Salmonella Isolates from Wild-Caught Tokay Geckos (Gekko gecko) Imported to the U.S. from Indonesia

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Abstract

Reptiles account for ~10% of live animal shipments imported to the United States (U.S.), the majority of which are sold in the pet trade. Characterizing Salmonella shedding by imported reptiles is of value to public health, the pet industry, and veterinary medicine. Here we report results of a pilot survey of Salmonella serotypes isolated from wild-caught Indonesian Tokay geckos (Gekko gecko) imported to the U.S. Upon arrival, the geckos were individually housed until a fecal sample was acquired for Salmonella culture. The geckos were divided into three groups with variable numbers to investigate density effects. A second group was imported after 3 months and combined with the previous groups. A total of 88 Salmonella isolates were obtained from 110 geckos surveyed, representing 14 serogroups and 17 unique serotypes. Group prevalence ranged from 31–73%. A significant increase in prevalence and a change in serotype richness were detected between the time of import and 6 months later at necropsy. Six isolates (6.8%) expressed resistance to more than one antibiotic. All S. enterica subsp. enterica Adelaide isolates were resistant to nalidixic acid and sulfisoxazole, one S. enterica subsp. arizonae 61:k:z35 isolate was resistant to ampicillin and sulfisoxazole, and another 61:k:z35 isolate was resistant to streptomycin and sulfisoxazole. Forty-three additional isolates expressed resistance only to sulfisoxazole. The mechanisms for increased prevalence and apparent change in serotype richness are unknown, but could be due to stress associated with trade, transport, and captivity, increased transmission from unnaturally high densities, or contact with other species shedding Salmonella along the trade route. Future studies to differentiate the physical, social, and physiological effects of trade-related conditions on Salmonella shedding and transmission among reptiles will benefit the industry by identifying ways to reduce mortality, and safeguard the individuals handling animals along the transport chain and other species encountered en route.

Key Words: Antibiotic resistance reptile—Gekko gecko—Salmonella—stress—Tokay gecko—trade.

Introduction

There are two known species of Salmonella: S. bongori and S. enterica. Salmonella enterica is comprised of six subspecies (designated by a roman numeral and name: I, enterica; II, salamae; IIIa, arizonae; IIIb, diarizonae; IV, houtenae; and VI, indica), 60 serogroups, and more than 2400 serotypes identified to date (Brenner et al. 2000). Forty percent of all Salmonella serotypes have been predominantly cultured from reptiles and are rare in other animals (Mermin et al. 2004). Salmonella is generally considered to be a part of the normal flora of the gastrointestinal tract (Jackson and Jackson 1971; MacNeill et al. 1986; Mader 1996; Jacobson 2007; Pedersen et al. 2009), and less frequently as a cause of salmonellosis in reptiles (Onderka and Finlayson 1985). Reptiles may become colonized or infected with Salmonella via the fecal-oral route through contact with other animals shedding the bacteria, or via ingestion of contaminated food or water (Mader 1996). Although uncommon, Salmonella can be a true pathogen in these hosts, and a number of serotypes have been associated with disease (e.g., septicemia, pneumonia, coelomitis, and isolated abscesses) (Onderka and Finlayson 1985;
Frye 1991; Mader 1996). Reptiles can be colonized with multiple serotypes simultaneously, and prevalence estimates vary considerably by species and animal history (e.g. free-ranging reptiles tend to have lower prevalence than captive reptiles) (Onderka and Finlayson 1985; Mader 1996; Pedersen et al. 2009). Nearly all serotypes cause illness in at least some species, and reptiles have been implicated as sources of Salmonella in livestock, poultry, and humans (Mader 1996; Mermin et al. 1997; Woodward et al. 1997; Olsen et al. 2001; Rodgers et al. 2002; Otokunefor et al. 2003; Schrötter et al. 2004; Wells et al. 2004; Fasman et al. 2005; Nagano et al. 2006; Futagawa-Saito et al. 2008; Sternberg et al. 2008; Pedersen et al. 2009; Van Meervenne et al. 2009; Harris et al. 2010). Because salmonellosis is a major public and veterinary health problem worldwide, the development of antibiotic resistance poses a problem. Especially concerning is the emergence of multi-drug resistance, often due to the use of antimicrobials therapeutically or prophylactically in food-producing animals or in human medicine (Threlfall, 2002). Specifically, in Southeast Asia, resistance against trimethoprim-sulfamethoxazole, chloramphenicol, streptomycin, tetracycline, and fluoroquinolones in Salmonella have been reported (Sanborn et al. 1975; Tjaniadi et al. 2003; Okeke et al. 2005).

Reptiles account for approximately 10% of live animal shipments imported to the U.S. yearly, the third most common imported taxonomic group after fishes and coral (Smith et al. 2009). Tokay geckos (Gekko gecko) are among the most common reptile species imported for sale as pets in the U.S. (Smith et al. 2009, unpublished data), and therefore characterizing their colonization with Salmonella is of value to public health, veterinary medicine, and the pet industry. Tokay geckos occur throughout the Indo-Australian Archipelago, but are imported to the U.S. largely from Indonesia, where they live in close proximity to humans and are commonly found within and around rural homes. The objectives of this pilot study were to (1) describe the prevalence and serotype identity of Salmonella isolated from wild-caught Indonesian Tokay geckos imported to the University of Georgia (UGA; Athens, GA) for a separate study, (2) determine if 3 months of captivity altered this prevalence or the composition and diversity of the serotypes isolated, and (3) determine antimicrobial susceptibility of the Salmonella subspecies isolated.

Materials and Methods

Animal collection and shipping

In 2009 we partnered with a legitimate professional international wildlife distributor (who will remain anonymous in this report) to conduct a pilot investigation of the parasites and other pathogens associated with wild-caught Tokay geckos from Indonesia. The geckos were captured by the distributor, following standard business practices, from rural locations outside Jakarta on the island of Java. The geckos were placed in 3×3-foot containers and transported to the distributor’s “farm” (some species are also bred there) within 24 h, where they were held for ~9 days in a single 15×15-foot enclosure. One hundred and fifty geckos, a mix of males and females, were captured and shipped as air freight, via Miami, to the UGA College of Veterinary Medicine in two batches. Batch 1 contained 60 animals that were captured and arrived at UGA in March 2009. Batch 2 contained 90 animals that were captured and arrived at UGA in July 2009. Batch 1 geckos were individually shipped in plastic bottles with air holes and shredded paper. Due to relatively high mortality rates resulting from dehydration in Batch 1, Batch 2 geckos were individually shipped in cloth bags suspended in a crate. Each bag contained shredded paper and several crickets; no mortality was noted in Batch 2. A total of 50 geckos from Batch 1 and 60 geckos from Batch 2 were analyzed for this study. The remaining 40 individuals died in transit or upon arrival, were morbid and excluded, or were used in other research.

Housing and sample collection

Upon arrival Batch 1 geckos were individually housed (10 days) until a fecal sample was acquired for Salmonella culture. Once a fecal sample was obtained, the individuals were randomly assigned to different cage groups to investigate the role of density on Salmonella prevalence and serotype diversity. Group A represented low density and contained 5 animals, Group B represented medium density and contained 15 animals, and Group C represented high density and contained 30 animals (Table 1). Batch 2 arrived in June and the geckos were again housed individually for fecal sample collection. Following sample collection, 15 geckos were assigned to each of the pre-existing Groups (A, B, and C) and a fourth, Group D, was created. Group D contained 15 animals (low density). All groups were housed in a single room in group cages that each measured 24×36×72 inches. The temperature and humidity were maintained at 26.6°C ± 4°C and 50–70%, respectively, with a 12-h light/dark cycle. The animals were monitored twice daily and fed a standard diet of crickets and mealworms. All procedures were reviewed and approved by the UGA Institute of Animal Care and Use (#A2008 12-051).

At the end of the study in September, all geckos were euthanized by first administering 30 mg/kg of ketamine intramuscularly, followed by intracardiac administration of sodium pentobarbital and decapitation. A fecal sample was collected directly from the gastrointestinal tract of each individual and submitted to the Athens Diagnostic Laboratory for isolation of Salmonella. We were unable to track Salmonella serotypes in individual animals once sorted into groups because attempts to mark the geckos with individual markers failed. As a result, the samples collected from individuals upon arrival in Batches 1 and 2 were combined in all analyses.

Salmonella isolation and identification

Fecal samples were enriched by inoculation in dulcitol selenite broth (prepared in house) and incubated at 41.5°C for 18 h. Enrichment broths were streaked onto Salmonella selective media XLT4, BGN, and MacConkey medium, followed by 37°C incubation overnight. Colonies with colony morphologies indicative of Salmonella were isolated and further characterized with biochemical tests. Those confirmed as Salmonella where further serogrouped. DNA was extracted from all initial serovar determinations and real-time PCR was carried out for the detection of Salmonella (Daum et al. 2002). A delayed-secondary enrichment was done for samples that cultured negative following primary enrichment and that were PCR-positive (Rigby and Pettit 1980). Salmonella isolates were forwarded to the National Veterinary Service Laboratory (NVSL) at Ames, Iowa, for definitive serotyping.

As part of a concurrent study on the diversity of Enterobacteriaceae of Tokay geckos, a loop of an aliquot of the
**Table 1. Salmonella enterica Isolated from Tokay Geckos (Gekko gecko) Imported from Indonesia**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Group</th>
<th>No. positive/no. sampled (%)</th>
<th>Salmonella species and subspecies</th>
<th>Serotypes (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (March, upon arrival)</td>
<td>A</td>
<td>4/5 (80)</td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. arizonae</td>
<td>Oslo (1)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4/15 (27)</td>
<td>S. enterica subsp. enterica</td>
<td>Adelaid (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. arizonae</td>
<td>Weltevreden (1)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>13/30 (43)</td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Oslo (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Apapa (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Bangkok (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Orientalis (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>Weltevreden (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>Rubislaw (1)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>21/50 (42)</td>
<td>S. enterica subsp. houtenae</td>
<td>11 unique serotypes</td>
</tr>
<tr>
<td>2 (July, upon arrival)</td>
<td>A</td>
<td>3/15 (20)</td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (2)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>4/15 (27)</td>
<td>S. enterica subsp. enterica</td>
<td>Apapa (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Weltevreden (1)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>7/15 (47)</td>
<td>S. enterica subsp. enterica</td>
<td>Newport (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>43:z4;23:- (4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>14/45 (31)</td>
<td>S. enterica subsp. houtenae</td>
<td>6 unique serotypes</td>
</tr>
<tr>
<td>1+2 (September at necropsy)</td>
<td>A</td>
<td>9/15 (60)</td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (4)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>9/17 (53)</td>
<td>S. enterica subsp. houtenae</td>
<td>43:z4;23:- (9)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>16/29 (55)</td>
<td>S. enterica subsp. houtenae</td>
<td>Newport (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>Apapa (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>Waycross and Rough_O:z4;23:- (1)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>34/61 (56)</td>
<td>S. enterica subsp. houtenae</td>
<td>8 unique serotypes</td>
</tr>
<tr>
<td>2 (July, upon arrival)</td>
<td>D</td>
<td>6/15 (40)</td>
<td>S. enterica subsp. enterica</td>
<td>Fresno (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>Eastbourne (1)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>3 unique serotypes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (September, at necropsy)</td>
<td>D</td>
<td>11/15 (73)</td>
<td>S. enterica subsp. enterica</td>
<td>Apapa (6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. arizonae</td>
<td>Rough_O:m,t:- (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>61:kz35 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>S. enterica subsp. houtenae</td>
<td>43:z4;23:- (2)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4 unique serotypes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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1 Thirty-four individuals in Batches 1 and 2 died between placement in groups and the end of the study in September.
identified as Rough serotypes. Four isolates lacked an O antigen and were used. Third-generation cephalosporins in developed and developing countries where some of the latest antimicrobials might not be available, and where older drugs (chloramphenicol, amoxicillin, and trimethoprim-sulfamethoxazole) are still commonly used.

**Results**

A total of 88 *Salmonella* isolates were obtained from the 110 geckos surveyed, representing 14 serogroups and 17 unique serotypes. Two different serotypes were recovered from three geckos (Table 1). Four isolates lacked an O antigen and were identified as Rough serotypes. Upon arrival at UGA, *Salmonella* was isolated from 21 of 50 (42%) Batch 1 geckos, and 20 of 60 (33%) Batch 2 geckos (Table 1). *Salmonella* DNA was detected via PCR from an additional three Batch 1 geckos and nine Batch 2 geckos upon arrival; however, the organism could not be isolated. Overall, the prevalence increased significantly between initial surveys done upon arrival at UGA and the end of the study (logistic regression: chi-square = 8.55, p = 0.0031). The prevalence of *Salmonella* in the 61 individuals that survived the study was 56%. Individuals in Groups A, B, and C exhibited a significantly higher prevalence at the end of the study (chi-square = 5.377, p = 0.0204), while Group D exhibited a moderate, but insignificant, increase in prevalence (chi-square = 3.394, p = 0.0654).

Geckos in Groups A, B, and C harbored 13 unique serotypes upon arrival, but only 8 at the end of the study; 5 serotypes were detected both upon arrival and at the end of the study (Fresno, Apapa, Newport, 43z4z23-, and 61zKz35). Nine serotypes originally recovered at arrival were not detected at the study’s close (Adelaide, Oslo, Weltevreden, Apapa, Bangkok, Orientalis, Rubislaw, and 44z4z32), but two new serotypes were (Waycross and Rough_O_4z4z23-). In contrast, only three serotypes were recovered from Group D geckos upon arrival, and five were recovered at the study’s close. Within Group D, serotype 43z4z23- was the only one recovered both at arrival and the close of the study. Fresno and Eastbourne were only detected at arrival, and Apapa, Rough_O_4z4z23-, Rough_O_m1z2-, and 61zKz35 were detected at the study’s end. Despite apparent shifts detected over the course of the study, serotype richness and composition did not significantly differ between arrival and the close of the study (richness: matched-pairs t-test: t10 = -1.00, p = 0.391; composition: multiple response permutation procedure (10,000 permutations): δ = 6.531, p = 0.171). There was no effect of group (cage density) on prevalence at the end of the study (logistic regression groups A, B, and C: chi-square = 0.442, p = 0.506).

Six *Salmonella* isolates (6.8%) expressed resistance to more than one antibiotic (Table 2). All Adelaide isolates were resistant to nalidixic acid and sulfisoxazole. Two S. enterica subsp. arizonae 61zKz35 isolates expressed resistance. One expressed resistance against ampicillin and sulfisoxazole, and the other isolate was resistant to streptomycin and sulfisoxazole (Table 2). Forty-three additional isolates expressed resistance against sulfisoxazole, and 53 isolates displayed a decreased susceptibility (MIC = 8) to chloramphenicol.

**Discussion**

Similarly to previous studies of some other reptile species, we detected a high prevalence and serotype diversity of *Salmonella* (Cambre et al. 1980; Onderka and Finlayson 1985;
Mader 1996; Pedersen et al. 2009). However, this study represents the first attempt to identify changes occurring after importation in *Salmonella* prevalence and serotype diversity associated with reptiles collected from the wild, shipped internationally, and held in captivity. Initially, we attempted to individually mark geckos by a variety of techniques; unfortunately all of them failed, and thus data were only analyzed by group. Despite this limitation, we isolated *Salmonella* from a high percentage of the geckos, and detected a significant increase in prevalence after time in captivity and a change in serotype richness.

Group prevalence ranged from 31–73%, which is relatively high compared to previous reports of *Salmonella* in Geckonidae (reported prevalence ranged from 2–48%) (Murphy and Myers 1993; Cyriac and Wozniak 2000; Callaway et al. 2011). Perhaps the most intriguing finding was the significant increase in prevalence from the time of arrival to the close of the study. Wild reptiles generally have a lower prevalence of *Salmonella* than those held in captivity (Mitchell et al. 2000; Saelinger et al. 2006; Scheelings 2008). Our findings confirm this pattern and further suggest that conditions associated with captivity (e.g., high density, stress, and temporary anorexia) may encourage shedding of *Salmonella* in reptiles. We did not find a significant effect of density on prevalence in this study; however, we believe this is probably the result of small sample sizes and a lack of replicates. Upon capture, geckos are immediately held at unnaturally high densities during transport to the farm, at the farm prior to export, and during shipping. We were unable to study the geckos in Indonesia prior to export, but suspect that the early stages of the trade route may be where the effects of density on both stress and infection are greatest. An explanation for the increase in the overall prevalence, but no increase in prevalence between different density treatments, may be that stress from cohabitating was enough to induce a change in the normal flora and promote *Salmonella* shedding. Indeed, research on poultry and livestock, for which *Salmonella* has been extensively studied, demonstrate that mixing of animals under dense conditions induces stress and causes increased fecal shedding of pathogenic bacteria (including enterotoxigenic *E. coli* and *Salmonella*) (Jones et al. 2001; Callaway et al. 2011). Hierarchical animals and those with largely solitary behaviors in the wild, like Tokay geckos, may be especially susceptible to density-induced stresses in captivity.

Fighting among pigs to establish a dominance hierarchy is common in high-density piggeries and is linked to reduced body weight and decreased resistance to bacterial infection (Kelley 1980; House et al. 1988; Morrow-Tesch et al. 1994). Tokay geckos are solitary in the wild, coming together only to mate, and males are renowned for their highly territorial and aggressive behavior. Shipping, handling, and housing such animals at high densities, a common practice to reduce costs in the pet trade, has the potential to induce significant stresses that increase shedding and ultimately the transmission of bacteria. Although no significant change in serotype richness and composition was detected, a change in serotype dominance was noted.

Certain reptile-associated serotypes (i.e., subspecies *arizonae* and *houtteui*) represented a higher proportion of the isolates at the end of the study compared with initial sampling at arrival. The reasons for this change are unknown, but it is possible that geckos arrived transiently-infected with serotypes that they acquired through close contact with other animals and/or humans over the trade route or their food source.

Many of the serotypes recovered from the geckos in this study have previously been identified in other hosts around the world. Serotype Newport is the third most common serotype associated with salmonellosis in humans in the U.S. (Centers for Disease Control and Prevention 2008), and has been commonly isolated from poultry sources, other food production animals, and humans (Baudart et al. 2000). Serotype Weltevreden is common in geckos, well water, animal products, and vegetables throughout Southeast Asia, Nigeria, and the Pacific region (Oboegbulem and Iseghohimhen 1985; Tsen et al. 1991; Murphy and Myers 1993; Thong et al. 2002; Ristori et al. 2007; Callaway et al. 2011). Weltevreden was the most common cause of human salmonellosis from 1993–2002 in Thailand (Bangtrakulnonth et al. 2004), and in the U.S. is typically associated with foods imported from Southeast Asia (Galalis et al. 2006; Zhao et al. 2006; Ponce et al. 2008). Serotype Adelaide has been cultured from poultry, Rubislaw has been found in food products ranging from black pepper to meat and fish, and Oslo appears most common in humans, but has also been detected in cockroaches (Chmel and Armstrong 1976; Onderka and Finlayson 1985; Devi and Murray 1991; Centers for Disease Control and Prevention 2008). Serotypes Houten, Apapa, and *Salmonella enterica* subspecies *arizonae* are more commonly associated with reptiles, but have been reported to cause salmonellosis in humans (Sanyal et al. 1997; Woodard et al. 1997; Willis et al. 2002; Cooke et al. 2009; Haase et al. 2011). Many reptile-associated serotypes reported in previous cases of human salmonellosis in the U.S. were not identified in this study, including *Salmonella* serotypes Nima, Paona, Sandiego, St. Paul, Tennessee, Typhimurium, IV 44:*Z*z1*, and IV 48:*Z*z2*:i:: (formerly S. Marina) (Mermín et al. 1997; Levy et al. 1999; Reporter et al. 2003; Weiss et al. 2011).

The higher number of serotypes in Batch 1 versus Batch 2 may be related to two factors. Each batch was collected from different locations outside Jakarta (though both were from rural villages), and Batch 1 suffered increased mortality and morbidity during shipment (apparently due to the packing method used). It is possible that the additional stress on Batch 1 resulted in an increased diversity of serotypes being shed at detectable levels. Future work on the impact of shipping stress or packing conditions on *Salmonella* prevalence and serotype diversity shed by reptiles should be conducted, especially given the potential public health implications for the various handlers along the trade route, and for consumers. Reptile-associated salmonellosis accounts for roughly 5% of all human cases in the U.S., 74,000 cases annually (Centers for Disease Control and Prevention 2003). In 1975 a commercial ban on the sale of turtles less than 4 inches in size was established to reduce the number of reptile-associated salmonellosis cases. This ban decreased the number of cases by an estimated 100,000 cases per year (Cohen et al. 1980). However, as the popularity of reptiles as pets has increased in recent years (Shepherd 2008), so has the incidence of reptile-associated salmonellosis (Mermín et al. 2004).

Worldwide, there is a wide range of antibiotic resistance patterns expressed by *Salmonella* species isolates. The geckos in this study may have been exposed to antimicrobial-resistant bacteria of domestic animals or humans in the peri-domestic setting in which they are typically found. In general, antibiotic resistance is a problem worldwide among the
family Enterobacteriaceae (Nordmann 2006). In Indonesia, antibiotics are readily available that may contribute to the selection of antibiotic-resistant strains (Simanjuntak et al. 2004), and there has been an increase in the prevalence of resistance against antibiotics for Salmonella and other Enterobacteriaceae (e.g., Klebsiella pneumoniae and Escherichia coli; Okeke et al. 2005; Hawser et al. 2009). In our study, the high prevalence of resistance to sulfisoxazole was not surprising, as other studies have commonly detected sulfonamide resistance (Corrente et al. 2004; Ebani et al. 2005; Zhao et al., 2005). We detected a high prevalence of decreased susceptibility to chloramphenicol, which has been reported to be common among some studies of reptile isolates (MacNiel et al. 2010), and rare among others (Ebani et al. 2005; Chen et al. 2010). The use of amphenicols is relatively high in Indonesia; chloramphenicol and thiampenicol represent 6% of courses taken by adults and 12% by children (Hadi et al. 2008), compared to their limited use in most developed nations due to resistance and health risks. In the current study, only a small number of isolates were resistant to multiple antibiotics. The four Adelaide isolates were resistant to nalidixic acid and sulfisoxazole. Nalidixic acid resistance has been reported at low prevalence among reptile Salmonella isolates (Corrente et al. 2004; Chen et al. 2010), and is important, given that nalidixic acid is a drug that is used to treat salmonellosis in adults. Interestingly, resistance to streptomycin was rare, which is in contrast to other studies of reptiles (Corrente et al. 2004; Chen et al., 2010).

Diagnostic testing to identify Salmonella in reptiles is widely recognized to be hampered by intermittent shedding of isolates, such that negative results do not necessarily indicate that the reptile is Salmonella-free. This, in addition to the frequent colonization of reptiles with Salmonella, suggests that detecting and eliminating the bacteria from pet reptiles is not a viable solution for preventing transmission to other pets, wildlife, livestock, or humans. Studies have examined oral vaccination and probiotics to treat Salmonella in reptiles, but significant advances in these areas have not yet been made (Mitchell et al. 2001; Holz and Middleton 2005). Ultimately, the pet industry and pet owners have to take responsibility for their own health and educate individuals to whom they sell reptiles or trade with. Salmonellosis is a major cause of illness in the U.S., and cases caused by reptile-associated serotypes are increasing in frequency, particularly among infants (Olsen et al. 2001). Guidelines have been established to help prevent salmonellosis in reptile owners (see those from the Association of Reptilian and Amphibian Veterinarians and the Centers for Disease Control and Prevention), and consumer education initiatives are reaching more groups (e.g., PetWatch and the CDC’s Healthy Pets Healthy People).

The increase in prevalence detected in this study and the apparent shift in serotype richness may be the result of several factors: increased shedding due to stress associated with trade, transport, and captivity; increased transmission resulting from unnaturally high densities of animals packed in shipments and held in cages post-arrival; or contact with other infected species (including humans) along the trade route. It is impossible to differentiate between these mechanisms in the present study, though each is likely to contribute to the changes in Salmonella colonization detected. Future studies to differentiate the physical, social, and physiological effects of trade-related conditions on Salmonella shedding and transmission among reptiles will benefit the industry by identifying ways to reduce mortality, and safeguard the individuals handling animals along the transport chain and other species encountered en route.

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Author Disclosure Statement

No competing financial interests exist.

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