Prevalence and antimicrobial-resistance profiles of *Salmonella* spp. isolated from green anoles (*Anolis carolinensis*) collected on the Haha-jima of the Ogasawara archipelago, Japan

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**ABSTRACT.** We investigated the prevalence of *Salmonella enterica* and its antimicrobial resistance from 79 green anoles, the invasive alien species inhabits Haha-jima of the Ogasawara archipelago. Samples were collected during the period between 2009 and 2010. The resistance of *S. enterica* of these samples against 12 common antimicrobial agents was also determined. *Salmonella* strains, including serovar Oranienburg and Aberdeen, were detected from the large intestines of 30.4% of 79 green anole samples. And 37.5% of which were resistant to Oxytetracycline. This study suggests that green anoles may play an important role of the infection of *S. enterica* on this island. Attention is needed from the aspect of public and ecological health.

**KEY WORDS:** green anole, Haha-jima, invasive alien species, *Salmonella enterica* Oranienburg, zoonosis
Colonies were screened using triple-sugar-iron and motility-lysine-indole media (Nissui Pharmaceutical Co., Ltd.) and were subsequently identified using the ID-Test EB-20 system (Nissui Pharmaceutical Co., Ltd.). Serotyping was carried out according to the Kauffmann-White scheme (Denka Seiken Co., Ltd., Tokyo, Japan).

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 susceptibility to antimicrobial drugs that are widely used in clinical and agricultural settings. The antimicrobial tested included ampicillin (ABPC), piperacillin (PIPC), cefozopran (CZOP), kanamycin (KM), gentamycin (GM), oxytetracycline (OTC), ofloxacin (OFLX), chloramphenicol (CP), nalidixic acid (NA), fosfomycin (FOM), sulfamethoxazole-trimethoprim (ST), and streptomycin (SM). The diameter (mm) of the zone of growth inhibition around each antimicrobial disk was measured using precision calipers and isolates were categorized as resistant or susceptible to each antimicrobial agent using standard methods [2]. Pulsed-field gel electrophoresis (PFGE) was performed using S. enterica serovar Braenderup H9812 as the standard [7] with seven and one samples from green anole and wild birds collected in Haha-jima, respectively, that were positive for S. enterica serovar Oranienburg. PFGE profiles were interpreted by visual analysis, and profile photographs were then scanned and analyzed using BioNumerics software (Applied Maths NV, Sint-Martens-Latem, Belgium). Similarities were determined using the Dice coefficient with clustering based on the unweighted pair group method using arithmetic averages [18].

Salmonella was identified in 24 (30.4%) of the green anole samples and two (6.1%) of the wild bird fecal swabs. Furthermore, 23 (95.8%) of the Salmonella isolates from green anoles and one (50.0%) from wild bird feces were identified as S. enterica serovar Oranienburg; a single Salmonella isolate (4.2%) from the green anole samples and a single sample (50.0%) from wild bird feces was identified as S. enterica serovar Aberdeen (Table 1). Nine strains (37.5%) from the green anole samples were resistant to
ORC, but no AMR was detected in the Salmonella isolated from the wild bird samples. The PFGE patterns of S. enterica serovar Oranienburg from samples collected on Haha-jima and Chichi-jima, and from green anole samples, wild bird fecal samples, and toilet bowl samples (green anole and toilet bowl samples from Chichi-jima were collected on 2007 to 2009 using our previously reports [17]), were identical (Fig. 2).

S. enterica serovar Oranienburg has been identified as the causative agent of food poisoning in humans and caused a large-scale outbreak in mainland Japan in 1999 [12]. Despite this one large outbreak, S. enterica serovar Oranienburg is an uncommon serovar of food poisoning in Japan [13]. However, a recent case report by the Society of Public Health, Social Welfare and Medical Service of Tokyo Metropolitan Government found that 29 of 32 food poisoning cases in Chichi-jima were attributed to S. enterica serovar Oranienburg, with just one being attributed to another S. enterica serovar (serovar Weltevreden) [8]. Furthermore, S. enterica serovar Oranienburg has been isolated from wild goats (93.0%), soil, water, and public toilets [17]. The infectious vector was unclear in these cases, but it was suggested that green anoles played an important role in the Salmonella infection cycle on Chichi-jima. There have been no reports of food poisoning on Haha-jima [8], but our results indicated that attention should be paid to green anoles on this island as they likely harbor pathogenic Salmonella. The PFGE pattern of S. enterica serovar Oranienburg isolated from green anoles on Haha-jima and Chichi-jima were identical, suggesting that this serovar was introduced to Haha-jima from Chichi-jima with green anoles at the same time. Furthermore, the PFGE patterns of isolates from wild bird fecal samples and green anole samples were the same, indicating that the infection cycle had been established among green anoles and wild birds in this area. It has been reported that green anoles are hunted by wild birds in both islands [21], which suggests a possible route for the spread of Salmonella infection. We suspect that similar interactions between green anoles and other infected animals and/or environments may not yet have occurred on Haha-jima, but further study is needed to verify this.

Recent studies have reported the emergence of AMR among bacteria that are carried by wild animals, possibly as a result of antimicrobial use in clinical and agricultural settings [1]. The S. enterica serovar Oranienburg identified in this study showed OTC resistance, suggesting a relationship between the use of these antimicrobials on livestock farms on this island. Further research will have to be performed to investigate the prevalence and identity of Salmonella in environmental samples (i.e., soil and water) and other animals on this island.

In this study, we determined the prevalence and AMR profiles of Salmonella associated with green anoles in Haha-jima and Chichi-jima. The population density of green anoles on Chichi-jima and Haha-jima is high (almost 1,000 heads/10,000 m² [14]) with the lizards roaming freely in and around houses. The human population density of Haha-jima is about one-fifth that of Chichi-jima, and Haha-jima also receives about one-fifth as many tourists as Chichi-jima (7,000 and 30,000 tourists visit Haha-jima and Chichi-jima, respectively, each year) [19]. However, green anoles harboring a high prevalence of Salmonella have multiplied, and it is therefore necessary to raise public awareness on both islands about the potential public health issue posed by these lizards.
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