Prevalence of Salmonella in green anoles (Anolis Carolinensis), an invasive alien species in Naha and Tomigusuku Cities, Okinawa Main Island, Japan

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ABSTRACT. Here, we investigated the prevalence of Salmonella enterica, with and without resistance to 17 common antimicrobial agents, in 706 green anoles (Anolis carolinensis) that were collected in Naha and Tomigusuku Cities, Okinawa Main Island, Japan, between 2009 and 2014. Salmonella strains, including S. enterica Weltevreden and Enteritidis serovars, were identified in the large intestinal content samples extracted from 15 (2.1%) of the analyzed green anoles. No antimicrobial resistance was detected. Thus, the present study demonstrates that although the prevalence of Salmonella and the risk of its transmission from the green anoles to humans or other animals on Okinawa Main Island are relatively low, the green anole population nevertheless represents a potential source of Salmonella infection that could affect human health in this region.

KEY WORDS: green anole, invasive alien species, Okinawa Main Island, Salmonella Enteritidis, Salmonella Weltevreden

The green anole (Anolis carolinensis) is a lizard that is native to North and Central America, but it has now spread to become an invasive species in several other countries [3, 10, 11]. It was introduced to the Chichi and Haha Islands, in the Ogasawara Archipelago of Japan, in the 1960s and 1980s, respectively, and to Okinawa Main Island in the 1990s [15, 18]. Its feeding behaviors caused the collapse of insect and other reptile populations on these islands; thus, it was listed as an Invasive Alien Species in Japan in 2005 [9].

Previous studies have shown that a proportion of the green anole populations in Florida (7.5%) [6], Guam (76.2%) [4], and Chichi Island (34.2%) carry Salmonella spp. [17]. However, to date, no study has investigated Salmonella infection rate in green anoles in Okinawa Main Island. Therefore, the aim of the present study was to investigate the prevalence of Salmonella in the green anoles that were collected on Okinawa Main Island over a 6-year period, to characterize Salmonella infection dynamics and support risk evaluation and pathogen management strategies in this region.

We analyzed 706 (frozen: −20°C) green anoles collected (by a company selected by the Ministry of the Environment) from the following three areas in Naha and Tomigusuku Cities, where the green anoles are frequently sighted: area A, a residential area besides Makabi Basin in Naha; area B, a residential area and city parks near the Japan Ground Self-Defense Force Camp in Naha; and area C, a residential area in Northwest Tomigusuku City in Okinawa Prefecture, between July 2009 and January 2015 (Fig. 1). These individuals were thawed to room temperature (the period of examination from the date of capture was 574.9 days on an average, with a minimum of 174 days and maximum of 1,202 days), and 0.1 g of their large intestinal content sample was extracted and incubated (37°C) in 5 ml of buffered peptone water (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for 24 hr. A 0.5 ml aliquot of each culture was then transferred into 4.5 ml of Rappaport Vassiliadis broth (Nissui Pharmaceutical Co., Ltd.) and incubated at 37°C for 20 hr, and then cultured at 37°C for 20 hr on Salmonella-Shigella agar (Nissui Pharmaceutical Co., Ltd.) as a secondary enrichment culture. Colonies (a single colony was picked from one sample) were screened using triple-sugar-iron and lysine-indole-motility media (Nissui Pharmaceutical Co., Ltd.), and subsequently identified using the ID-Test·EB-20 system (Denka Seiken Co., Ltd.). The χ² test was used to compare the prevalence of Salmonella in green anole samples obtained from the three areas.

The drug susceptibility of the isolates was determined as recommended by the Clinical and Laboratory Standards Institute [2].
Specifically, the Kirby Bauer disk diffusion method was used to determine the susceptibility of isolates to antimicrobial drugs that are widely used in clinical and agricultural (i.e., for domestic animal use and as feed additives) settings, comprising ampicillin, piperacillin, cefozopran, kanamycin, gentamicin, oxytetracycline, ofloxacin, chloramphenicol, nalidixic acid, fosfomycin, sulfamethoxazole-trimethoprim, streptomycin, cefazidime, ceftriaxone, cefotaxime, cephalexin, and cefturoxime (BD, Tokyo, Japan). The diameter (mm) of the growth inhibition zone around each antimicrobial agent disk was measured using precision callipers, and the isolates were categorized as resistant or non-resistant to each antimicrobial agent using standard methods [2].

We identified *Salmonella* in 15 (2.1%) of the 706 green anoles that were captured during the study, with 4% (7/176), 1% (1/101), 0% (0/129), 2% (2/100), 4% (4/100), and 1% (1/100) of the 15 individuals captured in 2009, 2010, 2011, 2012, 2013, and 2014, respectively. Furthermore, the *Salmonella* strain in nine green anoles (that were collected in 2009, 2010, 2012, 2013, and 2014) was identified as *S. enterica* serovar Weltevreden, whereas that in six green anoles (that were collected in 2009, 2012, and 2013) was identified as serovar Enteritidis (Table 1). The prevalence of *Salmonella* in area B was significantly low when compared with that in the other areas. *Salmonella* strains detected in all the 15 green anoles were sensitive to the tested antimicrobial agents.

The estimated prevalence of *Salmonella* in the green anole population in Okinawa Main Island was low (2.1%) compared with that in populations in North America (7.5%), Guam (76.2%), and Chichi Island (34.2%), and with that in wild and pet reptiles in Japan reported previously (25.3%, 74.1%) [4–6, 14, 17]. This is consistent with the fact that the green anole invaded Guam approximately 60 years ago, which is sufficient time to establish the infection cycle of *Salmonella* originating in the soil and/or stools of other wild animals [4]. Similarly, the green anole was introduced to the Chichi Island 30–50 years ago, and since then *Salmonella* has been isolated from wild goats and birds, as well as from public toilets [17]. The habitat of the green anole (and other reservoir animals) in these areas has been suggested to render them vulnerable to *Salmonella* infection. Our results suggest that similar interactions between the green anoles and other infected animals and/or environment have not frequently occurred in Okinawa Main Island. However, the prevalence of *Salmonella* was significantly high in areas A and C with more residential sites.

![Fig. 1. Collected areas of Green anole in Okinawa Main Island in this study. Area A and B were located in Naha City, and area C was located in Tomigusuku City.](image)

### Table 1. *Salmonella* isolates (including serovars) identified among green anoles collected on Okinawa Main Island, Japan

| Collection year | Sampling area | Ratio of positive/total samples (%) | Identified serovars | Number of positive samples collected (Area) |
|-----------------|---------------|-----------------------------------|---------------------|------------------------------------------|
|                 | A             | B                                 | C                   |                                          |
| 2009            | 6/57 (10.5)   | 1/119 (0.8)                      | 0/7 (0)            | 7/176 (4.0) S. Weltevreden 3 (A), 1 (B)     |
|                 | 1/25 (4.0)    | 0/69 (0)                         | 0/7 (0)            | 1/101 (1.0) S. Enteritidis 3 (A)           |
| 2010            | 0/68 (0)      | 0/59 (0)                         | 0/2 (0)            | 0/129 (0) S. Weltevreden 1 (A)             |
| 2011            | 0/14 (0)      | 1/66 (1.5)                       | 1/20 (5.0)         | 2/100 (2.0) S. Weltevreden 1 (C)           |
| 2012            | 1/38 (2.6)    | 0/38 (0)                         | 3/24 (12.5)        | 4/100 (4.0) S. Enteritidis 1 (B)           |
|                 | 0/20 (0)      | 0/60 (0)                         | 1/20 (5.0)         | 1/100 (1.0) S. Weltevreden 1 (C)           |
| 2013            | 8/222 (3.6)   | 2/411 (0.5)                      | 5/73 (6.8)         | 15/706 (2.1) S. Weltevreden 1 (C)          |
| Total           | 15/706 (2.1)  |                                   |                     |                                          |
than area B (where the green anoles were captured beside a force camp area, which is not a human residential area). Therefore, their presence in residential areas is a concern for public health.

*Salmonella enterica* serovar Weltevreden and Enteritidis, which were isolated from 15 green anoles in the present study, have been associated with cases of bacterial food poisoning in Okinawa Prefecture [12]. Among these cases of human food poisoning in Okinawa Prefecture, only two cases were due to *S. enterica* Weltevreden and the others were due to *S. enterica* Enteritidis [13]. On the contrary, studies on *Salmonella* prevalence in livestock and wild animals in Okinawa Prefecture have reported *S. enterica* Oranienburg in cattle, *S. enterica* Weltevreden in Muridae members, and *S. enterica* Enteritidis in mongoose [7]. Interestingly, *S. enterica* Oranienburg serovar that was frequently identified in the green anoles in Chichi Island (94.4%) was not identified in the present study [17]. This suggests that the green anoles analysed here were infected with *Salmonella* after their invasion to Okinawa Main Island. Further research is needed to determine whether the identified *Salmonella* serovars were originally carried by or, alternatively, gained by the green anoles when they invaded Okinawa Main Island, by comparing *Salmonella* genotypes in Okinawa Main Island with those in other areas.

Recent studies have reported the emergence of antimicrobial resistance (AMR) among bacteria that are carried by wild animals, possibly due to antimicrobial drug use in clinical and agricultural settings [1, 8]. For example, a previous study estimated the prevalence of *AMR Salmonella* to be 6.8% in wild Tokay geckos (*Gekko gecko*) imported to the US from Indonesia [16]. In the present study, we did not identify AMR *Salmonella*, suggesting that such factors have no effect on the green anole population in Okinawa Main Island. This indicates that the risk of spreading of AMR *Salmonella* from the green anoles to other wild animals and humans in this region is low.

We conclude that the prevalence of *Salmonella* was low and that there was no AMR *Salmonella* in the green anoles in Okinawa Main Island. However, notably, in the present study, we detected an *S. enterica* serovar that is well established as a cause of food poisoning in humans. Furthermore, the present findings support that while the green anoles may represent an important epidemiologic source of human *Salmonella* infections, they may conversely be negatively affected by human activities. Thus, continuous research efforts are essential to control *Salmonella* infection in the green anoles and to characterize the transmission mode of infections between human environments and wild animals, as it could affect both human and ecological health in Okinawa Main Island.

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