Six snakes with bony lesions at the beginning of the study died or were euthanized due to osteomyelitis during the study. The radiographic signs of osteomyelitis were progressive for five snakes that were serially radiographed. Only one snake with radiographic signs of osteomyelitis at the beginning of the study was still alive at the end of the study, and this animal's bony lesions were more extensive at the end. Thirty-nine intestinal *S. arizonae* isolates, representing 13 serotypes, were obtained from the 19 snakes. *Salmonella arizonae* serotype 56:Z4,Z23 was isolated only once from a cloacal culture, from a snake that had no radiographic bone lesions. Twelve extraintestinal *Salmonella* isolates, representing two serotypes, were isolated from six snakes. All extraintestinal isolates except one were of *S. arizonae* serotype 56:Z4,Z23, and all isolates from bone were of this serotype. One snake with characteristic bone lesions died, and *Providencia rettgeri* was cultured from each of the tissues cultured, whereas no *Salmonella* spp. were isolated from this snake. *Salmonella arizonae* serotype 56:Z4,Z24 appears to have a tropism for bone and other extraintestinal sites in *C. willardi* and may cause a progressive, ultimately fatal disease in this species.

Key words: *Crotalus willardi*, osteomyelitis, rattlesnake, *Salmonella arizonae*.

INTRODUCTION

Reptiles are well-known nonsymptomatic carriers of *Salmonella* spp.,^{4,8,12} but there are relatively few reports of these bacteria causing disease in reptiles.^{2,11,17,18} *Salmonella arizonae* has been cultured commonly from reptiles, most frequently from snakes,^{6,9,11,15,23} and of the *Salmonella* spp., the *arizonae* subgroup has been identified disproportionately as pathogens of snakes.^{2,13,17,18}

Salmonella arizonae serotypes comprise a major subgroup of *Salmonella* spp. (also referred to as group IIIa). Members of the *arizonae* subgroup are distinguished from other *Salmonella* by their ability to use malonate, liquefy gelatin, and their frequent ability to ferment lactose.²² Recent convention now identifies these organisms as *S. enterica* subspecies *arizonae*, with any serotype designation being denoted by letters and numbers after the subgroup

name.³ For simplicity, in this report, *S. arizonae* will be used to refer to all organisms from this subspecies.

Firm, irregularly shaped subcutaneous nodules were observed on two Arizona ridgenose rattlesnakes (*Crotalus willardi willardi*) housed at the Knoxville Zoological Garden (Tennessee, USA) in October and November 1990. There were six to nine nodules per snake, 1 × 1 × 1 mm to 10 × 10 × 5 mm in size, diffusely located on the snakes' bodies. An excisional biopsy of a rib nodule was obtained from each snake, and histopathology revealed bacterial pyogranulomatous osteomyelitis. Cultures of each biopsy grew *S. arizonae* as the dominant organism. One grew >1,000 colonies of serotype 56:Z4,Z23 in pure culture. Only nine colonies of *S. arizonae* grew from the other biopsy, and this isolate was not serotyped. Both snakes were euthanized in March 1991 because of lack of clinical improvement (pathology and bacteriology results included in Table 2).

The identification of an additional snake with *S. arizonae*-associated osteomyelitis in the fall of 1995 led to this prospective study of the Knoxville Zoo's *C. willardi* collection. This report describes the initial cases and the results of a 5-yr prospective study of the radiographic signs, intestinal *Salmonella* spp. carriage rates, and mortality.

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MATERIALS AND METHODS

A prospective study was conducted from 1 September 1995 to 31 December 2000. There were 19 snakes (15 *C. w. willardi* and four *C. w. silus*) in the collection at the beginning of the study period. Three females had been wild caught, and the remaining snakes were captive born.

The majority of the snakes (>80%) were housed individually on wood shavings substrate in glass terraria. A large terrarium with wood shavings substrate and pieces of wood was used when the snakes were grouped for breeding or parturition. Snakes were maintained from April to November at ambient daytime temperature of 21.1–26.6°C. For the remainder of the year, daytime temperatures ranged from 14.4°C to 18.3°C. Humidity was not measured. The snakes were fed killed mice at 10- to 20-day intervals from April to November.

The snakes were typically handled twice a year, once in spring and again in the fall. Radiographs were taken in October 1995 (19 snakes), October 1996 (10 snakes), November and December 1997 (13 snakes), December 1998 (12 snakes), and December 2000 (13 snakes).

Diagnosis of osteomyelitis was based on radiographic signs. All snakes that died or were euthanatized during the study period were necropsied, except one.

Cloacal cultures were obtained in April 1996, November or December 1997, December 1998, and December 2000. Specimens were collected with Culturett® swabs (Beckton Dickinson Microbiology Systems, Sparks, Maryland 21152, USA) and transported to the laboratory within 4 hr. Four fecal samples were obtained from feeder mice cages and cultured for *Salmonella* spp.

Salmonella screening procedures included direct culture on MacConkey and Hektoen-Enteric (HE) agars (Beckton Dickinson Microbiology Systems) and enrichment in Selenite F broth (Beckton Dickinson Microbiology Systems) for 18 hr followed by culture on HE. Cultures were incubated at 35°C. Intestinal tissue obtained at necropsy was similarly cultured. Extraintestinal tissues obtained by biopsy or necropsy were also cultured on Columbia Agar plates (Beckton Dickinson Microbiology Systems) containing 5% sheep blood, with and without colistin (10 µg/ml)–nalidixic acid (15 µg/ml) supplement and in fluid thioglycollate broth. *Salmonella* isolates were presumptively identified by their reactions on conventional biochemical tests followed by agglutination with polyvalent *Salmonella* grouping antiserum (Poly A-I & Vi, Difco, Detroit, Michigan 48232, USA) or automated biochemical iden-

tification (GNI card, Vitek-AMS, BioMerieux Vittek, Hazelwood, Missouri 63042, USA) (or both). *Salmonella* isolates were serotyped at the National Veterinary Services Laboratory, Ames, Iowa 50010, USA.

RESULTS

At the beginning of the study, seven of the 19 (37%) snakes had radiographic lesions compatible with osteomyelitis. None of the snakes with radiographic lesions were directly related. The median age of captive-born snakes with radiographic lesions was 9.5 yr (6.3–12.3 yr, $n = 4$). The three wild-caught *C. w. willardi* females with bony lesions in 1995 had each been in captivity for more than 5 yr (5.2–11.2 yr). The median age, in 1995, of the captive-born snakes without radiographic lesions was 4.5 yr (1.2–10.2 yr, $n = 12$).

At the end of the study, 10 of the original snakes (six captive-born *C. w. willardi* and four *C. w. silus*) remained in the collection. Nine births occurred in the colony in 1999, but the offspring were not included in this study.

Six snakes remained free of bony lesions suggestive of osteomyelitis throughout the study period. The median age of these snakes at the end of the study was 12.0 yr (6.5–15.4 yr). Two of these snakes that developed radiographic signs of osteoarthritis of the spine (but no rib lesions) were not believed to have osteomyelitis.

Three snakes that had no bony lesions in 1995 developed radiographic signs of osteomyelitis during the study period and were alive on 31 December 2000. Lesions were first identified when these snakes were 3, 9, and 13 yr of age. The youngest of these snakes was the offspring of a wild-caught female with osteomyelitis.

Three snakes that had no radiographic lesions in 1995 died during the study. One snake developed a questionable radiographic sign suggestive of osteomyelitis in 1997, but a full necropsy was not performed because of severe postmortem autolysis. Postmortem cultures were obtained from this animal. The other two snakes had no bony lesions identified by radiology or necropsy.

Six *C. w. willardi* with bony lesions in 1995 died or were euthanatized during the study. The radiographic signs of osteomyelitis were progressive for each snake for which serial radiographs were obtained ($n = 5$). The cause for euthanasia or death for each of these was debilitation due to osteomyelitis. Median age at time of death or euthanasia for the captive-born snakes was 16.7 yr (8.3–17.3 yr, $n = 3$). The three wild-caught snakes that died or were euthanatized had each been in captivity for at



Figure 1. Whole-body ventrodorsal radiograph of an Arizona ridgenose rattlesnake. Note the multiple bony masses associated with the ribs. There is also extensive new bone production associated with multiple sites in the vertebral column. *Salmonella arizonae* serotype 56:Z4,Z23 was isolated from the liver, ovary, and bone of this snake at necropsy.

least 5.2 yr at the time of death (5.2–7.0 yr). Only one snake with radiographic signs of osteomyelitis in 1995 was still alive on 31 December 2000. This snake's bony lesions were more extensive at the end of the study period than at the beginning.

Clinical signs varied depending on the severity of lesions. Snakes with only a few rib lesions had palpable, firm nodules but were otherwise normal. Typical nodules were 2–4 mm in height and 3–5 mm in diameter. Snakes with vertebral lesions limited to a few vertebrae showed no clinical signs. Snakes with more extensive vertebral lesions had either limited movement or no movement in the affected areas. The affected vertebral regions ranged from 3.4 to 22.0 cm in length. Restricted range of spinal motion could be palpated, but only two snakes had palpable nodules over the spine. Severely affected snakes could not balance on a hook, right themselves if placed in dorsal recumbency, or effectively strike at prey items, which led to the decision to euthanize.

Radiology

The most common bony abnormalities were associated with the ribs (Fig. 1). Radiographic abnormalities ranged from focal spherical enlargement of the ribs to linear reactions extending the full length of the rib (Fig. 2). The focal enlargements appeared to be due to enlargement of the medullary cavity with expansile remodeling of the surrounding cortex. Bony reaction would often fuse adjacent ribs. There were 1–16 affected ribs per snake.

Osteomyelitis of the spine started as an area of bony lysis within the medullary region of the vertebral body (Fig. 3). There was expansile enlargement of the surrounding cortex. In advanced stages of disease, periosteal new bone formed and often extended to adjacent vertebrae and proximal aspects of the ribs. Pathologic fractures frequently developed.

The vertebral column of two snakes with osteoarthritis had areas with indistinct bony margins.

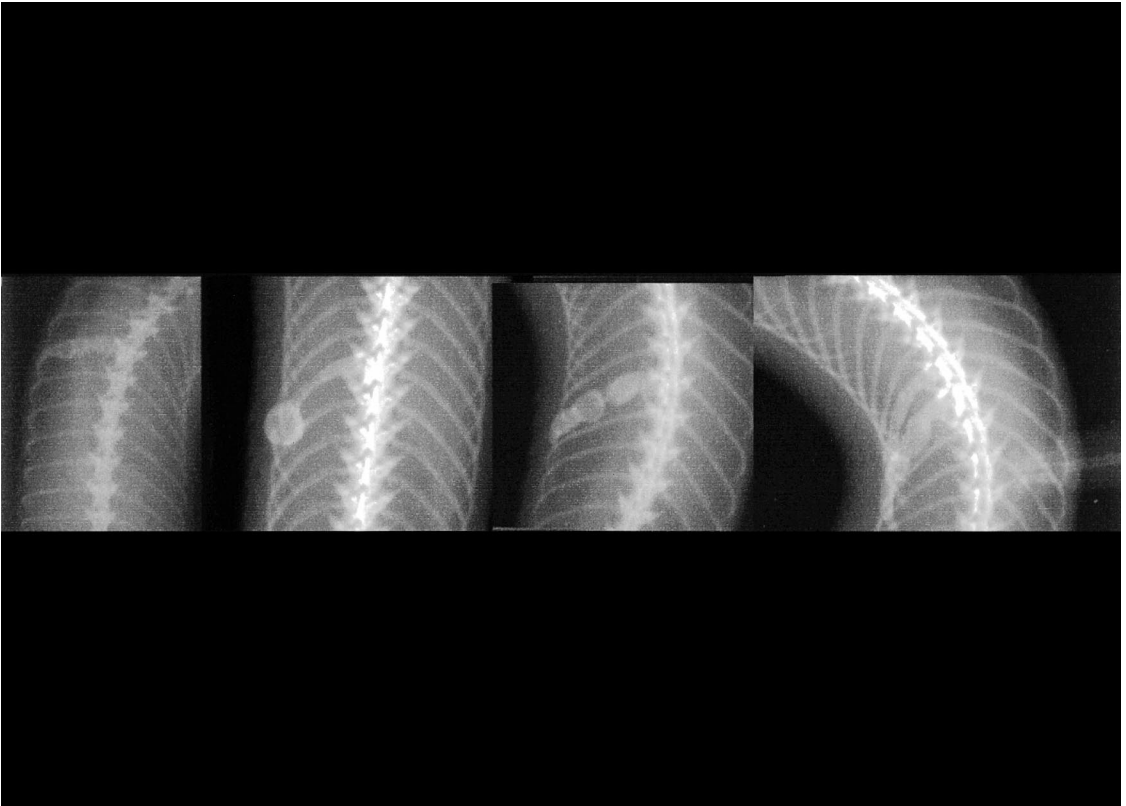


Figure 2. Composite image showing the varied appearance of osteomyelitis of the ribs of some ridgenose rattlesnakes. Bony reactions range from focal spherical enlargements (image second to the left) to linear enlargement of the rib along its entire length (image far right and left) or a combination of the two (image second from the right).

This was the result of mild bony proliferation associated with the dorsal lamina and edges of the vertebral bodies. The bony abnormalities did not increase in severity, and there was no evidence of bony lysis or expansile enlargement of the vertebral bodies characteristic of the snakes with osteomyelitis.

Pathology

Nine *C. w. willardi* died or were euthanatized during the study. One snake was too autolyzed for a full necropsy, but postmortem cultures were obtained. Two snakes died of necrotizing enteritis and had no bony lesions. *Salmonella arizonae* 50:K-Z was cultured from the colon of one of these snakes. The heterophilic, granulomatous, and necrotizing intestinal lesions were present in both the proximal small intestine and the colon.

Six snakes that died or were euthanatized during the study period had bony lesions. The necropsy results for these snakes are summarized in Table 1. The most common histopathologic lesions were in

the bones, intestinal tract, and gonads. The bony lesions showed a mixture of diffuse heterophilic-granulomatous osteomyelitis and discrete heterophilic granulomas. Osteonecrosis and sequestra were common in affected bones. In most cases, there was a development of periosteal woven bone in the form of an exostosis that contained diffuse or discrete foci of heterophilic-granulomatous inflammation. All the affected bones examined showed marked osteopenia of trabeculae and cortex.

Ova within the ovary and oviduct were commonly affected in female snakes. The lesions comprised heterophilic-granulomatous oophoritis and salpingitis, with occasional foci of necrosis, especially in the oviduct. The ova were often infiltrated by a mixture of heterophils and macrophages and contained numerous bacteria. *Salmonella arizonae* serotype 48:I-Z was cultured from one snake's testis. The testis had mild to moderate amounts of granulomatous infiltrates, but active spermatogenesis was present.

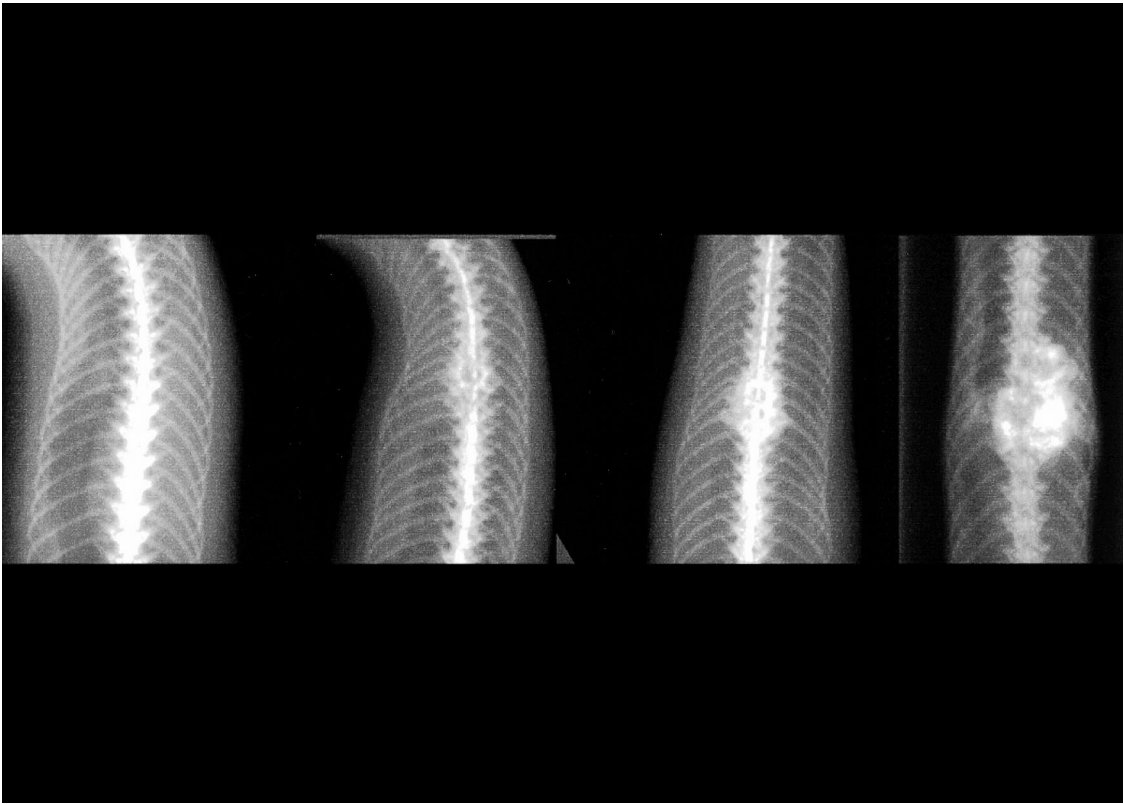


Figure 3. Composite image showing the varied appearance of vertebral osteomyelitis in ridgenose rattlesnakes. Severity of vertebral lesions depicted increases from left to right. Note the extensive new bone production associated with the affected vertebral bodies. There is also bony lysis within the medullary region of the affected vertebral bodies.

Bacteriology

Ten cloacal cultures were obtained in April 1996, 10 in November or December 1997, 12 in December 1998 and 10 in October 2000. Forty-one cloacal *S. arizonae* isolates, representing 13 serotypes, were obtained from 17 snakes (Table 2). At least one isolate was obtained from each snake cultured. Single isolates of *S. enterica* serotypes Aqua and Newport were the only nonarizonae *Salmonella* organisms isolated. *Salmonella arizonae* serotype 56:Z4,Z23 was isolated only once from a cloacal swab, from a snake that had no radiographic evidence of bone infection.

Since 1 September 1995, 12 extraintestinal *Salmonella* isolates, representing two serotypes, were obtained from six snakes. Six isolates were from bone lesions (from five different snakes). Three *Salmonella* isolates were also obtained from the gonads of snakes. The cultures of bone and lung from one snake were too contaminated to permit identification of any single organism, but *S. arizonae* was

isolated from a heavily overgrown culture of that snake's liver. *Salmonella arizonae* was also isolated from the liver of another snake with bony lesions. All extraintestinal *Salmonella* isolates except one were *S. arizonae* serotype 56:Z4,Z23 and were susceptible to amikacin, ampicillin, cefotaxime, chloramphenicol, enrofloxacin, gentamicin, and trimethoprim-sulfa. *Salmonella arizonae* serotype 56:Z4,Z23 was the only *Salmonella* serotype isolated from bone lesions. *Salmonella arizonae* 48:I-Z was isolated from the testis of one snake with bony lesions.

One snake with characteristic bone lesions died, and *Providencia rettgeri* was cultured from liver, heart blood, and two bone samples. No *Salmonella* spp. were cultured from any tissues.

Biopsies were not permitted on any snakes after 1995; therefore, no extraintestinal cultures were obtained from any living snakes with radiographic signs of osteomyelitis at the end of the study. *Salmonella* spp. were not cultured from any mouse fecal sample.

Table 1. A summary of the bone, intestinal, and gonad histological lesions and bacterial isolates from eight ridgenose rattlesnakes (*Crotalus willardi*) with osteomyelitis.

Snake (yr of death)	Histopathological diagnosis	Culture results
1004 (1991)	Biopsy: ribs and vertebral body: granulomatous osteomyelitis with multifocal heterophilic granulomas, osteonecrosis, and sequestra. Necropsy: rib, transverse process, and body of vertebrae: granulomatous and heterophilic osteomyelitis with endosteal granulomas.	<i>Salmonella arizonae</i> , 56:Z4,Z23, >1,000 colonies from bone biopsy
1003 (1991)	Biopsy: rib: granulomatous and heterophilic osteomyelitis. Necropsy: rib, transverse process, and body of vertebrae: granulomatous and heterophilic osteomyelitis with endosteal granulomas.	<i>Salmonella arizonae</i> , not serotyped, nine colonies isolated from bone biopsy
1005 (1997)	Rib: heterophilic and granulomatous osteomyelitis, osteonecrosis, and sequestra. Liver: multifocal, random granulomatous hepatitis and periportal fibrosis. Small intestine: acute, fibrinonecrotic enteritis.	<i>Salmonella arizonae</i> , 56:Z4,Z23, >100 colonies isolated from rib
368 (1997)	Rib exostosis: diffuse heterophilic and granulomatous osteomyelitis with osteonecrosis and sequestra.	<i>Providencia rettgeri</i> , >100 colonies isolated from bone, heart blood, and liver
1007 (1997)	Vertebral body: expansion of transverse processes by multifocal heterophilic granulomas with osteonecrosis and sequestra. Ovary: granulomatous and heterophilic oophoritis with multifocal lymphoid aggregates.	<i>Salmonella arizonae</i> , 56:Z4,Z23, >100 colonies isolated from liver, ovary, and bone
371 (1995)	Rib: diffuse granulomatous and heterophilic osteomyelitis with osteonecrosis and sequestra. Vertebral body: multifocal heterophilic granulomas and intervening diffuse granulomatous and heterophilic osteomyelitis. Ovary: multifocal heterophilic and granulomatous oophoritis with bacteria in ovum.	<i>Salmonella arizonae</i> , 56:Z4,Z23, >100 colonies from bone, >1,000 colonies isolated from ovum
911 (2000)	No histopathology performed on bone or gonad.	<i>Salmonella arizonae</i> , 56:Z4,Z23, >80 colonies isolated from vertebrae and rib, >10 colonies from gonad
912 (2000)	Rib: focal fragmentation and loss of cortical bone with abundant new (reactive, trabecular) bone formation. Multifocal, marked infiltrates of heterophils are present in the area of bone loss; heterophils in one region surround a focal aggregate of foamy macrophages (granuloma). Testes: multifocal, mild to moderate, interstitial granulomatous infiltrate, admixed with lesser numbers of heterophils, lymphocytes, and plasma cells is present.	<i>Salmonella arizonae</i> , 56:Z4,Z23, isolated from broth only from rib; one colony isolated from liver, 48:I-Z, >50 colonies isolated from testicle and intestine

Treatment

Two treatment regimens were initiated during the prospective study period. In the summer of 1996, three *C. w. willardi* with bony lesions, one *C. w. willardi* and one *C. w. silus* without lesions, and one *C. w. silus* without evidence of osteomyelitis

but with radiographic lesions of osteoarthritis were treated with oral enrofloxacin (Bayer Corp., Animal Health Division, Shawnee Mission, Kansas 66201, USA; 5 mg/kg, p.o., injected into dead feed mice). Because of their infrequent feeding schedule and occasional missed dosing, the snakes were medi-

Table 2. Serotypes of *Salmonella enterica* subspecies *arizonae* and other *Salmonella enterica* subspecies isolated from cloacal swabs of captive ridgenose rattlesnakes (*Crotalus willardi*).^a

Animal ID#	Osteomyelitis ^b	Serotype or isolate			
		Spring 1996	Fall 1997	Fall 1998	Winter 2000
368	+	48:1-Z	65:L, V-Z, rough O:K-Z35	dead	
369	+	NI	38:(K)-Z35	38:(K)-Z35	38:(K)-Z35
906	+	NC	NC	59:K-Z35	rough O:K-Z35
908	-	NC	50:K-Z	NI	50:K-Z
909	+	NC	48:1-Z35	NI	NC
910	-	NC	6,14:Z10-Z	48:1-Z, <i>Salmonella</i> Newport	48:1-Z
911	+	48:1-Z	NI	NI	65:L, V-Z
912	+	NI	48:1-Z	NI	48:1-Z
1005	+	38:(K)-Z35	dead		
1007	+	50:R-Z, 6,14:Z10-Z	dead		
1008	-	50:K-Z	dead		
1147	?	48:1-Z	NC		
1350	+	50:R-Z	38:(K)-Z35, 6,14:Z10-Z	dead	50:R-Z
1413	-	38:(K)-Z35	38:(K)-Z35	<i>Salmonella</i> Aqua, 50:R-monophasic	6,14:Z10-Z, 38:(K)-Z35
1414	-	38:(K)-Z35	NC	38:K-monophasic	38:(K)-Z35
1462	-	NC	NC	6,14:Z10-Z	38:(K)-Z35
1463	-	NC	38:Z35-monophasic	38:K-monophasic	56:Z4,Z23

^a NC, not cultured; NI, no *Salmonella* spp. isolated.^b Presence or absence of radiographic signs of osteomyelitis at anytime during study period (1995–2000).

cated only every 19–30 days, resulting in only four or five oral enrofloxacin treatments consumed by each snake. Twelve snakes, three with radiographic signs of osteomyelitis and nine without signs, were left untreated. Treated snakes showed no positive effects from therapy, and all three snakes with bony lesions showed progression of their bone disease between the 1995 and 1996 radiographic evaluations.

In April 1997, seven snakes (five *C. w. willardi* and one *C. w. silus* with radiographic lesions and one unaffected *C. w. silus*) were implanted with one or two ampicillin-impregnated methyl methacrylate beads. The beads were made by mixing 1 g of ampicillin powder (Amp-Equine®, SmithKline Beecham Pharmaceuticals, Philadelphia, Pennsylvania 19101, USA) with one 40-g package of methyl methacrylate powder (Surgical Simplex P®, Howmedica Inc., Pfizer Hospital Products Co., Rutherford, New Jersey 07070, USA) and delivering the mixture into a 8-Fr rubber feeding tube (Sovereign®, Sherwood Medical, St. Louis, Missouri 63103, USA). After hardening, the feeding tube was peeled off, and the rods were cut into 5-mm-long beads. These beads were gas sterilized. The areas over palpable bony lesions were swabbed three times with a tamed iodine solution (Betadine® solution, Purdue Fredrick Co., Norwalk, Connecticut 06850-3590, USA), and beads were aseptically placed subcutaneously directly over the bony lesions using a 12-gauge needle attached to a syringe designed for the delivery of identification transponders (Trovan Ltd., Douglas, Isle of Man, U.K.). Immediately after placement of the beads, the snakes were radiographed in an attempt to identify bead location. None of the snakes showed any changes in severity of bony lesions within 2.5 cm of the implants at the fall 1997 or fall 1998 radiographic evaluations. In the affected snakes, two implants had migrated >2.5 cm from the site of original placement, and one implant could not be found by palpation or radiology in the fall of 1997.

DISCUSSION

Salmonella sp. is one of the most commonly cultured Gram-negative bacteria from reptiles with osteomyelitis,¹¹ and *S. arizonae*-associated osteomyelitis is an endemic disease of *C. willardi* at the Knoxville Zoo Garden. *Salmonella arizonae* osteomyelitis has been observed in another collection of *C. willardi*,¹³ and *Salmonella* osteomyelitis has also been observed in similar captive rattlesnakes species (B. Raphael, Wildlife Conservation Society, 1999, pers. comm.). *Salmonella* spp. appear to be important pathogens for captive rattlesnakes.

Although many serotypes of *S. arizonae* were cultured from the cloaca of this colony's ridgenose rattlesnakes, serotype 56:Z4,Z23 was isolated only once from a cloacal swab. In contrast, this serotype was associated with osteomyelitis and nonenteric sites in all but one snake with bony lesions. It appears that this serotype has tropism for extraintestinal sites in this species. The serotyping of all *arizonae* isolates from Knoxville colony was important to our identifying this relationship.

The National Veterinary Services Laboratory's database of over 200,000 *Salmonella* isolates from 1996 through 2001 shows only three isolates of serotype 56:Z4,Z23 from sources other than present study's colony: a dog in Texas, an unknown source in California, and a snake in North Dakota (Kathleen Ferris, National Veterinary Services Laboratory, Ames, Iowa, pers. comm.). There is only one record of an isolation of this serotype from a person (source: heart blood) in a 10-yr review by the U.S. Center for Disease Control.²² The latter study showed serotype-specific infection patterns, suggesting distinct differences in virulence among *arizonae* serotypes.²²

Salmonella arizonae infections in humans have been associated with ingestion of rattlesnake meat. Dried rattlesnake meat is consumed as a folk remedy for a variety of illnesses in the southwestern United States and Mexico.^{1,16,19,21} *Salmonella arizonae* has been cultured from blood, joints, bones, and urine from immunocompromised individuals who ingested desiccated rattlesnake meat in powder or capsule form, and it has also been cultured from the powder and capsules.¹⁶ *Salmonella arizonae* serotype 56:Z4,Z23 was cultured from the blood of one individual who consumed rattlesnake products.¹ Snake-origin *Arizona hinshawii* has also been implicated as the cause of osteomyelitis in a person.⁷

The exact mode of transmission of *S. arizonae* 56:Z4,Z23 in the present study's colony is unclear. Ingestion is the primary method of transmission of *Salmonella* spp. in mammals and is probably the most important route in reptiles.¹⁰ Fecal *Salmonella* shedding in snakes is well documented; in this study, the frequency of *Salmonella* isolation from individual cloacal samples was 84%. There was, however, very little opportunity for fecal–oral transmission of the 56:Z4,Z23 serotype among the snakes in this colony because this serotype was identified from only one of 44 cloacal cultures. Additionally, the snakes in this colony usually cohabitated only very briefly. It is possible that the cages and the substrate became contaminated and acted as fomites, presenting the organisms to animals when they ingested substrate while eating. Alter-

nately, the feed mice may be a source of the pathogenic serotype; however, the cultures of fecal samples from the feeder mice cages failed to grow any *Salmonella* spp. It is interesting that all the three wild-caught snakes in the colony were affected, so these snakes may have been infected before capture.

Salmonella can be transmitted vertically in poultry. *Salmonella* spp., including the *S. arizonae* 56:Z4,Z23 of the present study, have been cultured from the reproductive tract of turtles and snakes, suggesting the potential for vertical transmission in reptiles as well.^{5,14} Additionally, *S. arizonae* has been cultured from the testis of three other captive *C. willardi*. (J. Jarchow, pers. comm.). Two wild-caught female snakes, with bony lesions in 1995, had produced a total of six offspring that were present in the colony at the beginning of the study. Two of these six snakes developed bony lesions during the study period. The other four snakes remained free of bony lesions. Five years may be too short a study period to thoroughly investigate vertical transmission because the snakes with radiographic lesions tended to be older than those without lesions.

The culture of *P. rettgeri* from bone and other tissues of one snake, and the failure to culture *S. arizonae* from it, indicates that at least one other organism may produce similar bony lesions or that heavy growth of *P. rettgeri* may interfere with the recovery of smaller numbers of tissue-associated *Salmonella* spp.

Osteoarthritis and osteomyelitis were distinguished radiographically in this study because biopsies were not obtained. A recent report of 15 cases of spinal lesions in snakes commented that a histologic distinction between spinal osteoarthritis and inflammatory osteomyelitis can be difficult.¹¹ It is possible that the two snakes diagnosed with spinal osteoarthritis may have harbored low-grade infections of the vertebral column. However, a distinctive feature of the infections in the present report was the presence of rib lesions in all confirmed cases. The relationship between the spinal osteoarthritis and osteomyelitis in this study will remain unclear until all snakes are necropsied.

Osteomyelitis can be difficult to treat. Treatments during the present study were unsuccessful. The antibiotic regimen used in 1990 and 1991 was based on personal experience and dosages were based, in part, on metabolic scaling. One snake was treated with parenteral ceftiofur (Naxcel®, Pharmacia and UpJohn, Kalamazoo, Michigan 49001, USA; 0.82 mg/kg i.m. q. 48 hr for 12 wk, then 1.5 mg/kg i.m. q. 48 hr for 8 wk). The other was treated with cef-

tiofur (1.0 mg/kg i.m. q. 48 hr for 8 wk, then 2.5 mg/kg i.m. q.48 hr for 8 wk) and trimethoprim-sulfadiazine (24% injectable, no longer available; 14.4 mg/kg, i.m. for 2 days, then q. 48 hr for 4 wk) concurrently. Neither therapeutic effort was successful.

The choice of the therapeutic regimens during the prospective study was based on the unsuccessful experience with the 1990–1991 cases and the desire to minimize handling of these venomous and occasionally fractious animals. As the disease appeared slowly progressive, causing few clinical signs, the use of novel therapeutic choices seemed, at least initially, appropriate. The veterinary staff were unaware that these snakes fed so infrequently, and it was not until treatment sheets were collected that it was discovered that so few oral treatments had been administered. The infrequent feeding and short feeding season were most likely the reasons for the failure of the oral medication regimen. Antibiotic-impregnated polymethyl methacrylate (AIPM) beads have been used to treat deep-tissue bacterial infections in a variety of domestic animals and offer the potential for long-term, localized treatment of infections.²⁰ No reports could be found on the use of AIPM beads in reptiles, and some unique aspect of the physiology of these snakes, such as low body temperature, may be responsible for the failure of this therapy. Additionally, although all *Salmonella* isolates were susceptible to ampicillin, it is possible that too little antibiotic was incorporated into the beads or that a different antibiotic may have been more successful. The loss or movement of the beads is another explanation for the lack of therapeutic effect.

Osteomyelitis associated with *S. arizonae* serotype 56:Z4,Z23 is an important disease of the Knoxville Zoo ridgenose rattlesnake colony. Lesions develop slowly over years and are difficult to treat. Stringent efforts to prevent infection, by limiting contact between affected and unaffected snakes, may be more successful than attempts to treat affected individuals. Further investigation of the possibility of vertical transmission of this pathogen also seems merited.

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