The Journal of Veterinary Medical Science

NOTE

Wildlife Science

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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J. Vet. Med. Sci.
82(10): 1558–1561, 2020
doi: 10.1292/jvms.19-0632

Received: 25 November 2019
Accepted: 21 August 2020
Advanced Epub:
16 September 2020

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

The green anole (*Anolis carolinensis*) is a lizard native to North and Central America, but it has become an invasive species in several other regions of the world [4, 10, 11]. It was also introduced to Chichi-jima and Haha-jima of the Ogasawara archipelago of Japan in the 1960s and 1980s, respectively, and likewise, to Okinawa Main Island in the 1990s [15, 20]. Its feeding behaviors have caused the populations of insects and other reptiles to collapse on these islands; thus, it was listed as an invasive alien species in Japan in 2005 [9].

Furthermore, anoles may also introduce infectious diseases to human and native fauna by acting as reservoirs for pathogens [3]. Invasive species have been known to introduce pathogens that result in the extermination of native species. For example, in the British Isles, the red squirrel (*Sciurus vulgaris*) population is in long-term decline because of a parapoxvirus introduced by invasive grey squirrels (*Sciurus carolinensis*) [16]. Hence, attention should be paid to the pathogens carried by invasive species to protect public and ecological health [3].

Previous studies have shown that a proportion of green anole populations in Florida (7.5%) [6], Guam (76.2%) [5], and Chichi-jima (34.2%) carry *Salmonella bacteria* [17]. Furthermore, in Chichi-jima, the pathogenic *Salmonella enterica* serovar Oranienburg was isolated from feral goats and toilets at rates of 22.0% and 2.6%, respectively [8, 17], indicating that the *Salmonella* infection cycle is already established among wild animals and humans on this island.

However, few studies have investigated *Salmonella* infection rates among green anoles on Haha-jima. Therefore, the aim of the present study was to investigate the prevalence and antimicrobial resistance (AMR) profiles of *Salmonella spp*. isolated from green anoles collected on Haha-jima over two years. This survey characterized the *Salmonella* infection dynamics on the island and the results can inform risk evaluation and pathogen management strategies in this region.

We analyzed 79 frozen (−20°C) green anoles captured by the Japan Wildlife Research Center, with the permission of the Ministry of the Environment, as well as 33 fecal swabs of wild birds (species unknown) collected in Haha-jima, Ogasawara archipelago, Tokyo, between 2009 and 2010 (Fig. 1).

Samples were thawed at room temperature (the period of examination from the date of capture was about 5–30 days), and 0.1 g of the large intestinal contents were extracted and incubated at 37°C in 5 m*l* Enterobacteriaceae Enrichment Mannitol broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) for 24 hr, especially bird fecal samples were incubated one swab directly. A 0.5 m*l* aliquot

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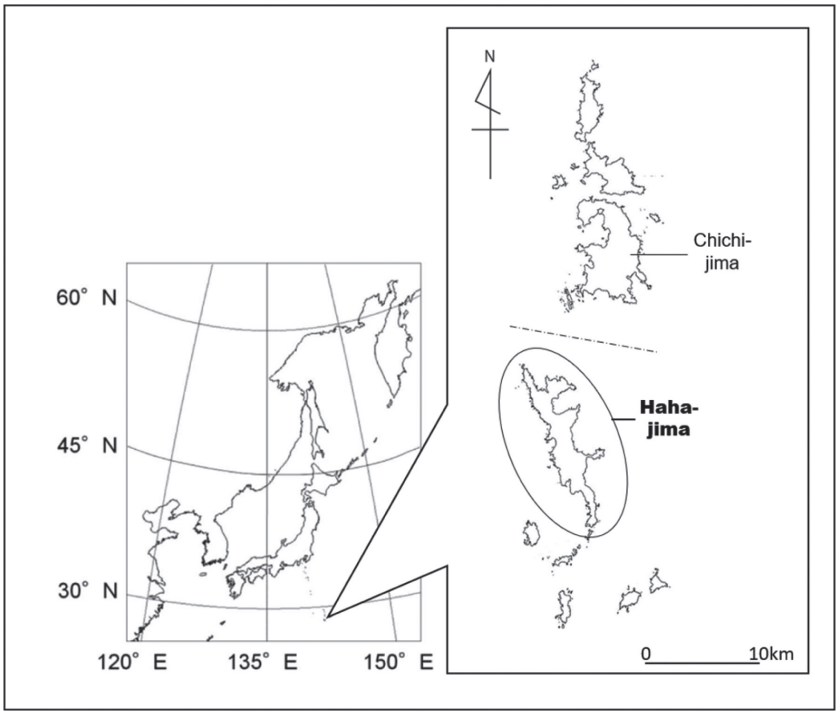

Fig. 1. Location of Haha-jima island with the Ogasawara archipelago. The distance between Haha-jima to Chichi-jima is about 35 linear kilometers (broken line).

Table 1. Salmonella isolates (including serovar) identified among green anoles and wild birds on Haha-jima island

Sample	Ratio of positive/ total samples (%)	Identified serovar	Number of positive samples collected
Green anole	24/79 (30.4)	*S.* Oranienburg	23
		S. Aberdeen	1
Wild bird	2/33 (6.1)	*S.* Oranienburg	1
		S. Aberdeen	1

of each culture was then transferred to 4.5 m*l* of Rappaport-Vassiliadis broth (Nissui Pharmaceutical Co., Ltd.) and incubated at 37°C for 20 hr. Aliquots of these secondary enrichments were cultured on CHROMagar™ Salmonella agar (BD, Tokyo, Japan). 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

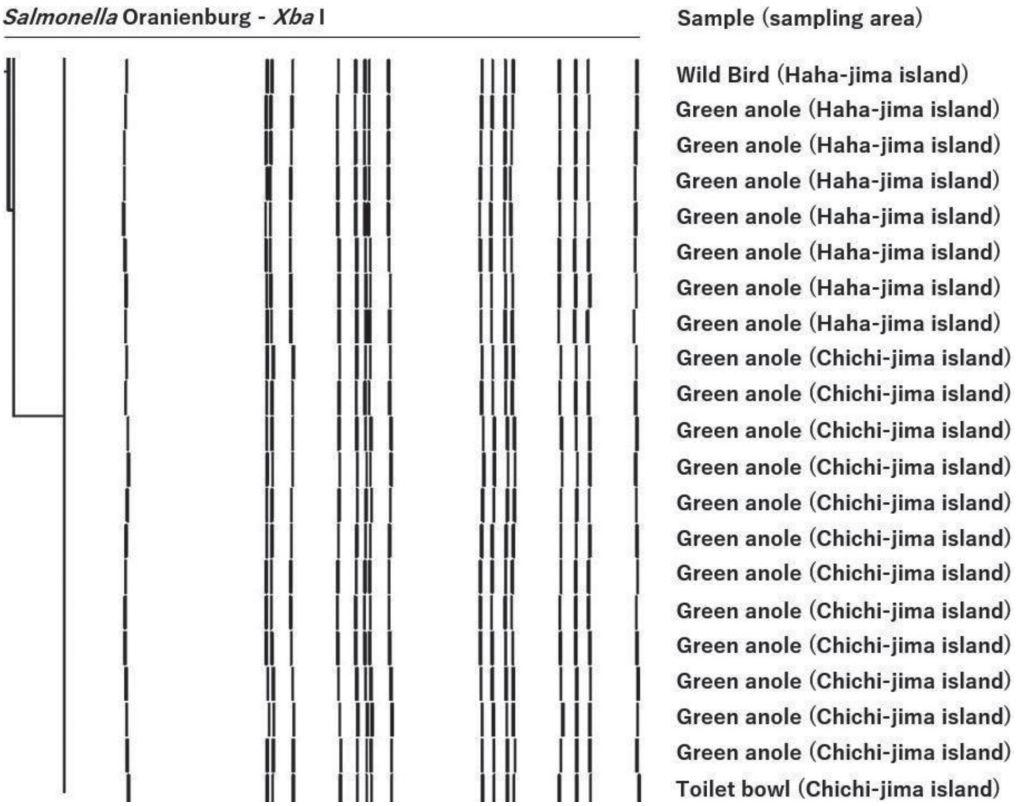

Fig. 2. Pulsed-field gel electrophoresis (PFGE) pattern of *Salmonella* Oranienburg strains cleaved with restriction enzyme *Xba I.*

OTC, 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 m^2 [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.

ACKNOWLEDGMENTS. We thank Mr. Masaaki Takiguci, Mr. Yuji Takafuji, and Mr. Hideaki Mori of the Japan Wildlife Research Center for helping capture and sample the green anoles. We thank Dr. Masafumi Nukina of Kobe Municipal Environmental Hygiene Research Center for assistance with *Salmonella* serotyping and PFGE analysis.

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