Enterobacteriaceae Isolated from Iguanid Lizards of West-Central Texas

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The prevalence of members of the family Enterobacteriaceae in the intestines of seven species of iguanid lizards native to west-central Texas was determined. Of the 67 lizard specimens examined, 48.7% were infected with Salmonella and 9% were infected with Salmonella arizonae. Two lizard species (Sceloporus olivaceus and Crotaphytus collaris) were shown to have a 100% prevalence of Salmonella.

Salmonella and Salmonella arizonae have long been known to occur in reptiles. In 1939, bacteria described as Salmonella spp. were isolated from a horned lizard (Phrynosoma solare), a Gila monster (Heloderma suspectum), and a chuckawalla (Sauromalus ater) (4). These cultures were later determined to be S. arizonae (6). The next isolation of Salmonella from a reptile was from a bull snake (Pituophis catenifer) in 1944 (8). The presence of these enteric pathogens was known as early as 1939, but the extent of their occurrence and their relationship to human disease were not known at that time.

Although Salmonella was reported from turtles as early as 1946 (14), such isolations were not recognized as important until 1953, when a turtle was implicated as the vector in a case of human salmonellosis (3). The fact that Salmonella could be transmitted to humans by turtles resulted in extensive research on the relationship between Salmonella and reptiles, especially turtles.

Lizards have also been found to harbor Salmonella in their intestines (5, 7, 10, 11, 13, 17). In a few cases the transmission of Salmonella from lizards to humans has been documented (1, 2). The other intestinal Enterobacteriaceae of reptiles in general and lizards in particular seem largely to have been ignored, possibly because they seem to lack the definite connection which Salmonella has to human disease.

The present study was initiated for two reasons. First, none of the lizard species native to west-central Texas had ever been cultured for Salmonella. Second, very little data existed on the occurrence of other members of the Enterobacteriaceae in the intestines of any lizard species. Thus, the purpose of this study was to isolate and identify all species of the family Enterobacteriaceae from the intestinal tracts of the iguanid lizards of west-central Texas and to determine the prevalence of each enteric species in each lizard species examined.

Materials and Methods

Lizard specimens. The seven species of iguanid lizards that commonly occur in Tom Green and Irion Counties of Texas were cultured for enteric bacteria. The lizard species studied were: the rusty lizard (Sceloporus olivaceus), the spiny crevice lizard (Sceloporus pointsettii), the eastern fence lizard (Sceloporus undulatus), the Texas horned lizard (Phrynosoma cornutum), the collared lizard (Crotaphytus collaris), the Texas earless lizard (Cophosaurus texana), and the tree lizard (Urosaurus ornatus). All specimens of the protected species of P. cornutum were collected under a Texas scientific collecting permit.

All the lizard specimens were captured alive between May and October 1976. The lizards were captured from locations throughout the two counties. They were not from a single population except for S. pointsettii, which occurs in a single area in these counties. Only apparently healthy native lizards were used.

Upon capture each specimen was placed in a separate sterile container for transport to the laboratory and to prevent bacterial cross-contamination between specimens. In the laboratory each lizard was sacrificed and cultured within 48 h of capture to minimize any change in the intestinal microflora from that found in the wild state.

Isolation. Lizard specimens were dissected under strict aseptic conditions in a hood sterilized by ultraviolet light (Lab Con Co., Kansas City, Mo.) with dissecting instruments sterilized by autoclaving at standard conditions. The intestine was removed from just below the stomach to just above the anus. The entire intestinal tract was then placed in a sterile petri dish and cut into fine pieces with a sterile scalpel. These pieces were aseptically transferred to a sterile tissue grinder containing 5 ml of sterile polikilothermal saline solution (0.65% NaCl), and the contents were finely ground.

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A small portion of this intestinal suspension was streaked onto the primary plating media, MacConkey agar (Difco Laboratories, Detroit, Mich.) and Hektoen enteric agar (BBL Microbiology Systems, Cockeysville, Md.). One milliliter of this suspension was also added to 9 ml of tetrasionate broth (BBL) for enrichment culture of Salmonella. These cultures were incubated at 35°C for 24 h. After incubation the enrichment broth was streaked onto another MacConkey agar plate and another Hektoen enteric agar plate, which were then incubated at the same conditions.

After the incubation periods, the two MacConkey agar plates and the two Hektoen enteric agar plates were carefully examined for different bacterial colony types. A representative isolated colony of each type was found and picked off each plate, aseptically transferred to a Trypticase soy agar slant (BBL), and allowed to grow at 35°C for 24 h.

Preliminary identification. An oxidase test was performed on each isolate, using 1% aqueous tetramethyl-p-phenylenediamine dihydrochloride (Marion Scientific Corp., Kansas City, Mo.). Since all Enterobacteriaceae are oxidase negative, any isolate that was oxidase positive was discarded. Each oxidase-negative culture was then presumptively identified by the method of Johnson et al. (12).

The results of the preliminary identification tests of all isolates obtained from the same lizard were compared. From those cultures from a single lizard that were found to be identical on every reaction, one was selected for final biochemical identification. Any culture that was different on even a single reaction was also subjected to final biochemical identification.

Biochemical identification. The biochemical tests were done with the API 20 Enterobacteriaceae system (Analytab Products, Inc., Plainview, N.Y.). The API 20 Enterobacteriaceae system is very accurate in identifying enteric bacteria (15, 18, 19) and even more accurate when the API Profile Register is applied to the results (16).

Serological confirmation. All isolates identified as species of Salmonella were confirmed serologically with Salmonella somatic polyvalent antiserum (BBL). When agglutination occurred in the polyvalent antiserum, it was considered a positive test. When both a positive serological test and a characteristic biochemical profile were obtained for an isolate, this culture was considered to be Salmonella.

RESULTS

In this study 316 enteric isolates were cultured and identified from 67 iguanid lizard specimens representing seven lizard species. Table 1 indicates the number of specimens positive per total specimens of a lizard species for a particular enteric species along with the percent positive of that lizard species. When more than one isolate of the same bacterial species was cultured from a single lizard specimen, these isolates were considered as a single isolation of that bacterial species from that particular lizard.

Salmonella was isolated from 47.8% of the 67
lizard specimens. It was cultured from all lizard species except *U. ornatus*. Two lizard species, *S. olivacea* and *C. collaris*, were found to have *Salmonella* in all the specimens cultured. *S. arizonae* was isolated from 36.4% of the *S. poiney* specimens and 25.0% of the *C. texana* specimens.

*Enterobacter cloacae* was cultured from all seven lizard species and was found in 41.8% of the lizard specimens. *Escherichia coli* was isolated from 19.4% of the lizards.

**DISCUSSION**

Roggendorf and Muller (17) in Germany attempted to determine the prevalence of members of the family *Enterobacteriaceae* in 39 lizards of different species. They found more *Escherichia* (50%) than was found in the present study (19%). In addition, more *Enterobacter* was found in the lizards from Texas. On the other hand, the German study found higher incidences of *Citrobacter*, *Klebsiella*, and *Proteus*. They also found low occurrences of *Proteus inconstans* and *Edwardsiella*. These organisms were not isolated from the Texas lizards. Roggendorf and Muller found that 50% of the 39 lizards that they studied harbored *Salmonella*, whereas in the present study 47.8% of the 67 lizards contained this pathogen. *S. arizonae* was cultured more frequently from the Texas lizards (9%) than from the lizards in Germany (3%).

The percent infection of lizards with *Salmonella* reported in the literature varies greatly. The following values have been reported: 12% by Hinshaw and McNeil (9), 62% by Lee and Mackerras (13), 11.2% by Collard and Montefiore (5), 77% by Iveson et al. (11), 2 and 9% by Hamel and McInnes (7), and 8% by Hoff and White (10). Although all of these studies were concerned with the occurrence of *Salmonella* in lizards, they are not directly comparable.

The differences in percent infection with *Salmonella* may reflect several variables. Some of these studies were done with captive or zoo specimens, whereas other studies were done with wild or native lizards. Lizards in these two different conditions are likely to have different bacterial flora. Many studies have lumped the different lizard species together when compiling results. The present study and others (5, 7) have shown great differences in intestinal flora, both qualitatively and quantitatively, between different lizard species. Many different methods of isolation and identification have been used and are also likely to affect the results.

This study attempted to determine the prevalence of all *Enterobacteriaceae* in all species of iguanid lizards occurring in a particular geographical area. The identity and prevalence of the enteric bacteria present in native lizards must be established before the question of their significance to the lizards or to humans can be considered. The present study has shown which members of *Enterobacteriaceae* are present and in what prevalence in the iguanid lizards of Tom Green and Irion Counties of Texas.

In conclusion, lizards are a significant reservoir of *Salmonella* and *S. arizonae* in west-central Texas. Of the 67 lizards examined, 47.8% harbored *Salmonella* and 9% yielded *S. arizonae*. None of these lizards had any apparent symptoms of salmonellosis. In two species of lizards (*S. olivacea* and *C. collaris*), *Salmonella* was cultured from every specimen collected. These isolations suggest that *Salmonella* may be normal flora for these two lizard species.

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**LITERATURE CITED**


