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RESEARCH AND PRACTICE

Evaluation of Prevalence, Transmission, and Prevention Methods of Salmonellosis from Pet Turtles in Michigan

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ABSTRACT:

The sale and distribution of turtles with a carapace smaller than four inches in diameter was banned by federal law in 1975 on the grounds that such animals were frequently associated with human *Salmonella* infections. However, the popularity of these small turtles as pets has resurfaced, and in many places in Michigan they are being sold illegally. This study was conducted in Michigan to determine the prevalence of *Salmonella* spp. in the most popular pet turtle species, *Trachemys scripta elegans* commonly known as red-eared sliders, and to evaluate the compliance of pet stores with laws concerning the sale of these animals. One hundred and fifty pet stores nearest to the center of five large cities in Michigan (30 each for Lansing, Detroit, Flint, Kalamazoo, and Grand Rapids) were contacted by phone.

During this study, it was found that 41% (7/17) of the stores that indicated that they sold turtles also sold small turtles. Those seven stores were visited and sanitary conditions, animal housing, client education offered, and requirements for sale were observed. Each of these stores sold the authors a small turtle without asking any questions. One store required the authors to sign a form stating that they were buying the turtle for educational purposes, but did not require any verification. Each of the turtles bought was tested for *Salmonella* carriage. Six out of seven (86%) purchased turtles were positive for *Salmonella* spp. Among the positive turtles, the serotypes found were *S. Litchfield* (2/7), *S. Norwich* (1/7), and *S. Welteverden* (1/7), one was confirmed as *Salmonella* but untypable and another was contaminated upon arrival at NVSL and not serotyped.

This study showed that the nationwide ban on the sale of turtles with carapaces smaller than four inches in diameter is not being adequately enforced in Michigan, and that the turtles being sold have a high prevalence of *Salmonella* carriage.

Key words: public health, epidemiology, reptile

INTRODUCTION:

Salmonellosis can be a serious disease, especially in infants, young children, the elderly, and the immunocompromised. Complications of this disease can be severe and can lead to septicemia and meningitis. The Centers for Disease Control and Prevention estimates that three to five percent of the up to six million cases of human salmonellosis each year may be attributed to exposure to reptiles (CDC, 2006). *Salmonella* infection has been long associated with turtles (Chionidi and Sundberg, 1981). Infected turtles are almost always totally healthy, but still carry the *Salmonella* organism and can transmit it to humans.

The sale and distribution of turtles with a carapace smaller than four inches in diameter was banned by law in 1975 by the U.S. Food and Drug Administration (FDA) (CFR—Title 21, Part 1240, Sec. 1240.62 (b)) because of growing evidence that it may be associated with human *Salmonella* infections, especially in children. It is believed that turtles of this size pose a higher risk of infecting children with *Salmonella* spp. because they can easily fit the animals in their mouths. The CDC estimates that since this ban was established, approximately 100,000 cases of salmonellosis are prevented annually. In recent years the popularity of these small turtles as pets has resurged (AVMA, 2007), and in many places small turtles are being sold illegally in spite of this regulation (CDC 2004, 2007, 2008). Recently, there has been an increase in reports of salmonellosis associated with exposure to turtles and other exotic reptiles (CDC, 2005, 2006, 2008; JAVMA News, 2001), including a multistate outbreak of *Salmonella* Paratyphi B var. Java associated with exposure to turtles during October 2007-January 2008. As of January 18, 2008, a total of 103 cases from 33 states had *Salmonella* isolates indistinguishable from the ones found in six turtles or the water from their habitats in the homes of case-patients in California, North Carolina, Ohio, and Wisconsin (CDC, 2008).

Salmonella commonly lives in the intestine of vertebrates and has been frequently reported in reptiles (Hidalgo-Vila *et al.*, 2006). Turtles sold as pets often come from breeding farms where turtles are housed in crowded ponds and nesting areas in a manner that often fosters *Salmonella* transmission (D'Aust *et. al.*, 1990). Also, uninfected turtles can become infected while housed with other turtles at the breeding farm, during shipment, at the pet store, or even at a pet owner's home. Despite the health risks, turtles have become increasingly popular as pets in recent years, perhaps due to a popular cartoon TV show regarding a group of young adult turtles with reputed martial arts training.

During the early 1970's, about 14 percent of *Salmonella* infections came from small pet turtles. In 1999, the CDC estimated that pet reptiles or amphibians were the source of about 93,000 *Salmonella* cases a year or 7 percent of all cases (CDC, Healthy PETS Healthy people).

The objective of this study was to determine how many Michigan stores sold turtles and under what conditions they were offered for sale. The authors sought to determine the prevalence of *Salmonella* spp. in the turtles, the most popular of which is the *Trachemys scripta elegans*, commonly known as red-eared sliders. Federal law states that viable turtle eggs and live turtles with a carapace length of less than 4 inches shall not be sold to the public; with the exception of animals used for bona fide scientific, educational, or exhibition purposes, or animals intended for export only. This law excludes marine species (families Dermachelidae and Chelonidae).

METHODS:

Pet Stores: One hundred and fifty pet stores nearest to the center of five large cities in Michigan (30 each city: Lansing, Detroit, Flint, Kalamazoo, and Grand Rapids) were located in an online directory and contacted by telephone. The following basic questions were asked during the phone calls:

- “Do you sell turtles?”
- “What kind of turtles do you have?”
- “What is their price?”
- “What size are they?”

The conversation varied slightly each time, depending on what the person at the pet store responded, but the same basic questions were always asked.

Following contact by phone, seven stores were selected for a visit because they claimed to have red-eared sliders for sale with a carapace length smaller than four inches. The following conditions were observed during the store visits:

- Accessibility of visitors to handle animals
- Turtle housing
- Hand cleaning facilities
- Posters or signs explaining risks or conditions for selling turtles smaller than four inches in length
- Client education regarding hygiene
- Conditions of sale
- Forms used, if any, before selling the turtles

Turtle Housing and Sample Collection: One turtle was purchased from each of the seven pet stores. The turtles’ carapace length was measured in inches from nuchal to supracaudal scute. Six of the animals were individually housed in 5x3x2 inches sterile plastic containers with distilled water to prevent cross-contamination and one larger turtle with a carapace length of 3.25 inches was housed in a 9x13x3 inches container under the same conditions. Because *Salmonella* shedding is intermittent, each turtle was housed for five days in this enclosure without a water change. After the five-day housing period, a 10 mL water sample was then collected aseptically for *Salmonella* culturing.

Culture, Isolation, and Serotyping: Each water sample was enriched in Tetrathione Broth (Difco™, Becton, Dickson and Company, Sparks, MD 21152, USA) at 37° C for 24 hours, cultured in XLT4 (Difco™, Becton, Dickson and Company, Sparks, MD 21152, USA) agar

medium at 37° C for 24 hours, and biochemically confirmed based on color change and the pattern of these changes in inoculated Triple Sugar Iron Agar slants (Difco™, Becton, Dickson and Company, Sparks, MD 21152, USA) after incubation at 37° C for 24 hours. The colonies suspected as being *Salmonella* based on morphology and color on Triple Sugar Iron Agar were tested serologically using group-specific *Salmonella* antisera (Becton, Dickson and Company, Sparks, MD 21152, USA) according to the instructions stated by the manufacturer.

Serotyping was performed by the United States Department of Agriculture, Animal and Plant Health Inspection Service's National Veterinary Services Laboratory (NVSL) in Ames, Iowa, USA.

RESULTS:

Seventeen of the 150 pet stores contacted by phone indicated that they sold turtles. Seven of these claimed to have red-eared sliders for sale that had a carapace length smaller than four inches. Those seven stores were visited and sanitary conditions, animal housing, client education offered, and requirements for sale of a small turtle were observed. The species of animals sold, other than turtles, was noted in order to determine if the retailer would require licensure by the United States Department of Agriculture, Animal and Plant Health Inspection Service under the Animal Welfare Act (AWA) (7 U.S.C. 2131 et seq.), and therefore receive regular veterinary inspections.

Each of these stores sold the authors a small turtle without asking any questions. None of these stores offered a verbal warning regarding the health risks or laws governing the sale of turtles. Only three of them posted warnings about the requirements for the sale of small turtles. Store #2 had a sign which stated "Less than 4 inches not sold as pets". Store #3 had a sign above the tank stating "Must be 18 and for educational purposes only, please ask" and required the signing of a form stating that the turtle was being purchased for educational purposes, but no verification of this was requested. Store #5 had a sign close to the tank stating "Must be older than 18, less than 4 inches only sold for education or exhibition".

As shown in Table 1, the animals sold were observed in order to determine if the store fell under USDA/APHIS jurisdiction for reasons other than the turtles sold.

Table 1
Comments and Observations

Store	Comments and Observations		
	Animals sold in the store	Turtles housed per tank	Information required to sell turtle with carapace smaller than 4 inches in diameter
1	Freshwater fish, birds, rodents, and reptiles including turtles.	7	None, turtle sold immediately.
2	Puppies, rodents, ferrets, fish, insects, crabs, snails, and reptiles including turtles.	10 (3 different species) *	None, turtle sold immediately.
3	Puppies, fish, birds, rodents, and reptiles including turtles.	12	Sold after signing health advisory sheet stating that the purchase was for educational purposes.
4	Fresh and saltwater fish and reptiles including turtles.	1	Sold turtle that measured 3.25 inches, as if it was bigger than 4.0 inches and stated "we can't sell them any smaller".
5	Rodents and reptiles including turtles.	5	None, turtle sold immediately.
6	Rodents and reptiles including turtles.	7	None, turtle sold immediately.
7	Puppies, kittens, amphibians, fish, rodents, insects, birds, and reptiles including turtles.	5	None, turtle sold immediately.

**Yellow-bellied slider (Trachemys scripta scripta), red-eared slider (Trachemys scripta elegans), map turtle (Graptemys spp.) all smaller than four inches and housed in same tank.*

Each of the turtles bought was tested for *Salmonella* carriage. Six of the seven turtles (86%) were positive on *Salmonella* culture according to color change when grown on Triple Sugar Iron Agar (Difco™). Two isolated colonies from each positive water/turtle sample were selected and serogroups were determined by slide agglutination. Two of the six positive turtles yielded two different serogroups each, for a total of eight isolates. Seven of these isolates were sent to NVSL for confirmation and serotyping; the other positive isolate was obtained at a later date and was not serotyped. For Store/Turtle #2, serogroups A and B were detected in the laboratory and were believed to be two different isolates. However, NVSL confirmed that both isolates yielded *S. Litchfield*. For Store/Turtle #3, serogroups B and C were detected in the laboratory. The

serogroup B isolate was confirmed as *Salmonella* by NVSL but they were unable to identify its serotype, while the serogroup C isolate was contaminated upon arrival at NVSL and not serotyped.

Table 2
Individual Results for Turtles Tested

Store/ Turtle	Turtle Size (in)	<i>Salmonella</i> Culture Result	Serogroup	Specific Serotype
1	1.25	Positive	B	Litchfield
2	1.15	Positive	A	Litchfield
			B	Litchfield
3	1.5	Positive	B	<i>Salmonella</i> (untypable)
			C	Contaminated
4	3.25	Positive	B	Norwich
5	1.25	Negative	-	-
6	1	Positive	A	Welteverden
7	1.25	Positive	E	Not serotyped

Educational materials or verbal communication of the risks of handling reptiles were scarce. Forty-two of the 150 stores contacted which did not have small turtles for sale stated that they did not have them because it was illegal to sell them (42/150, 28%), and five stated that the reason was because they posed a risk of diarrheal illness (5/150, 3%).

DISCUSSION:

The modern turtle farming business reportedly started in Louisiana during the depression with turtles captured from the swamps (Hidalgo-Villa *et. al.*, 2006). Since the mid 1980s, more than 95 million pet turtles have been sanitized in the egg with antibiotics in these farms and exported for the pet store trade to other states and countries. This operation is certified *Salmonella*-free by the Louisiana Department of Agriculture & Forestry. D’Aoust and collaborators examined turtles imported into Canada from Louisiana for the presence of *Salmonella*. Of 28 lots of allegedly “*Salmonella*-free” turtles, 21% harbored *Salmonella*. The widespread use of gentamicin on turtle farms to produce *Salmonella*-free eggs for export has apparently encouraged development of antibiotic resistance in bacterial strains. Of 37 *Salmonella* strains isolated, 30 (81%) were gentamicin-resistant (D’Aoust *et. al.*, 1990). This finding of antimicrobial resistant strains of *Salmonella* is a serious public health issue that makes the high *Salmonella* prevalence an even greater public health hazard.

Turtles that are successfully sanitized with antibiotics while in the egg can nevertheless become contaminated with *Salmonella* shortly after birth. Previous studies have shown that the efforts to make turtles *Salmonella*-free have been ineffective (D’ Aoust *et. al.*, 1990), and perhaps have even given customers a false sense of security. In addition, the turtles that were observed for sale at pet stores were housed with many other turtles, possibly from various sources, and the aquatic medium has proven to be a favorable environment for *Salmonella* persistence,

transmission (Polo *et. al.*, 1999), and proliferation (Unpublished data, Ballester *et. al.*). Therefore, all turtles housed in the tank at that time or after the last thorough sanitation of the tank will essentially be exposed to the same aquatic microflora.

Attempts to culture reptiles for *Salmonella* are sometimes not successful because of intermittent shedding. However, the *Salmonella* organism can reportedly remain viable in the environment for up to 30 months and, in general, the aquatic medium is considered a favorable environment for its transmission (Polo *et. al.*, 1999). These factors combined with inappropriate husbandry and sanitation can lead to high-risk environments for acquiring salmonellosis.

For this study, only stores near the center of five major Michigan cities were contacted. Seventeen (11%) of the stores contacted indicated that they sold turtles. From these, nine (53%) sold small turtles, but only seven (41%) of the stores had them for sale during the months they were contacted for this study. Also, multiple websites were found where individuals can readily purchase small turtles in a very convenient fashion. The authors suspect that in Michigan small turtles could be even more accessible than this study determined.

Salmonella serotypes isolated from the turtles in this study were *S. Litchfield*, *S. Norwich* and *S. Welteverden*. Woodward *et. al* conducted a study in Canada from 1991-1996 in which they studied the association of exotic pets with human cases of salmonellosis, and they also isolated *S. Litchfield* from one of the patients who was associated with a pet turtle. Although *S. Litchfield* has been repeatedly implicated in *Salmonella* outbreaks in produce (CDC, 2008 a & b), the authors were unable to find any other reports of *S. Litchfield*, *S. Norwich*, or *S. Welteverden* associated with turtles.

CONCLUSION:

This study showed that the nationwide ban on the sale of turtles with carapaces shorter than four inches is not being adequately enforced in Michigan. Even though small turtles were only found for sale in seven (41%) of the 17 stores that indicated they sold turtles, the purchase of these animals was largely without restrictions and only in one instance involved a very limited amount of education.

The following are simple steps recommended by the CDC (CDC, 2009) that should be taken to reduce the chances of acquiring salmonellosis from a pet turtle:

- Observe thorough sanitation while handling a turtle:
 - Wash hands immediately after handling a turtle or being exposed to its environment and before contacting any other surfaces, including your body.
 - Clean the turtle's tank and change its water frequently.
 - Do not change the water in the same area where food is prepared for human consumption.
 - Do not allow the turtle and the turtle's water near your mouth.
- Do not have a turtle in a household with immunocompromised individuals such as children under five years old, the elderly, or people who have lower natural resistance to disease due to pregnancy, cancer, AIDS, chemotherapy, organ transplants, diabetes, liver problems, or other diseases.

In many households, children will be held accountable for the caretaking of their pets and cannot be expected to follow these rules every time. Taking this into account and the fact that *Salmonella* spp. can live on the surface of these animals, the CDC recommends that households with young children or immunocompromised individuals do not keep reptiles or amphibians as pets (CDC, 2009).

During this investigation, it was observed that enforcement of current laws regarding the sale of pet turtles in Michigan is deficient and that small turtles can still be acquired at pet stores locally and can also be bought online. In Michigan, the responsibility for enforcing this regulation falls on the Michigan Department of Agriculture. Their inability to properly enforce this law may likely be due to an increase in enforcement responsibilities while undergoing repeated major budget cuts. Based on this study's findings, the authors recommend that resources be provided so that current laws and health recommendations regarding pet turtles be enforced.

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