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Transovarian Passage, Visceral Distribution, and Pathogenicity of Salmonella in Snakes†

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Transovarian passage of salmonella was evaluated in snakes by cesarean delivery and subsequent bacteriological examination of fetuses. In all cases, the same *Salmonella* serotype was isolated from the feces of gravid females and their fetuses. The visceral distribution of salmonella in normal snakes was found to involve almost all visceral organs. Of nonenteric organs examined, salmonella was recovered most often from the livers and ureters. Experimental infections with *Salmonella typhimurium* and *Salmonella arizonae* were established by oral, intracardial, and intracoelomic routes. Animals infected orally shed the organism in feces, but did not develop humoral antibodies or any detectable adverse effect. Animals injected by the intracardiac and intracoelomic routes developed antibody titers of 1:256 to the respective salmonella serotypes, but remained normal throughout the experiment. On the basis of results, salmonella was regarded as an opportunistic organism in reptiles.

Reptilian salmonellosis has recently been reviewed (3). Although salmonella is a well-recognized pathogen in mammalian and avian species, its actual role as an etiological agent of disease in reptiles continues to be an area of controversy. As many as 94% of all reptiles harbor *Salmonella* spp. in their gastrointestinal tracts, without apparent adverse effect. No acceptable mode of transmission has been identified to account for this large percentage of *Salmonella* carriers. Stagnant breeding ponds, egg penetration, fecal contamination from other animals, and food sources have all been suggested as sources, but do not adequately explain the high infectivity rate in wild, free-ranging reptiles (3). Kaufman et al. (8, 9) documented the isolation of *Salmonella* from ovarian tissue and the egg of a turtle, suggesting transovarian passage, but these findings were not reproducible.

The isolation of *Salmonella* from nonenteric sites in reptiles is not uncommon. *Salmonella arizonae* (*Arizona hinshawii*) appears to have a greater tendency for visceral migration, and some early investigators suggested pathogenicity based exclusively on their isolation from nonenteric sites (3). Lesions are rarely, if ever, observed. Salmonella gastroenteritis and septicemia have been described; in all cases, poor husbandry, maladaptibility, and malnutrition may have been predisposing factors (3). Experimental infections in snakes (7), tortoises (5), and lizards (6) by oral, subcutaneous, intracardiac, and intraperitoneal inoculations have all failed to produce disease. Despite the failure of experimental infections and continued suggestions that salmonella is an opportunistic organism in reptiles (1, 3), reports continue to appear implicating salmonella as a primary agent of reptilian disease (10).

The present investigation was designed to determine (i) the role of transovarian passage of salmonella, (ii) the visceral distribution of salmonella in normal snakes, and (iii) the effects of oral, intracardiac, and intracoelomic infection of salmonella.

MATERIALS AND METHODS

Animals. Snakes were captured in several areas of Connecticut and New York state and housed separately at room temperature. Snakes of a given species were usually captured from the same area. All brown snakes and garter snakes were naturally infected with *Salmonella muenchen* and *Salmonella carrau*, respectively, before experimental inoculation. Animals appeared clinically normal, and antibodies to the natural or experimental serotypes were not detected before experimental inoculation. Treatment was not attempted. Natural serotypes were distinguished from experimental organisms by serological techniques (salmonella antisera; Difco Laboratories, Detroit, Mich.). Fecal specimens were collected by cloacal swabbing for at least 5 consecutive days. Cesarean sections were performed on four Northern water snakes (*Natrix sipedon* subsp. *sipedon*) as previously described (2). The total of 92 fetuses were examined. Five brown snakes (*Sonnoria dekayi*) and five garter snakes (*Thamnophis sirtalis*) were subjected to euthanasia

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and aseptically dissected, and each organ was cultured for bacteriological examination. Experimental infections were established in 28 snakes by either the oral, intracardiac, or intracoelomic route. Oral infection with *S. arizonae* was established in two brown snakes, and two other brown snakes were orally inoculated with *Salmonella typhimurium*. For intracardiac and intracoelomic inoculations, three garter snakes and three brown snakes were inoculated with *S. typhimurium*, and three brown snakes and three garter snakes were inoculated with *S. arizonae*. Orally inoculated snakes were killed 28 days post-inoculation, and one brown snake and one garter snake from the intracardiac and intracoelomic group were killed 15, 30, and 45 days after inoculation. Seven snakes of each species were left uninoculated, and one snake from each species was killed whenever experimental animals were examined. Whole blood and serum samples were obtained from the caudal vein. Sections of major organs were fixed in buffered 10% Formalin, processed for routine paraffin embedding, sectioned at 6 μm, and stained with Harris hematoxylin and eosin, and Brown and Hopps modified Gram stain.

Organisms. *S. typhimurium* was obtained from the National Animal Disease Laboratories, Ames, Iowa. This organism had been isolated from heart blood of an Eastern kingsnake (*Lampropeltis getulus*) (1). *S. arizonae* 9a9b:31,33,36 was obtained from the liver of a reticulate python (*Python reticulatus*) submitted for postmortem examination at our laboratory.

Culture procedures. All media were obtained from Difco Laboratories, Detroit, Mich., except for the gram-negative broth, which was obtained from BBL Microbiology Systems, Cockeysville, Md. Feces and gastrointestinal sections were cultured for salmonella by standard methods with selenite brilliant green broth, gram-negative broth, MacConkey agar, xylose-lysine-deoxycholate agar, and brilliant green agar. Organisms from visceral organs and fetuses were isolated by using gram-negative broth, tryptose broth, xylose-lysine-deoxycholate agar, and blood agar. Blood was examined by using tryptose broth and blood agar. Organisms were identified by standard biochemical testing or a commercially available enteric identification system (API-20E; Analytab Products, New York, N.Y.) or by both. *Salmonella* spp. were serotyped by the Connecticut State Department of Health, Hartford, or the Center for Disease Control, Atlanta, Ga. Cultures for experimental infections were grown in tryptose broth for 18 to 24 h, sedimented by centrifugation at 4,340 × *g*, and resuspended in phosphate-buffered saline to a concentration of 10^{12} viable cells per ml. Approximately 10^{12} cells were inoculated for each experimental infection.

Serology. Whole cell antigens were prepared by growing the organism on tryptose agar, harvested, fixed in 0.25% Formalin, and standardized by spectrophotometry at 600 nm. Sera were preserved with 1:10,000 merthiolate and diluted with buffered NaCl solution. Antibody titers were determined by standard agglutination techniques.

RESULTS

Transovarian passage. Results of culture from feces of gravid female snakes and their fetuses are presented in Table 1. *Salmonella* was isolated from three snakes, and the same serotype was isolated from their respective fetuses. In the case of one of the snakes excreting *S. muenchen* and *S. arizonae*, only *S. muenchen* was recovered from the fetuses. *Salmonella* was not isolated from one of the gravid females, but *S. arizonae* was isolated from all of her fetuses. In all cases, organisms isolated from gravid snakes were biochemically and serologically identical to those isolated from their fetuses.

Visceral distribution. *Salmonella* organisms were consistently isolated from all segments of the gastrointestinal tract posterior to the esophagus, and occasionally from other visceral organs (Table 2). The liver was a common site of isolation, with 80% of the snakes having positive cultures. Ureters were positive in 70% of the snakes, and kidneys were positive in 30%. *Salmonella* was isolated from the ovaries of two garter snakes undergoing oogenesis at time of necropsy. The uterus, testes, heart, and lungs were negative for *Salmonella* in all snakes. The garter snakes in this study were naturally infected with *S. carrau*, and the brown snakes were infected with *S. muenchen*. On microscopic examination of visceral organs, there were no lesions, and bacterial organisms could not be demonstrated. Serological techniques failed to detect any antibodies to the salmonella isolates.

Experimental infections. *Salmonella* organ-

TABLE 1. Salmonellae recovered from the feces of gravid female snakes and organisms isolated from their fetuses[a]

Snake	Fecal isolates	Fetal isolates
1	*S. enteriditis* serotype *muenchen* *S. arizonae* 20:23;21	*S. enteriditis* serotype *muenchen* *Klebsiella oxytoca*
2	*S. enteriditis* serotype *carrau* *S. arizonae* "O" rough:31;33	*S. enteriditis* serotype *carrau* *S. arizonae* "O" rough:31;33
3	*S. arizonae* 20:23;21	*S. arizonae* 20:23;21 *Providencia rettgeri*
4		*S. arizonae* 24:33 (monophasic)

[a] The numbers after the species names represent subspecies based on somatic (O) and flagellar (H) antigens.

TABLE 2. Visceral distribution of *Salmonella* in normal snakes

Organ	No. of *Salmonella* isolations in[a]:	
	Garter snakes	Brown snakes
Esophagus	0	1
Stomach	4	2
Small intestine	5	3
Colon	4	3
Cloaca	3	4
Liver	5	3
Ureters	3	4
Kidneys	0	3
Ovaries	2	0

[a] Number of isolations per 5 organs tested.

isms were repeatedly isolated from feces of orally inoculated snakes. Adverse effects were not observed. At postmortem examination, there were no gross or histological lesions, and antibodies to *Salmonella* did not develop during the experiment. Nonenteric cultures were negative except for the livers, where the natural serotype of *S. meunchen* was isolated from one snake and the experimental serotype of *S. arizonae* was isolated from another. The small intestine, colon, and cloaca were positive for the experimental serotypes.

In animals inoculated by the intracardiac route, blood cultures were positive for the respective experimental serotypes throughout the experiment. Two brown snakes, one infected with *S. typhimurium* and the other infected with *S. arizonae*, developed clinical signs 6 days after inoculation. There was a progressive loss of righting reflex until death on days 8 and 10 post-inoculation. Lesions were limited to the heart; however, salmonella was recovered from all organs. Cardiac lesions consisted of an acute suppurative pericarditis and myocarditis, apparently the result of injection technique.

All other snakes inoculated by the intracardiac route remained normal throughout the experiment. Immunoglobins developed by day 12 post-inoculation and rose to a maximum titer of 1:256. Colony-forming units developing on blood agar from a single drop of blood (approximately 10 μl) decreased from an average of 45 to an average of 15 colony-forming units at the termination of the experiment. In all cases, regardless of the time of euthanasia, *S. typhimurium* or *S. arizonae* was isolated from all visceral organs, but not from feces. Lesions did not develop in these snakes, but gram-negative bacilli were present within macrophages of the liver.

Animals inoculated by the intracoelomic route remained normal throughout the experiment. Adverse effects were not apparent. *Salmonella* was recovered from blood by day 6 post-inoculation, but became negative by day 25. Antibodies developed by day 14 and rose to a maximum titer of 1:256. *Salmonella* was isolated from all visceral organs of animals killed at 15 days, but gross or histological lesions were not present. By 30 days, there was a mild chronic inflammation of the coelom, consisting of a diffuse accumulation of mononuclear leukocytes and granulocytes. After 45 days post-inoculation, there was a decrease in inflammatory cells and an increase in fibrotic tissue surrounding the coelomic cavity. When selective and enrichment media were used, all organs were positive for *Salmonella*; however, organisms could not be isolated on primary plating on blood agar. Similar to the results obtained in animals inoculated by the intracardiac route, experimental *Salmonella* serotypes could not be isolated from feces of snakes infected by the intracoelomic route. Lesions were not observed in control animals, and all tissues were negative for *S. typhimurium* and *S. arizonae*.

DISCUSSION

Results of cesarean section and subsequent bacteriology have shown that snake fetuses are not sterile; they may be born infected with salmonella or other enteric organisms. The isolation of *Salmonella* from whole fetuses does not necessarily mean that they will excrete the organisms. Results of experimental infections presented here show that snakes with systemic or intracoelomic infections do not excrete salmonella in feces; therefore, unless fetal infection were enteric, salmonella might not be excreted. The failure to isolate *S. arizonae* from one of the gravid female snakes which had positive fetuses is not unexpected, since variability in fecal excretion rates often results in consecutive negative cultures (3).

Salmonella can occur in almost any visceral organ of snakes that appear clinically healthy. The livers and urinary tracts were the most common nonenteric organs infected. A single brown snake was found to be negative for *Salmonella* which was an unusual finding, since all brown snakes were captured from the same small area and presumably were exposed to the same opportunity for infection. Perhaps some snakes develop resistance to infection; it has been observed that different *Salmonella* serotypes are rarely transmitted from one reptile to another (3). A common source of infection is suggested by the occurrence of *S. carrau* in garter snakes and *S. muenchen* in brown snakes, since snakes of the same species were captured in the same habitat. Of the snakes examined in this study, 55 of 56 snakes (98%) were naturally infected with salmonella.

The results of my experimental infections were similar to those originally reported by Dimow and Slawtschew (5–7). Orally infected animals excreted *Salmonella* in the feces, but did not develop antibodies or any illness. Animals inoculated by the intracoelomic and intracardiac routes developed antibodies and a mild or undetectable inflammatory response. Antibody titers appeared high in view of the fact that environmental temperatures were maintained at approximately 25°C, a temperature known to inhibit humoral antibody formation (4). The reduction in colony-forming units with time in animals inoculated by the intracardiac route suggests a declining septicemia or partial elimination of infection. Cardiac lesions which occurred in two of the experimental animals were probably traumatic and incidental, more related to how the injection was given than to the pathogenicity of salmonella.

The results reported here suggest that *Salmonella* is an opportunistic organism and possibly part of the normal bacterial flora in reptiles. Oral, systemic, and intracoelomic infections were established at immunosuppressive temperatures and at cell concentrations 10^6 times the infective dose for humans (3), and yet, overt disease was not observed. It would thus appear that snakes are very resistant to disease caused by bacteria of the genus *Salmonella*. The distinction between infection and disease is of paramount importance. Clinical salmonellosis in reptiles probably only occurs as a manifestation of the maladaptability syndrome, a condition resulting from anorexia, malnutrition, emaciation, breakdown in immune mechanisms, and secondary invasion by opportunistic organisms. I have often observed this type of secondary salmonellosis at our laboratory. The significance of recovering these organisms at necropsy needs to be tempered by appreciation for the multifocal salmonella distribution in normal snakes. Caution needs to be exercised in clinical and postmortem diagnosis of salmonellosis in reptiles.

Since 1944, reptiles have been shown to be a reservoir of salmonellosis and have been implicated in transmitting the agent to humans, livestock, and companion animals. The importance of the high salmonella carrier rate in reptiles on the ecology of salmonella and zoonosis has been previously discussed (3).

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LITERATURE CITED

1. **Cambre, R. C., D. E. Green, E. E. Smith, R. J. Montal, and M. Bush.** 1980. Salmonellosis and arizonosis in the reptile collection at the National Zoological Park. J. Am. Vet. Med. Assoc. **177**:800–803.
2. **Chiodini, R. C., and J. P. Sundberg.** 1980. Cesarean delivery in the snake. J. Vet. Med. Sm. Anim. Clin. **76**:1605–1608.
3. **Chiodini, R. J., and J. P. Sundberg.** 1981. Salmonellosis in reptiles: a review. Am. J. Epidemiol. **113**:494–499.
4. **Cohen, N.** 1971. Reptiles as models for the study of immunity and its phylogenesis. J. Am. Vet. Med. Assoc. **159**:1662–1671.
5. **Dimow, I.** 1966. Versuche, landschildkroten der arten *Testudo graeca* und *Testudo hermanni* mit salmonellabakterien zu infizieren. Zentralbl. Bakteriol. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **199**:181–184.
6. **Dimow, I.** 1966. Versuche zur kunstlichen infektion von eidechsen (*Lacerta muralis*) mit salmonella und arizonabakterien. Zentralbl. Veterinaermed. Reihe B **13**:587–590.
7. **Dimow, I., and R. Slawtschew.** 1967. Versuche der experimentalinfiziering von schlangen *Vibera ammodytes ammodytes* mit salmonella-und arizonabakterien. Pathol. Microbiol. **30**:495–497.
8. **Kaufmann, A. F., M. D. Fox, G. K. Morris, B. T. Wood, J. C. Feeley, and M. K. Frix.** 1972. Turtle associated salmonellosis. III. The effects of environmental salmonellae in commercial turtle breeding ponds. Am. J. Epidemiol. **95**:521–528.
9. **Kaufmann, A. F., and Z. L. Morrison.** 1966. An epidemiologic study of salmonellosis in turtles. Am. J. Epidemiol. **84**:364–499.
10. **Marcus, L. C.** 1981. Veterinary biology and medicine of captive amphibians and reptiles. Lea & Febiger, Philadelphia.