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Preliminary findings of *Salmonella* spp. in captive green iguanas (*Iguana iguana*) and their environment

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Abstract

Captive reptiles are routinely identified as reservoirs of *Salmonella* spp. and reports of reptile-associated salmonellosis are increasing. Unfortunately, little is known about the epidemiology of *Salmonella* spp. and green iguanas. We did a limited survey of a green-iguana farm in El Salvador to identify sources of *Salmonella* spp. in green iguanas and their environment. A limited number of samples for microbiological culture were collected from iguanas (adult, hatchling, and embryos) and their environment (food, water, soil, shelter, insects, and wild-caught lizards). *Salmonella* spp. was isolated from the intestine of both adult (3/20) and hatchling iguanas (8/20). There was no evidence of *Salmonella* spp. in the reproductive tracts of female iguanas (0/10). *Salmonella* spp. was isolated from the surface of 40% (7/16) of the egg surfaces tested. *Salmonella* spp. was not identified from the externalized yolk-sac of the iguana embryos tested. Soil samples from a breeding pen and a nest were both positive for *Salmonella* spp. Eight different *Salmonella* spp. serotypes were identified in this survey. These results suggest that horizontal transmission of *Salmonella* spp. is a potential source of exposure to hatchling iguanas at this facility.

Keywords: Green iguana; *Iguana iguana*; *Salmonella* spp.

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1. Introduction

Reptiles constitute the fastest-growing sector of the pet market in the USA. A 1994 National Pet Owner's Survey recorded over 7.3 million pet reptiles in the USA. US Fish and Wildlife Service statistics confirm that the green iguana is becoming an increasingly popular pet as evidenced by the importation of over 640,000 immature animals in 1997.

Captive propagation of green iguanas occurs primarily in Central and South America with the largest numbers shipped to the USA. Commercial breeding stock is maintained in open earthfloor pens. Male and female iguanas are housed together from November to January during which mating occurs. Eggs are deposited in soft soil between January and March in 60–80 cm deep nests. The adults are then removed from the pens to prevent damage to the nests. Incubation time and hatching are influenced by environmental conditions including soil moisture and temperature, but the hatching season begins in late March and continues through June. Mature iguanas and hatchlings are fed with a protein concentrate supplemented with a variety of vegetables and fruits (depending on seasonal availability). The hatchlings are held in the breeding pen for a minimum of seven days until consigned to wholesalers.

Salmonella spp. infection was first identified in snakes in 1944 (Hinshaw and McNeil, 1945), and in turtles and lizards in 1946 (McNeil and Hinshaw, 1946). Until the 1960s, reports of reptile-associated salmonellosis were rare. During the 1970s, approximately 4% of US households owned pet turtles and these animals accounted for 14% (300 000) of all US reported cases of salmonellosis in children under 10 years of age in the USA (Gangarosa, 1985). In 1975, the US Food and Drug Agency implemented an interstate ban on commerce in turtles. This effectively halted the sale and ownership of turtles within the continental US and markedly reduced the number of turtle-associated cases of salmonellosis cases in the USA (Cohen et al., 1980).

More recently, reports of salmonellosis from non-turtle reptile reservoirs have gained national attention (Centers for Disease Control, 1992a; Centers for Disease Control, 1992b; Ackman et al., 1995). In most documented cases, the strain of *Salmonella* spp. isolated from the patient was common to a pet reptile — confirming the source of infection (Meehan, 1996). The US Centers for Disease Control has estimated that in 1996, there were over 50 000 cases of reptile-associated salmonellosis (Meehan, 1996).

The increased popularity of the green iguana with attendant risks of salmonellosis in owners and contacts of both clinically affected and normal green iguanas merits study. There have been no documented epidemiological investigations to demonstrate when and how the commercial green iguana becomes infected with *Salmonella* spp. The purpose of this study was to identify potential sources of *Salmonella* spp. contamination in commercial production of the green iguana.

2. Materials and methods

In March 1998, field investigations were initiated at a commercial iguana farm producing 200 000 hatchlings annually located in Costa Del Sol, El Salvador. The facility comprised 25 pens with a combined area of 8 ha. The breeding colony comprise

approximately 11 000 females and 4000 males held in earthen pens. Shelter was provided by concrete-block and bamboo-roofed structures.

Non-chlorinated water was supplied to the animals in a cement basin located in each pen. Water was replaced every 48 h. Iguanas were fed with a commercial concentrate (Blue Diamond Iguana Diet, Fluker Farms, Port Allen, LA, USA) and a mixture of vegetables and fruits (prepared daily). Vegetables and fruits were stored in an open shelter protected by a 1.2 m fence. The dietary concentrate, vegetables and fruits were mixed and ground to a homogenous coarse consistency. A mineral supplement was added to the feed weekly.

Specimens for microbiological examination were collected from eggs in nests and from hatchling and adult iguanas. Samples were collected by the El Salvadorian representative from the Ministry of Agriculture. Sample collection was not based on a formal randomization technique. Samples also comprised food components including dietary concentrate, mineral supplement, vegetables, fruits, and feed at the preparation site. Environmental specimens included soil, dried egg shells, fecal material, water, insects, and free-living lizards captured on the farm. Replicate food and environmental samples were collected on two consecutive days.

Mature breeding iguanas and hatchlings were euthanized by cervical dislocation and specimens collected at post-mortem examination using appropriate sterile technique. Blood specimens were collected from adult iguanas by intracardiac puncture using a 22 gauge 2.5 cm needle on a disposable 3 ml syringe. Blood samples were collected in sterile tubes devoid of anti-coagulant. Specimens of liver, colon contents, ovary, oviduct, and yolk-sac (hatchlings) were collected for microbiological examination. Approximately 1 g of liver was obtained by excision and placed into a sterile Whirl-pak® bag (Nasco, Cedarsburg, WI, USA). The tissue was macerated and added to 10 ml of selenite broth. Samples of colon contents and mucosa were obtained by incision of the organ and swabbing with a Mini-tip culturette® (Becton Dickinson, Cockeysville, MD, USA), which was introduced into the lumen of the colon. Ovaries were excised using sterile technique, placed into a sterile Whirl-pak® bag, macerated, and added to 10 ml of selenite broth. The oviducts were excised using sterile technique and a Mini-tip culturette® introduced into the incision to swab the mucosa. Samples of internalized yolk were obtained by incision of the yolk-sac and a Mini-tip culturette® introduced into the sac to obtain a representative specimen. Selenite suspensions were held at 20°C before and during transport by air to the Louisiana State University School of Veterinary Medicine. Samples were incubated at 37°C for 24 h within 48 h of collection.

Two sites within a breeding pen designated No. 4 (a convenience selection) were excavated to 77 cm. Embryos were fully developed within the eggs, but the yolk-sac had not been internalized. Eight eggs were sampled from each nest. The external surface of the eggs was swabbed using a Mini-tip culturette® which was then transferred to a tube containing 10 ml of selenite broth. One side of the egg was decontaminated with 70% isopropyl alcohol, which was allowed to dry, and the area was incised. A Mini-tip culturette® was used to sample the embryonic yolk-sac and was placed into 10 ml of selenite broth.

Samples of the dietary concentrate and mineral supplement were collected from unopened 50 kg sacks as delivered from a commercial feed mill. Approximately 20 g of

concentrate and supplement were obtained by aseptic technique and added to 10 ml of selenite broth. Representative 20 g samples of cabbage and mango were collected from the storage site, minced, and added to 10 ml of selenite broth. A mixed feed sample (20 g) was collected from the grinder and added to 10 ml of selenite broth. The wooden cutting table used for feed preparation and the feed grinder were sampled using a sterile Mini-tip culturette®.

Soil samples were collected from each of the breeding pens (No. 4 and 5) near the water basins and from two excavated nests. Approximately 20 g of soil was obtained using a sterile tongue depressor and was transferred to 10 ml of selenite broth. Approximately 2 g of dried egg shell collected from the surface of an excavated nest with a sterile tongue depressor was transferred to 10 ml of selenite broth. Fecal material was collected using a sterile tongue depressor near the water basin in a specific pen housing breeder animals. Approximately 10 g of fecal material was transferred to 10 ml of selenite broth. Water samples were collected from both an active and a stagnant well, and the water basins in the pens. Water was collected into sterile plastic Whirl-pak® bags, mixed by rotating and 1 ml transferred to 10 ml of selenite broth. Ten house flies (*Musca* spp.) captured in the vicinity of the feed preparation area were ground in a sterile mortar and added directly to 10 ml of selenite broth. Fifteen ants collected near a water basin in a breeding pen were ground in a sterile mortar and added to 10 ml of selenite broth. Two pooled fecal samples, representing six animals in each pool, from wild rainbow lizards (*Cnemidophorus lemniscatus*) were collected using a Mini-tip culturette® and added directly to 10 ml of selenite broth. The selenite cultures were held at 20°C before and during transport by air to the Louisiana State University School of Veterinary Medicine. Samples were placed in an incubator at 37°C for 24 h within 48 h of collection.

After incubation, the enriched selenite cultures were mixed on a Vortex agitator for 5 s. A heat-sterilized bacterial loop was used to transfer an aliquot of enriched broth to the surface of a petri dish containing xylose–lysine–desoxycholate agar (XLD, Remel, Lenexa, KS, USA). Streaked plates were incubated at 37°C for 24 h under aerobic conditions. Presumptive *Salmonella* spp. colonies were evaluated on indicator media including urea, lysine iron agar (LIA), and triple iron agar (TSI). A heat-sterilized bacterial loop was used to streak a portion of a colony onto slants of urea, LIA and TSI agar. The preparations were incubated aerobically at 37°C for 24 h. The presence of *Salmonella* spp. on urea slants were denoted by a red color change. *Salmonella* spp. on LIA slants were alkaline over alkaline with gas production. Positive TSI samples were alkaline over acid. Samples that were urea negative, LIA positive and TSI positive were further evaluated using API 20E Test Strips® (bioMerieux Vitek, Hazelwood, MO, USA). A heat-sterilized bacterial loop was used to transfer sample colonies from the TSI slant to 10 ml of 0.85% saline to attain a concentration equivalent to a 0.5 McFarland's equivalence turbidity standard (Remel, Lenexa, KS, USA). The samples were placed into the designated receptacles on the test strips in accordance with the manufacturer's directions and incubated aerobically at 37°C for 24 h. The bacterial reactions were interpreted using the appropriate key.

Exact 95% binomial confidence intervals (CIs) were calculated using Epi Info 6 version 6.04b (Centers for Disease Control, 1997).

3. Results

Salmonella spp. subgroup 3 was isolated in 25 (13%) of 190 samples derived from iguanas and their environment. Three of 20 intestinal samples (15%; CI: 3%, 38%) and two of 20 liver specimens (10%; CI: 1%, 32%) from adult iguanas yielded a *Salmonella* spp. There was no evidence of *Salmonella* spp. in the blood, ovary or oviduct of adult females (CI on 0/10 for each tissue: 0%, 31%). Eight of 20 intestinal samples (40%; CI: 19%, 64%) and one of 20 internalized yolk samples (5%; CI: 0%, 25%) from hatchling iguanas yielded a *Salmonella* spp. The organism was isolated from the exterior of 7 of 16 egg shells (44%; CI: 20%, 70%). It was not possible to detect *Salmonella* spp. in the embryonic yolk-sac prior to hatch (0/16; CI: 0%, 21%). The results of the cultures of 38 environmental samples are reported in Table 1. There was no evidence of *Salmonella* spp. contamination of feed or water in this limited survey, but none to the contrary either (all CI had upper limits above 50% for such samples). *Salmonella* spp. was isolated from the soil surface in a breeder pen and from a nest. Insects found in the iguana pens and around the food preparation area did not yield *Salmonella* spp., but the organism was isolated from the pooled feces of rainbow lizards captured on the farm.

Nineteen of the 25 *Salmonella* spp. isolates were submitted for serotyping (National Veterinary Services Laboratory, Ames, IA, USA, Table 2) and eight different *Salmonella* serotypes were identified. The remaining six samples were lost during transport. Five of the six (83%) external egg isolates were identified as *Salmonella* 48: I-Z *arizona*. The *Salmonella* isolated from the rainbow lizards and nest soil were serotype 44: Z4, Z23. The two liver isolates were *Salmonella* 11: Z4, Z23. Three different serotypes (*S. senftenberg*, *S. havana*, and *S. saint-paul*) were isolated from the intestines of hatchling

Table 1
Salmonella spp. isolations from environmental samples collected from a commercial *Iguana iguana* farm in Costa Del Sol, El Salvador

Sample	Number	
	Positive	Total
Water samples	0	5
Adult concentrate	0	3
Juvenile concentrate	0	3
Mixed feed sample	0	2
Vegetables	0	2
Feed preparation site	0	2
Mineral supplement	0	2
Lizards	2	2
Insects	0	3
Shelter	0	2
Soil from pens	1	6
Nest soil	1	2
Dried egg shells	0	2
Fecal material pen No. 4	0	2

Table 2
Salmonella serotypes isolated from *Iguana iguana* and their environment in Costa Del Sol, El Salvador

Serotype	Sample
S. 48: I-Z (*arizona*)	Egg surface (5), hatchling internal yolk
S. 44: Z4, Z23 (*arizona*)	Adult male liver, adult female liver, adult female intestine
S. 11: Z4, Z23	Nest soil, rainbow lizards (2), egg surface
S. senftenberg	Adult male intestine, hatchling intestine
S. newport	Pen No. 4 soil
S. havana	Hatchling intestine
S. matadi	Adult female intestine
S. saint-paul	Hatchling intestine

green iguanas that were of age for export to the US. *S. saint-paul* has been implicated in reptile-associated zoonotic salmonellosis (Lamm et al., 1972).

4. Discussion

The purpose of this study was to evaluate the status of *Salmonella* spp. in green iguanas and their environment in a commercial breeding farm exporting to the USA. A specific question relating to the epidemiology of the infection concerned the potential for vertical and horizontal transmission.

The presence of *Salmonella* spp. in the intestine of both hatchling and adult iguanas is consistent with previous reports of isolates from both cloacal and fecal specimens (Centers for Disease Control, 1992b). The absence of *Salmonella* spp. in feed suggests that diet was not a source of infection in this commercial operation on the days of sampling; however, a larger sample of feed stuffs would be required to determine the true *Salmonella* spp. status of the diet. The green iguana is geophagic and coprophagic and juveniles may acquire intestinal microflora from droppings of mature individuals (Sokol, 1971). Under natural conditions, the hatchling iguanas would consume adult fecal material older than 90 days. Unless weather conditions were extreme, *Salmonella* spp. would be expected to survive over this period of time (Morse, 1974). Current practices of iguana farms do not include fecal removal from the pens after the breeding animals have been removed. Isolation of *Salmonella* spp. in the soil of the breeding pen near the water basin suggest that regular removal and disposal of fecal material might reduce exposure.

Salmonella spp. in the 5–7 day old hatchlings is an important finding. Reptiles absorb the internalized yolk as a source of nutrients and probably do not consume food within the first week after hatching. The tongue of the iguana is a chemosensory organ and oral exposure to *Salmonella* spp. could occur from contact with infected soil in nests or pens while investigating their environment. Although *Salmonella* spp. was not isolated from water, insects, or structures within pens, in this limited survey, environmental contamination still should be considered as a possible source of infection.

One of 20 juvenile iguanas (5%) had *Salmonella* spp. in the internalized yolk-sac. It is possible that contamination of the umbilicus occurred in the nest after hatching. Abrasion

of the external yolk-sac by soil after emerging from the egg may facilitate penetration by *Salmonella* spp. It is also possible that contamination occurred following contact with soil or egg-shell remnants in the nest. The presence of *Salmonella* spp. in soil could infect hatchlings.

The relatively high presence of *Salmonella* spp. detected on the external surface of the egg in contrast to the failure to isolate the organism from the yolk supports the theory that eggs are contaminated in passing through the proctodeum (which is in close proximity to the coprodeum) and that surface infection of the shell is a mechanical process dependent on colonization of the terminal intestinal tract.

The presence of *Salmonella* spp. in the rainbow lizards captured on the farm represents a potential reservoir of infection for the breeders and their progeny. Although these lizards were captured outside the iguana pens, workers reported observing these lizards within the pens. Other potential sources of environmental contamination include free-ranging birds and rodents. Although traps were set for these animals, none was captured. Vermin were not considered a problem on the farm since initiating control methods in 1996.

The failure to recover *Salmonella* spp. from the ovaries, oviduct and yolk-sac of embryos might suggest that this organism is not transmitted vertically in the green iguana; however, a larger sample size would be required to confirm this. In other captive reptiles, including red-ear sliders (*Trachemys scripta elegans*), *Salmonella* spp. has been isolated from the oviduct and interior of the egg (Kaufman and Morrison, 1966).

Because of the high carriage risk of *Salmonella* spp. in green iguanas, potential owners should be informed of the potential risks associated with zoonotic infection. Washing of hands after handling iguanas is strongly recommended (Centers for Disease Control, 1999). Providing an appropriate environment and adequate nutrition for the pet iguana is also important to maintain health. Iguanas are inappropriate pets for immunocompromised owners and in households with young children (Centers for Disease Control, 1999).

5. Conclusions

This survey represents the first documented investigation of the epidemiology of *Salmonella* spp. in commercial green iguanas. This limited study was undertaken because the opportunity existed to collect samples during a routine governmental inspection. Our findings in this study provide some valuable insight into the status of this particular farm; however, a more rigorous study should be performed to determine the true status of this population.

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