Salmonella in Reptiles

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The popularity of reptiles as pets and exhibit animals in zoologic gardens continues to increase. As long as these animals remain popular, veterinarians will be asked to field questions regarding the *Salmonella* in reptiles. This article will present an overview of the taxonomy of *Salmonella* and the virulence factors associated with these bacterial organisms. Microbiological culture is the standard method for isolation of *Salmonella*, and the various culture techniques used to isolate *Salmonella* will be presented. *Salmonella* is routinely isolated from apparently healthy reptiles, however, *Salmonella* can also cause significant pathology in reptiles. This article will present an overview of the current literature. The major concern facing most veterinarians working with reptiles is handling reptile-associated salmonellosis (RAS). A historical review of RAS will be discussed.

Key words: Reptile, *Salmonella*, zoonosis.

Taxonomy

Salmonella is a gram-negative, facultative anaerobe from the family Enterobacteriaceae. Most of the described species are pathogenic.[1] *Salmonella* are typically nonlactose fermenters, although *S. arizonae* routinely ferment this sugar.[2] Hydrogen sulfide is a hallmark of this group of bacteria, although several strains of *S. paratyphi* and *S. cholerasuis* do not produce it.[2] Several different sugars may be used as a carbon source, although *S. arizonae*, which is routinely isolated from reptiles, uses malonate as a primary carbon source.[2]

The DNA of *S. typhimurium* is approximately 90% homologous to *Escherichia coli* DNA.[3] This homology allows for chromosomal transfer between the 2 organisms. The acquisition of plasmids that confer antibacterial resistance or biochemical characteristics are frequently transferred by this mechanism. Veterinarians should recognize that bacteria can readily transfer resistance mechanisms and inappropriate antimicrobial use in veterinary medicine contributes to the growing problem of antimicrobial resistance.

The DNA homology shared by organisms within the family Enterobacteriaceae has led to confusion among microbiologists regarding taxonomy. The current classification of the genus *Salmonella* includes 2 species, *S. enterica* and *S. bongori*, and 6 subspecies of *S. enterica* including *enterica* (subspecies I), *salamae* (subspecies II), *arizonae* (subspecies III), *diarizonae* (subspecies IIIb), *houtenae* (subspecies IV), and *indica* (subspecies V).[4] Subspecies I is routinely isolated from humans, whereas the other subspecies are frequently isolated from poikilotherms and the environment. Serotype identification for subspecies I are designated by the geographic location from which the serotype was initially isolated.[5] The serotypes of the remaining subspecies are designated by their antigenic formula. There are currently 2,435 described *Salmonella* serotypes and the majority (1,435) are classified under subspecies I.[4] The *arizona* and *diarizonae* subspecies have 94 and 321 serotypes, respectively.

There are 3 antigens used to serotype *Salmonella*, including the O (heat stabile somatic) antigens, Vi (heat labile capsular) antigen, and H (flagellar) antigens. The Kauffman-White scheme is used to list the antigenic formulae, which are expressed as O antigen(s), Vi antigens (when present), H antigen(s) (phase 1), and H antigen(s) (phase 2, when present).[5] Organisms with O antigens in common are placed into similar O groups and arranged alphabetically by H antigens. The specificity of the O factors is determined by the composition of the polysaccharide and may be altered by mutation or bacteriophage conversions.[6]

Lysogenization by phages may change the O antigen formulae for an organism. Phages can be differentiated from one another serologically

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and the factors associated with phage conversion are underlined when using the Kaufmann-White scheme. Currently, phage typing is limited to a few serovars, including *S. typhi, S. typhimurium, S. dublin, S. enteritidis, S. heidelberg,* and *S. schottmuelleri.*[7] Identification of these phage types may be important in epidemiologic studies. Antibiotic resistance patterns, biotyping, and plasmid profile analysis are other diagnostic techniques that may be used to identify a *Salmonella* organism beyond the serovar. These techniques are routinely used in epidemiologic studies to determine the source of a pathogen in a disease outbreak.

Salmonella Pathogenesis

Virulence Factors

Infection in animals and humans with *Salmonella* may result in serious disease or give rise to a reservoir for other species and contacts within that environment. The interaction of *Salmonella* with a host gives rise to a number of clinical presentations including inapparent infection, recovered carrier state, enteritis, septicemia, and combinations of disease syndromes.[7]

A number of potential virulence factors have been identified in *Salmonella* to enable the organism to invade and infect a host. The majority of *Salmonella* are motile using a flagella to contact enterocytes, which are natural host cells.[8] Flagellae may be organized into a bundle at one pole, creating a smooth swimming activity, or project from the organism, resulting in a tumbling pattern. *S. typhimurium* with the single flagella are more invasive than those that project from the organism.[9] Virulence factors, such as flagella, are the basis of immunologic diagnostic tests that offer sensitivity, specificity, and rapid identification after enrichment culture. An enzyme-linked immunosorbent assay (Salmonella ELISA 96/1) offered by Bioline (Vejile, Denmark) uses affinity purified rabbit antibody specific to *Salmonella* spp. flagellar antigens.

Siderophores are iron scavenging chelators produced by bacteria when iron concentrations are low within a host. Bacteria excrete siderophores into the host tissues to chelate iron, which is incorporated into the bacteria through specific outer-membrane receptor proteins produced in response to a low concentration of iron.[10] The exact role for scavenging systems in an intracellular pathogen is under debate. *Salmonella typhimurium* produces a phenolate siderophore (enterochelin) considered to be a virulence factor, however, it may not be essential for virulence.[11]

Lipopolysaccharide (LPS) is a major determinant of virulence in *Salmonella.* The LPS is composed of an internal Lipid A embedded in the outer membrane core region and an antigenic O region.[7] Organisms that lack the O core region are classified as "rough" mutants and are less virulent than organisms with an intact O core region ("smooth"). The lack of the O core region results in an increased susceptibility of the rough mutant. The O core region protects the organism by increasing the distance between the cell membrane and complement-mediated mechanisms. The chemical composition of the O antigen is also an important consideration in activating complement by the alternate pathway; this may affect the rate of phagocytosis by macrophages.[12] The endotoxic properties of LPS also contribute to virulence. Bacterial LPS is capable of stimulating a cascade of inflammatory mediators and immunoregulatory cytokines, leading to vascular damage and thrombosis.[13] Many of the clinical signs associated with *Salmonella* infection in humans, including cramps and fever, are attributed to LPS.

Many enteric pathogens rely on invasin genes to penetrate host enterocytes. Invasin genes are believed to mediate an extensive actin rearrangement in the host cell, resulting in a distortion of the cell membrane, enabling the organism to invade. Disruption of the invasion A gene in a strain of *S. typhimurium* prevents the organism from invading enterocytes. The invasion genes (A-H) are highly conserved among the *Salmonella.* The conservation of these genes across the genus has resulted in the development of improved molecular techniques (polymerase chain reaction [PCR]) to diagnose *Salmonella* infection.[14]

Isolation of *Salmonella*

Microbiologic culture is conventionally used to detect *Salmonella* in various tissues and excretions.[1] The standard microbiologic method incorporates a highly selective enrichment broth that inhibits genera other than *Salmonella.* Pre-

enrichment media, containing lactose, are used to provide additional energy to injured bacteria and increase the probability of recovery. Siebling et al[15] reported that pre-enrichment with lactose broth before enrichment in tetrathionate broth reduced the recovery of *Salmonella* from turtles. The 4 most commonly recommended enrichment media are tetrathionate broth with or without brilliant green, modified semisolid Rappaport-Vassiliadis, and Selenite broth. Enrichment media should be selected based on the subspecies of *Salmonella* to be isolated. Tetrathionate may inhibit the multiplication of certain *Salmonella* serotypes if the inoculum is small.[16] Selenite F is toxic to *Salmonella* Cholerae-suis.[17] Rappaport enrichment broth has been used to consistently isolate different subgenera of *Salmonella*, however, only 27% (3/11) of the strains of subgenus III were subcultured from Rappaport.[18] Samples are routinely incubated under aerobic conditions at 37°C in the enrichment media for 18 to 24 hours. After enrichment, the sample is plated onto a selective medium for isolation. Media, such as MacConkey agar and eosin methylene blue, are of low selectivity. Media of intermediate to high selectivity include desoxycholate citrate, xylose-lysine-desoxycholate, xylose-lysine tergitol 4, Rambach, and *Salmonella-Shigella* agar. Suspect colonies may be inoculated onto a selective screening media such as lysine iron agar and triple iron agar. Lysine iron agar is used because *Salmonella* decarboxylate lysine and produce hydrogen sulfide (H_2S). On the triple iron agar media, *Salmonella* ferment glucose and produce gas and H_2S. There are other biochemical tests or a slide agglutination test with antisera for *Salmonella* O groups that may be used to confirm *Salmonella*.

The microbiological techniques used to isolate *Salmonella* from reptiles are based on those developed for *Salmonella* subspecies from endothermic animals. *Salmonella* isolates from reptiles are commonly classified in subspecies III and IIIb and may use different biochemical pathways than other subspecies. Comparative studies to evaluate isolation success for reptile *Salmonella* serotypes using available enrichment broths and selective media are limited.[19,20]

A study was performed at the Bristol Zoological Gardens to evaluate isolation techniques using the feces material from 20 different reptile species.[19] Approximately 2 g of feces was divided equally and placed into either selenite F enrichment broth or Muller-Kauffmann tetrathionate broth followed by incubation under aerobic conditions at 43°C for 24 hours. A sample from each of the enrichment broths was subcultured onto 3 selective agars including brilliant green MacConkey agar, desoxycholate citrate agar, and de Loureiro's three-stock solution modification of bismuth sulfite agar, and incubated at 37°C for 24 hours. *Salmonella*-suspect colonies were further evaluated using appropriate biochemical tests. Sixteen different *Salmonella* serotypes were identified. *Salmonella* were more consistently isolated from selenite F (81%) than Muller-Kauffmann tetrathionate (63%). These findings suggest that selenite F would be the preferred enrichment broth for reptile serotypes, however, the sample size in this study was limited (N = 20). The selectivity of an enrichment broth may restrict the multiplication of certain *Salmonella* serotypes. When the results of the 2 enrichment broths were combined in this study, the number of isolates increased by 38%. Reptiles may harbor multiple serotypes, and the use of multiple enrichment broths may increase the recovery rate of salmonellae. Delayed culture may also increase the recovery rate of certain *Salmonella* serotypes. Subculture of selenite F and Muller-Kaufmann tetrathionate at 24 and 48 hours increased the chances of recovery of multiple serotypes.[19]

Kodjo et al[20] investigated the use of two different enrichment broths with 3 selective agars. Fecal samples were collected from a population of 32 chelonians comprising 9 species and assayed for *Salmonella*. Feces were enriched in either selenite or tetrathionate and incubated at 37°C for 24 hours. A sample was collected from each of the enrichment broths and plated on to either Rambach, *Salmonella-Shigella*, or xylose-lysine-tergitol 4 agar and incubated at 37°C for 24 hours. *Salmonella*-suspect colonies were further characterized using various biochemical tests. Thirteen of the 32 samples were *Salmonella*-positive. Thirteen isolates were recovered from selenite broth, compared with 11 using tetrathionate. All 13 isolates grew on Rambach and *Salmonella-Shigella* agar, compared with 12 on XLT-4.

Delayed secondary enrichment (DSE) is a technique used in the poultry industry to increase recovery rates of *Salmonella* in diagnostic

and environmental samples. Delayed secondary enrichment may be beneficial to *Salmonella* organisms that are damaged by antibiotics, require additional time for multiplication because of low numbers, or if competing bacteria are present in the sample. Waltman et al[21] collected 4,377 samples from poultry, including yolk sacs from 1-day-old chicks, tissues from Pullorum-typhoid reactors, and environmental swabs to evaluate 2 different selective medias and the recovery rates associated with a 24-hour enrichment period to a 5-day DSE. Samples were initially inoculated into tetrathionate broth and incubated for 24 hours at 37°C. Samples were plated onto brilliant green agar or brilliant green agar with novobiocin and incubated at 24 hours at 37°C. The remaining tetrathionate broth was retained at room temperature for 5 days. After this period, 1.0 mL of the suspension was added to a fresh tube of tetrathionate broth and incubated for 24 hours at 37°C. An aliquot was then collected from the sample and plated onto brilliant green agar with novobiocin and incubated for 24 hours at 37°C. Presumptive *Salmonella* colonies were characterized using biochemical tests. *Salmonella* were isolated from 464 samples (11%). Two hundred sixty-nine (58%) *Salmonella* were isolated after the 24-hour enrichment and 421 were isolated after the 5-day DSE. Forty-three (9%) of the *Salmonella* were isolated after the 24-hour enrichment and not the DSE, compared with 195 that were isolated after the DSE. The delayed secondary enrichment improved overall recovery of *Salmonella* by approximately 64%. Waltman et al[22] also evaluated a 3-day DSE and found that the combination of a 24-hour and 5-day DSE provided the most optimal results.

In a questionnaire administered to veterinary and independent diagnostic laboratories in the United States, 51% of respondents confirmed the use of a 24-hour enrichment period for culturing *Salmonella*.[22] These findings suggest that many laboratories may misclassify *Salmonella*-negative samples.

Although microbiologic culture is considered to be the standard in clinical microbiology, the true test characteristics (sensitivity, specificity, positive-predictive value, and negative-predictive value) are unknown. The reliability of culture may be affected by several factors, including the method used to collect the sample, quantity of sample collected and submitted, temporal and seasonal variation in shedding, and the method of culture.[14]

The PCR technique is a method of gene amplification that can be used to improve diagnostic capabilities. PCR is more sensitive and can provide a diagnosis within 24 to 48 hours. Six serotypes collected from green iguanas (*Iguana iguana*) in El Salvador were evaluated against 2 oligonucleotide primers. The first primer defined the amplified region of a 496 base pair (bp) conserved segment off the histidine operon gene of *S. typhimurium*.[14] The second primer sequence defined the amplified region of a 457 bp invasion protein (InvA) of *S. typhimurium*.[23] All 6 serotypes produced the appropriate 496 bp and 457 bp bands on their respective gels, suggesting that PCR may be useful to identify these serotypes in the green iguana.[24]

A follow-up study was performed to determine the sensitivity and specificity of microbiological culture and PCR for *Salmonella* detection in the absence of a gold standard.[25] Two different populations of hatchling green iguanas were used for the study. The prevalence of *Salmonella* in the 2 populations were 94% (population 1) and 67% (population 2). The PCR test sensitivities were 93% (population 1) and 91% (population 2) and the test specificities were 95% (population 1) and 96% (population 2). The microbial culture test sensitivities were 55% (population 1) and 70% (population 2) and the test specificities were 92% (population 1) and 95% (population 2). The results suggest that PCR is more sensitive than culture. The test sensitivity of culture in this study was low and the results suggest that approximately 30% to 45% of the *Salmonella*-positive animals being screened in these populations using culture alone would not be detected. To reduce the number of false-negative cases, a combination of diagnostic tests, such as PCR and microbial culture, could be used to reduce misclassification.

Reptile Salmonellosis

Salmonella was first isolated from a lizard (*Heloderma suspectum*) in 1944, although an earlier unconfirmed report of an organism was consistent with the biochemical attributes of *Salmonella* isolated from 3 dead wild-caught Gila monsters in 1939.[26,27] *Salmonella* was first isolated from a gopher snake (*Pituophis catenifer deserticola*) killed

on a turkey farm for eating turkey poults, and it harbored 3 serotypes: *Salmonella panama, S. meleagridis,* and an unidentified paracolon type.[28] This was also the first report of multiple serotypes being isolated from the same reptile. Snakes were considered a reservoir for the *Salmonella* and implicated as the source of the *Salmonella* outbreak in the turkey flock. The first report of a *Salmonella* from a chelonian involved *Salmonella newport,* which was isolated from the liver, spleen, lungs, and intestine of a Galapagos tortoise (*Geochelone elephantopus*) at necropsy.[26]

Clinical signs of *Salmonella* infection in reptiles are variable. In a group of 5 Gila monsters inoculated intracoelomically with *Salmonella,* 4 of the animals died between 18 hours and 3 months.[27] *Salmonella* was reisolated from 2 of the dead Gila monsters, but no gross lesions were observed in any of the lizards. The fifth lizard was unaffected. In a study on horned lizards *(Phrynosoma solare),* 5 animals were infected with *Salmonella* resulting in death in all 5 animals within 12 days. Splenomegaly was a common finding, and *Salmonella* was isolated from all 5 animals at necropsy.

Onderka and Finlayson[29] sampled 150 reptiles at necropsy for *Salmonella.* Forty-six (51%) of the snakes, 22 (48%) of the lizards, and 1 (7%) of the chelonians yielded *Salmonella.* Thirty-one serotypes were isolated from the sampled population. Death in 15 (17%) of the snakes and 5 (11%) of the lizards was attributed to salmonellosis. Pure cultures of salmonellae have been isolated from snakes with subacute necrotizing enteritis.[29] *Salmonella* has also been identified as the primary pathogen in a boa constrictor (*Constrictor constrictor*) with pneumonia. Reptiles that undergo bacteremia may develop visceral lesions. Hepatitis has been observed in snakes, and nephritis, oophoritis, myocarditis, and aortic valvular endocarditis have been diagnosed in green iguanas with *Salmonella* bacteremia at postmortem.[29]

Salmonellosis has been reported in farm-reared crocodiles.[30,31] Affected animals were lethargic and anorectic. *Salmonella* septicemia has been associated with acute death in farm-raised Nile crocodiles. High stocking density, contaminated diets, and deficiencies in hygiene were considered to be factors predisposing to infection and clinical severity.[30]

Treatment of *Salmonella*-positive reptiles is controversial. *Salmonella* are capable of developing resistance to antimicrobials through a number of mechanisms, and there is concern that the administration of antimicrobials may result into the development of resistant organisms. Unfortunately, this theory is routinely accepted as dogma. Veterinarians use antimicrobials to treat a variety of non-*Salmonella* bacterial maladies in reptiles. How do antimicrobials affect the *Salmonella* that may also reside in these animals? In theory, if an antimicrobial is selected based on an antimicrobial sensitivity profile, the drug is administered for an appropriate length of time, and necessary hygiene and environmental control methods are initiated, then eradication of a specific organism should be possible.

In a recent study to evaluate *Salmonella* clearance after enrofloxacin (Bayer Corp, Shawnee Mission, KS) (10 mg/kg orally once a day for 14 days), administration in *Salmonella*-positive, enrofloxacin-sensitive green iguanas showed 100% (N = 20) of the animals treated with enrofloxacin were cleared and 100% (N = 20) of the controls remained *Salmonella*-positive. Cloacal samples were collected from the test subjects every 5 days for 15 days and samples of the liver, gallbladder, colon, small intestine, spleen, and blood were tested using culture and PCR (Mitchell, unpublished data, December, 1999). In a follow-up study to assess the long-term effects of this treatment protocol, 65% of the animals given enrofloxacin (10 mg/kg orally SID for 14 days) were *Salmonella*-negative after 70 days (Mitchell, unpublished data, July, 2000). The *Salmonella* isolates from the iguanas that remained positive were all still sensitive to enrofloxacin. Failure to clear these animals may have been associated with duration of treatment, effective dose, or sequestration of the organism, but it was not the development of resistance. Further studies are required to evaluate the long-term effectiveness of various *Salmonella* control methods.

Salmonella as a Component of the Indigenous Gastrointestinal Flora

Salmonella are routinely isolated from apparently healthy reptiles. Refai and Rohde[32] examined 25 fecal samples from reptiles in the Gizeh Zoological Gardens for *Salmonella.* Fourteen of

the fecal specimens yielded *Salmonella.* Five of the fecal samples contained 2 serotypes and 1 fecal sample contained 3 serotypes. Zwart[33] examined samples from wild and captive reptiles in Ghana and found that the prevalence of *Salmonella* in lizards (37.5%) was greater than in snakes (29.6%). Delage et al,[34] in a study from Morocco, reported that tortoises (70.2%) were more likely to harbor *Salmonella* than snakes (45%). A collection of reptiles comprising 11 snakes and 3 crocodilians maintained in a natural museum were examined for salmonellae. Six of the snakes yielded *Salmonella* and the crocodilians were all free of infection.[35] Kourany and Telford[36] collected intestinal samples from 447 wild lizards in Panama. One hundred thirty-one (29.4%) were *Salmonella*-positive and 36 different serotypes were identified.

One hundred twenty-seven chelonians from the reptile collection at the Bronx Zoo were examined for *Salmonella.*[37] Thirty-seven of the chelonians were *Salmonella*-positive for *S. durham.* The prevalence in this population is similar to those described for chelonian collections at the Basel, Bern, and Zurich Zoological Gardens.[38] The prevalence reported in the chelonian collection at the Frankfurt Zoological Park was higher (50.5%) than those reported from other institutions.[39] Jackson and Jackson[40] sampled chelonians (N = 124) from 9 different zoologic parks in the United States and reported an overall prevalence of 12.1%. The differences between these populations may be attributed to methods of sample handling and isolation and environmental exposure and dietary contamination.

These findings suggest that *Salmonella* may be routinely isolated from captive or wild reptiles. Animals that are not displaying clinical signs associated with salmonellosis should be left untreated. If animals are being used in an zoologic exhibit or area frequented by children, they should be removed. Animals that are *Salmonella*-positive do not need to be killed; instead they should be placed with a responsible caretaker that is aware of the zoonotic potential.

Reptile-Associated Salmonellosis

The first case of turtle-associated salmonellosis in humans was reported in 1943 and the frequency of cases increased over the next 20 years.[41,42] It was not until 1963 that the first case of turtle-associated salmonellosis in a child was reported.[43] *Salmonella hartford* was recovered from a 7-month-old infant with diarrhea, vomiting, and fever. An investigation of the environment of the infant resulted in the isolation of the same serotype from the family's pet turtle. The increased frequency of turtle-associated salmonellosis in children was of concern to both state and federal health officials.

On January 1, 1968, the Washington State Board of Health issued a regulation restricting the sale of turtles that were not certified *Salmonella*-free and effectively halted the pet turtle retail trade in the state.[44] The number of turtle-associated salmonellosis cases decreased in the Seattle, Washington, area after the policy was instituted. Salmonellosis attack rates for children under 10 years of age in Washington were lower after 1968 compared with 1966 and 1967, whereas the attack rate for children in this same age group in the United States increased.[45]

In an attempt to estimate the magnitude of turtle-associated salmonellosis in the United States, a series of retrospective surveys of laboratory confirmed cases were conducted to determine frequency of turtle ownership using data from various state and county health agencies including Utah; Atlanta, Georgia; Santa Clara County, California; and Seattle, Washington.[45] A retrospective case-control study was also conducted on cases reported in Connecticut. Twenty-four percent of the salmonellosis cases in Connecticut were associated with an exposure to a turtle, compared with 2% of the controls. The findings in the other parts of the country varied, with salmonellosis cases in Santa Clara, California, reporting an association with turtles in 18% of the cases, 15.6% in Utah, 11.6% in Seattle, and 10.9% in Atlanta.[45] This information was than combined with previous turtle-associated salmonellosis reports in an uncontrolled study in Minnesota (turtle exposure, 25%)[42] and a controlled study in New Jersey (turtle exposure cases, 22.6%; controls, 5.7%).[46] An estimate of the number of turtle-associated salmonellosis cases in the United States was then developed. The proportion of cases of juvenile salmonellosis associated with exposure to turtles was averaged among the 7 sites to yield a mean of 18.2%. This average was than applied to the estimated number of salmonellosis cases in the

United States (2,000,000) assuming that turtles were present in 4.2% of U.S. households.[47] The estimate of the total number of households at risk was determined by a calculation derived from the number of turtles sold in a given year (1971, 15,000,000) and the number of households in the U.S. (1971, 60,000,000). The estimate of 4.2% corresponded to the number of households that maintained a turtle in the Connecticut and New Jersey studies.[45] Using these reference parameters, approximately 14% (280,000) of the salmonellosis cases in the U.S. in 1971 were turtle-associated.

The Federal government realized the significant health risk associated with pet turtle ownership and enacted regulatory measures similar to those enforced in Washington. In 1972, the Food and Drug Administration (FDA) required certification verifying salmonella-free status for the interstate transport of pet turtles. This program was found to be ineffective. A study conducted by the Centers of Disease Control concluded that 38% of the animals certified to be *Salmonella*-free were contaminated.[48] In 1975, the FDA banned the interstate shipment of all turtle eggs and live turtles with a carapace length of less than 10.2 cm. The decision to restrict the sale of turtles with a carapace length less than 10.2 cm was based on the assumption that these animals would be less desirable by young children. The enforcement of this policy resulted in a 77% reduction of the incidence of cases in those states without indigenous production of turtles.[49] The number of cases in Louisiana and Mississippi, 2 prominent turtle-producing states, remained unchanged during 1970 to 1976, contrasting with a significant decrease in other non–turtle producing states.[49]

The federal ban did not restrict the exportation of turtles from the U.S., and *S. pomona* was isolated from turtles shipped from Louisiana to Guam, Yugoslavia, Japan, Great Britain, France, and Israel.[50-54] In 1984, an illegal shipment of turtles to Puerto Rico was responsible for an outbreak of *S. pomona*. Health officials estimated that 15% of all infant salmonellosis was attributed to the illegal shipment.[55]

Exceptions to this 1975 law were made for marine turtles and educational and scientific institutions. Violators of this law are provided a written demand to destroy the animals under FDA supervision within 10 days. Violators are also subject to a fine not more than $1,000 and/or imprisonment of not more than 1 year for each violation. Herpetoculturists that breed exotic chelonians have avoided prosecution by selling animals under the guise of educational animals. Currently, there are hundreds of reptile swap meets a year in the U.S. where chelonians with a carapace length less than 10.2 cm can be purchased.

Attempts to reduce or eliminate *Salmonella* in turtles using antimicrobials was initiated after the FDA ban was implemented in 1975. Treatment of hatchlings using oxytetracycline in their tank water for up to 14 days alleviated shedding in treated turtles, but did not affect systemic infection.[56] Treatment of the freshly laid eggs with oxytetracycline or chloramphenicol using a temperature differential egg dip method was successful at eliminating *Salmonella* in eggs less than 1 day old, but did not clear eggs greater than 2 days old.[15] Large-scale experimentation on commercial turtle farms using surface decontamination and pressure or temperature differential treatment of eggs with gentamicin dip solutions for eggs greater than 2 days old and hatching the eggs on *Salmonella*-free bedding substantially reduced *Salmonella* infections and shedding rates in hatchling turtles.[57] Forty percent of the eggs not treated with the gentamicin were found to harbor *Salmonella* spp, whereas only 0.15% of the treated eggs were positive. Legislative implementation of this concurrent method of surface decontamination and gentamicin treatment by the Louisiana Department of Agriculture in 1985 was hailed as a victory by the turtle farmers. Unfortunately, the use of gentamicin and the other antimicrobials has led to an even greater concern through the development and persistence of antimicrobial resistant strains of *Salmonella*. Many of the antimicrobial resistance problems encountered in the turtle industry are directly related to placing these compounds into the hands of inexperienced individuals.

The occurrence of *Salmonella* in red-eared turtle (*Pseudemys scripta elegans*) eggs imported to Canada from 4 different Louisiana turtle farms in 1988 were examined, and of the 28 lots tested, 6 (21%) lots from 3 of 4 exporters were *Salmonella* positive.[58] Of the 37 *Salmonella* strains isolated, 30 (81%) were gentamicin resistant.[58] Similar results have been reported in which

environmental samples and live hatchlings were collected directly from 2 Louisiana turtle farms.[59] Isolates of *S. arizonae* and *S. poona* collected from the turtles at one of the farms were resistant to erythromycin, gentamicin, tetracycline, and triple sulfa. Pond water samples from both farms showed similar antimicrobial resistant patterns to erythromycin. In 1988, 115 batches of turtle hatchlings were submitted from 28 farms to the Louisiana Department of Agriculture and Forestry for analysis.[59] Five (4.3%) *Salmonella* isolates were obtained. Four of the organisms were submitted for serotyping; 3 were *S. arizona* and 1 was *S. poona.* All 4 isolates were resistant to erythromycin, gentamicin, tetracycline, and triple sulfa. These findings suggest that these animals may pose a significant human health risk and further marketing of these animals should be curtailed.

Reports of certified *Salmonella*-negative hatchling turtles testing *Salmonella*-positive when arriving in the importing country have been on the rise since the advent of egg sanitation and antimicrobial dip treatments.[58] A reason for this discrepancy has been attributed to the sample size required for testing.[59] In 1972, United States federal regulations were established to screen hatchling turtles for *Salmonella,* and the testing protocol required that 60 turtles be submitted for culture to a certified diagnostic laboratory. At the time of the regulations, the prevalence of *Salmonella* in turtles was approximately 40%, however, the advent of egg sanitation and antimicrobial treatment methods reduced the apparent prevalence below 0.2%.[49,57] Using a standard normal approximation of a binomial distribution to estimate the sample size has shown that there is only a 26% probability of detecting *Salmonella* in a population of turtles with a prevalence of 0.5%.[59] These findings suggest that a larger sample size of turtles should be submitted to ensure that false-negative batches are not exported.

Reptile-Associated Salmonellosis: 1990-Present

The incidence of reptile-associated salmonellosis cases in humans has increased dramatically during the past decade.[60] In 1996, the Centers for Disease Control (CDC) estimated that reptiles accounted for 3% to 5% of the 2 million to 6 million cases of human salmonellosis in the U.S.[61] In most documented reptile-associated cases of salmonellosis, the strain of *Salmonella* isolated from the patient was common to the pet reptile, suggesting the source of infection.[60] The increased incidence of reptile-associated salmonellosis has been associated with the increased popularity of these animals as pets during the past decade. From 1989 to 1993, imports increased 82%, from 1.1 million to 2.1 million animals (United States Fish and Wildlife Service, 1993). Green iguanas accounted for the largest proportion with imports increasing by 431% from 143,000 to 760,000.

In October 1995, a 3-week-old infant died in Indiana from *S. poona.* The same serotype was isolated from a pet iguana. The parents claimed that the infant did not have direct contact with the pet. This was the first reported case of a human fatality attributed to a *Salmonella* isolate from a pet iguana. Since this report was published, additional cases involving infants, children, immunocompromised individuals, and healthy adults have been described. During 1994 to 1995, health departments in 13 U.S. states reported over 60 cases of individuals with unusual serotypes of *Salmonella* in which patients had either direct or indirect contact with reptiles. The increased number of reports created a special concern for public health officials and prompted further study into the association of reptile-specific *Salmonella* serotypes with cases of human salmonellosis.

Epidemiology of Reptile-Associated Salmonellosis

Atypical serotypes of *Salmonella* have been anecdotally associated with reptile ownership. Cieslak et al[62] defined and described the epidemiology of reptile-associated serotypes (RAS) in the United States. RAS of *Salmonella* are defined as those in which reptilian sources composed the majority of the reports of nonhuman isolates reported to the CDC between 1981 and 1990. Human isolates of RAS reported to the CDC between 1970 and 1992 were analyzed, and incidence rates were calculated by age, sex, state, and year. A 1991 American Veterinary Medical Association Survey was used to compare reptile ownership to rates of RAS isolation by state. The annual incidence of RAS increased from $2.4/10^7$

persons per year in 1970 to 8.4/10^7 persons per year in 1992. This rise in cases represents a total of 150 new cases per year based on U.S. population estimates. Pet reptile ownership has been reported to be increasing (7.3 million reptiles), however, estimates of turtle sales in 1971 (15,000,000 turtles) far exceed these more recent estimates. The increased incidence of RAS may be associated with improved clinical diagnostic techniques, a higher carriage rate in squamates (nonchelonian reptiles), or the duration of exposure to the pet. The average life span of an aquatic turtle in captivity in 1971 was estimated to be less than 2 months when these pets were marketed before the 1975 FDA ban on interstate transport.[45] The average longevity of squamates in captivity in 1999 is likely to be greater than 2 months because of improved husbandry techniques. The 17 states with the highest incidence of RAS isolates from humans also had the highest rate of reptile ownership. The incidence of infection was higher in infants (66.1/10^7/yr) than in individuals greater than 1 year of age. Infants also accounted for a greater number of RAS isolates (27.2%) than other *Salmonella* serotypes (18.8%). The higher incidence of infection in infants is contrary to the findings with turtle-associated salmonellosis cases during the early 1970s. In the turtle-associated cases, case-patients were between 1 to 9 years of age and had direct contact with the reptile, whereas in the more recent cases (1990s), infants are reported to have no direct contact with a nonchelonian pet reptile.

In 1993, 3 green iguana–associated salmonellosis cases were reported to the New York State Department of Health (NYSDH) during a 2-month period.[63] The cases were notable because they involved rare *S. matadi* and *S. poona*. The *S. matadi* was isolated from the stools of 2 teenage boys that had handled a green iguana. The *S. poona* was isolated from the cerebrospinal fluid of a 6-week-old infant. These cases prompted health officials to question whether specific *Salmonella* serotypes are associated with exposure to reptiles.

A matched case-control study was performed using the 1993 New York State *Salmonella* surveillance data base.[63] Case selection included those individuals with *Salmonella* serotypes common to reptiles or characterized in reports of reptile-associated salmonellosis. Reptile-associated serotypes of *Salmonella* were again defined as those which reptilian sources composed the majority of the reports of nonhuman isolates reported to the CDC between 1981 and 1990.[62] The RAS included 35 serotypes of *Salmonella* type I; all serotypes in II, III, and IV; and 10 other serotypes linked to human RAS cases. Each case was matched by age (<5 years of age within 2 years; 5 to 21 years of age within 3 years; and >21 years of age within 10 years) and date of diagnosis (within 30 days) to 1 or 2 controls, comprising cases of *Shigella* spp. infection reported to the NYSDH in the same year. Telephone surveys were conducted on all available cases and controls to acquire data on symptoms, hospitalization, pet ownership, exposure to reptiles, and dietary habits. Of the 1,362 *Salmonella* spp. serotypes, 42 (3%) were considered RAS, of which 24 (57%) interviews were conducted. Twelve (50%) of the cases reported reptile ownership, compared with only 2 of 28 controls (matched odds ratio [OR], 6.6; 95% confidence interval [CI], 1.4 to 31.0). Ten of the cases had specific contact with iguanas, in contrast to controls, all of which reported no contact with an iguana. It has been estimated that approximately 5% of all salmonellosis cases are reported.[64] Using this estimate, there would have been in excess of 700 RAS cases in New York State during 1993. The findings suggest that reptile-associated salmonellosis is more than an incidental occurrence.

Outbreaks of RAS are rare, despite the fact that 95% of the 174 zoos and aquariums in North America affiliated with the American Zoo and Aquarium Association exhibit reptiles.[65] In January 1996, *Salmonella enteritidis* was isolated from the feces of several children living in Jefferson County, Colorado.[66] The only common link among these patients was that they had visited an exhibit of Komodo dragons held over a 9-day period at the Denver Zoological Gardens. An epidemiologic investigation (matched case-control study) was conducted to assess the extent of the outbreak.

There were 39 culture-confirmed cases and 26 suspect cases. Forty-eight case individuals, comprising 33 culture-confirmed and 15 suspects, were the first to become ill in their households. The median age of the patients was 7 years (range, 3 months to 48 years); 53 (82%) were under 13 years old and 34 (55%) were male. The median time until the onset of disease

was 3.5 days. *Salmonella enteritidis* was isolated from only 1 of the 4 Komodo dragons at the exhibit. The phage type 8 isolate was common to the case patients. The same organism also was isolated on 3 occasions from the barrier wall that separated the animals and the visitors. Visitors were allowed to rest their hands and elbows on the barrier surface, which was also accessible to the animals. Direct contact with the reptiles was limited with only 2 controls touching an animal.

Twenty-six cases were matched to 49 controls for the case-control study. There was a significant risk associated with touching the barrier that housed the dragons (OR, 4.0; 95% CI, 1.2 to 13.9). Hand washing after visiting the exhibit or before the next meal was found to be protective (OR, 0.1; 95% CI, 0.02 to 0.47). There was no difference between the groups when comparing the risk of eating food purchased at zoo concessions or touching a reptile skin on display at the exhibit. This report described the first known outbreak of reptile-associated salmonellosis at a zoologic park and reinforces the concern that transmission of *Salmonella* from reptiles to humans may occur thorough environmental contamination.

Veterinarians working in private practice and in zoologic institutions can serve a vital role in the prevention of reptile-associated salmonellosis. Pet reptile owners and visitors to zoologic parks should be informed of the potential risks of maintaining and handling these animals. Immunocompromised individuals and young children should avoid contact with reptiles. Hand washing should be recommended following any contact with a reptile or reptile enclosure.

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