

# *Salmonella* Isolates in the Introduced Asian House Gecko (*Hemidactylus frenatus*) with Emphasis on *Salmonella* Weltevreden, in Two Regions in Costa Rica

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## Abstract

The Asian house gecko *Hemidactylus frenatus* has been widely introduced in Costa Rica and tends to establish in human settlements. Some studies in other invaded countries have suggested that this gecko plays a significant role in the epidemiology of salmonellosis and it is of value to public health. To our knowledge, no studies have examined *Salmonella* from this species in Costa Rica. Therefore, we collected 115 geckos from houses in two Costa Rican regions. We examined gut contents for *Salmonella* through microbiological analysis. Presumptive *Salmonella* spp. were sent to a reference laboratory for serotyping and antimicrobial susceptibility testing. Molecular typing was also conducted with the main *Salmonella* isolates of zoonotic relevance in Costa Rica. *H. frenatus* was found in 95% of the houses surveyed. *Salmonella* was isolated in 4.3% of the samples, and four zoonotic serovars were detected. None of the isolates were resistant to the antibiotics most frequently used for salmonellosis treatment in Costa Rica. All *Salmonella* isolates from the lower gut of *H. frenatus* are associated with human salmonellosis. Pulsotypes from *Salmonella enterica* serotype Weltevreden were identical to the only clone previously reported from human samples in Costa Rica. Molecular typing of *Salmonella* Weltevreden suggested that *H. frenatus* harbors a serovar of public health importance in Costa Rica. Results demonstrated that *H. frenatus* plays a role in the epidemiology of human salmonellosis in two regions of Costa Rica. However, more detailed epidemiological studies are needed to understand better the role of the Asian house gecko with human salmonellosis, especially caused by *Salmonella* Weltevreden, and to quantify its risk in Costa Rica accurately.

**Key Words:** Introduced species—Reptiles—Salmonellosis—Zoonotic disease.

## Introduction

THE NONTYPHOIDAL *Salmonella* spp. (*Salmonella enterica*) can cause acute gastroenteritis (human salmonellosis) and represents a public health burden in both developing and developed countries (Majowicz et al. 2010). There are >2500 serovars of *S. enterica* and most of them are zoonotic (Barua et al. 2014). Although *Salmonella* infection in humans is usually caused by consumption of contaminated food (i.e., meat, poultry, or eggs), it can also occur after direct or indirect contact with animals (Ebani et al. 2005, Hoelzer et al. 2011). Many reptiles are known to carry multiple types of *Salmonella* in their gastrointestinal tracts and they may therefore shed these bacteria intermittently in their feces

(Burnham et al. 1998, Mermin 2004, Pedersen et al. 2009). Forty percent of *Salmonella* serovars have been cultured from reptiles and are rarely found in other animals or humans (Mermin 2004). Some studies have suggested that many *Salmonella* serovars are probably commensal organisms in reptiles and retain their pathogenicity for warm-blooded animals (Chiodini and Sundberg 1981, Corrente et al. 2006); however, there is still some debate about the role of *Salmonella* in reptiles (Hidalgo-Vila et al. 2008). Reptiles, and particularly those kept as pets, have been recognized as a source of human salmonellosis, which is responsible for approximately 6% of human *Salmonella* spp. infections (Woodward et al. 1997, Corrente et al. 2004, Mermin 2004). More specifically, introduced house geckos have been

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implicated as a source of *Salmonella* infections (Helm 1981, Steele 2008).

The Asian house gecko, *Hemidactylus frenatus*, is a very successful invasive reptile (Rödger et al. 2008, Hoskin 2011). It is native to Asia and the Indo-Pacific and has colonized many tropical and subtropical areas of the world (Savage 2002). It is remarkably well adapted to and regularly establishes in human settlements and highly human-modified habitats (Lever 2003). Some studies of *H. frenatus* have reported *Salmonella* serovars that are associated with human salmonellosis and have suggested that this gecko plays a significant role in the epidemiology of salmonellosis in invaded places (Chan 1982, Callaway et al. 2011, Nwachukwu et al. 2014). Because this gecko can harbor *Salmonella* associated with human salmonellosis and is commonly found in houses, there is substantial opportunity for disease transmission to humans through contaminated gecko droppings (Singh et al. 2013, Nwachukwu et al. 2014).

In Costa Rica, the Asian house gecko was introduced more than two decades ago; it now appears widespread in most of the country and tends to establish in human settlements (Abarca 2006). Nonetheless, Costa Rican scientists have shown little concern about the introduction of this gecko and its potential risks for human health. According to recommendations of the Office International des Epizooties (OIE), it is important to analyze not only the risk of a nonnative animal becoming invasive but also the risk of pathogens being introduced with the animal (Office International des Epizooties 2012).

To explore the gecko's potential role in the epidemiology of human salmonellosis, the main objectives of this study were to determine the prevalence of *Salmonella* spp. from the lower gut of *H. frenatus* from two regions of Costa Rica, identify the serotypes of *Salmonella* isolates, determine their susceptibility to antimicrobials, and establish the pulsotype of the main *Salmonella* isolates of zoonotic relevance in Costa Rica.

## Materials and Methods

### Sampling and sample collection

We aimed to collect ~120 geckos from five rural towns from the North Pacific and Northwest Caribbean region of Costa Rica (~60 individuals per region; see below) between June, 2013, and February, 2014. These regions were chosen because a previous study had confirmed the isolation of *Salmonella* Weltevreden from human infections in both regions (Bolaños et al. 2012) and the geckos were known to be present. We selected three towns in the North Pacific region: Sardinal, Palmira, and San Blas, and two towns in the Northwest Caribbean region, Puerto Viejo de Sarapiquí and La Virgen. In each region, we identified each neighborhood block from the towns, numbered them, and randomly selected 60 blocks. Then, from each selected block we randomly selected one house to collect one gecko and perform a 10-min survey.

We captured geckos by hand using a new pair of disposable gloves for each captured individual and placed them in individual sterile plastic bags with breathing holes. Captured geckos were transported to the Laboratory of Veterinary Bacteriology at the National University of Costa Rica (UNA) within 2 days of capture. Upon arrival, geckos were eutha-

nized by intracardiac administration of 0.05 mL of T-61 Euthanasia Solution (Hellebrekers 1990). The lower gut was removed aseptically, and culturing procedures for *Salmonella* (see below) were conducted within 1 h of removal.

### Sample size

We selected a sample size of 60 individuals per region using the methods described in Lwanga and Lemeshow (1991) with an estimated proportion of 0.07, a confidence level of 95%, and a precision value of  $\pm 0.065$ . We hypothesized that *Salmonella* proportion (prevalence) would be around 7% on the basis of a previous study of *H. frenatus* in Northern Australia by Callaway et al. (2011). We calculated the sample size using Piface software version 1.76 (Lenth 2007).

### Salmonella isolation

The lower gut of each gecko was weighed and processed separately. Tissues were first homogenized in buffered peptone water solution (BPW) in a 1:10 ratio. Samples were processed using two protocols in parallel, direct plating and with enrichment. For direct plating, we streaked a swab of the mixture onto agar plates with *Salmonella* selective media (Brilliant Green Agar [BGA], and Xylose Lysine Tergitol 4 [XLT4]) and then incubated plates for 24–48 h at 37°C. For the enrichment method, 40  $\mu$ L of the mixture was inoculated into 3 mL of BPW and incubated for 24 h, along with the directly streaked plates. After BPW incubation, 100  $\mu$ L and 1000  $\mu$ L were transferred into 10 mL of Rappaport-Vassiliadis (RV) broth and 10 mL of tetrathionate broth, respectively. Both tubes were then incubated for 24 h at 42°C, after which a swab from each selective-enrichment broth was inoculated onto BGA and XLT4 plates and incubated for 24–48 h at 37°C. After incubation, plates from both protocols were examined for typical *Salmonella* colonies and tested, with the biochemical screening tests Christensen urea, triple sugar iron (TSI), and lysine iron agar (LIA). Strains with a biochemical profile consistent with *Salmonella* were then tested serologically with *Salmonella* Omnivalent antiserum (Denka), and biochemical identification was confirmed by Vitek 2 using a GN ID Card.

### Serotyping, antimicrobial susceptibility testing, and molecular typing

*Salmonella* isolates were sent to the National Bacteriology Referral Center (Centro Nacional de Referencia en Bacteriología [CNRB]) of the Costa Rican Institute for Research and Teaching in Nutrition and Health (Instituto Costarricense de Investigación y Enseñanza en Nutrición y Salud [INCIENSA]), in Cartago, Costa Rica, for serotyping and antimicrobial susceptibility testing (Kirby Bauer/Clinical and Laboratory Standards Institute [CLSI]). The isolates were assigned to different serovars according to the White–Kauffmann–Le Minor Scheme (Grimont and Weill 2007). The results of the antimicrobial analysis were interpreted using guidelines of the CLSI (CLSI M100-S23, 2013). The antimicrobial agents used for sensitivity testing were ampicillin, trimethoprim–sulfamethoxazole, ceftazidime, chloramphenicol, ciprofloxacin, cefotaxime, nalidixic acid, tetracycline, streptomycin, sulfonamides, piperacillin, piperacillin–tazobactam, and nitrofurantoin. The main *Salmonella* isolates of zoonotic relevance in Costa Rica were

TABLE 1. SEROVARS OF *SALMONELLA ENTERICA* SUBSP. *ENTERICA* ISOLATED FROM THE LOWER GUT OF ASIAN HOUSE GECKOS (*HEMIDACTYLUS FRENATUS*), COSTA RICA

Region	Town	Serovar	No. of samples collected	No. of positives
Pacific	Sardinal	Abaetetuba	36	2
	Sardinal	Weltevreden	36	1
	San Blas	Glostrup	13	1
	Bagaces	Adelaide	10	1
Caribbean	Puerto Viejo	Weltevreden	31	1

selected for molecular typing with pulsed-field gel electrophoresis (PFGE) according to the standardized protocol of the PulseNet Latin America and Caribbean Network (Ribot et al. 2006) using the restriction enzyme XbaI. PFGE profiles were analyzed by using Bionumerics software version 5.1 (Applied Maths). The dice coefficient and unweighted pair group with arithmetic mean (UPGMA) with 1.5% band position tolerance were applied to compare the genetic relatedness between isolates.

#### Salmonella prevalence

The prevalence of *Salmonella* spp. from the lower gut of geckos and the 95% confidence interval (CI) was calculated using the package “Prevalence” (Devleeschauwer et al. 2014) in R version 3.1.1 (R Development Core Team 2014).

#### Ethics and permissions

The animal handling and procedures were in accordance with the guidelines established by the Comité de Bienestar Animal y Bioética de la Escuela de Medicina Veterinaria de la Universidad Nacional de Costa Rica and in agreement with the corresponding law Ley de Bienestar de los Animales of Costa Rica (Law 7451 on Animal Welfare). This study was conducted under research permission of the Ministerio de Ambiente y Energía (069-2013-SINAC).

#### Results

A total of 121 houses were visited across the two study regions and 115 geckos were collected. Sixty-three individuals were collected from Sardinal ( $n=36$ ), San Blas ( $n=13$ ),

and Palmira ( $n=14$ ) in the Pacific region. In the Caribbean region, 52 individuals were collected from La Virgen ( $n=21$ ) and Puerto Viejo de Sarapiquí ( $n=31$ ). Collected individuals included 60 females and 55 males. The average mass of the lower guts of geckos was  $0.13 \pm 0.01$  gram (mean  $\pm$  standard error [SE], range 0.12–0.28 gram).

The prevalence of *Salmonella* spp. from the lower gut of the geckos in the Pacific region was 6.5% (4/63; 95% CI 3–12%), in the Caribbean 2% (1/52; 95% CI 0–6%), and for both regions 4.3% (5/115; 95% CI 1–8%). The serotyping of *Salmonella* revealed four serovars from *S. enterica* subsp. *enterica* (Table 1). In anecdotal sampling outside of our study region in Bagaces, Guanacaste, we also isolated *Salmonella* Adelaide from one of 10 geckos collected from a single house, but these individuals were not included in the prevalence analysis.

Two isolates of *Salmonella* Weltevreden were obtained from different regions that showed indistinguishable PFGE patterns (Fig. 1). Both isolates showed the pulsotype CRINJQPX01.0002 using the restriction enzyme XbaI.

None of the *Salmonella* isolates were resistant to the antibiotics commonly used to treat human salmonellosis in Costa Rica, such as trimethoprim–sulfamethoxazole, ciprofloxacin, or cefotaxime (Table 2). However, 50% of isolates showed intermediate resistance to streptomycin and resistance to sulfonamides.

#### Discussion

In this study, 95% of the houses we surveyed were inhabited by Asian house geckos. Individuals were found in different places in houses, such as bedrooms, living rooms, and kitchens, confirming that this species has invaded practically all of our study areas and has settled efficiently in houses. Thus, human residents of these areas live in close relationship with *H. frenatus*, as was also seen in Northern Australia (Callaway et al. 2011).

To our knowledge, this is the first study examining *Salmonella* in an introduced and peridomestic species in Costa Rica. This study is important because along with other studies conducted in other invaded countries, it demonstrates the potential of *H. frenatus* as a reservoir of a bacteria that is of public health relevance in developing and developed countries (Chan 1982, Callaway et al. 2011, Singh et al. 2013, Nwachukwu

Similarity: 100.00% (weight 30)

Dice (Opt:1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%) [0.0%-100.0%]

PFGE

PFGE-XbaI

Site

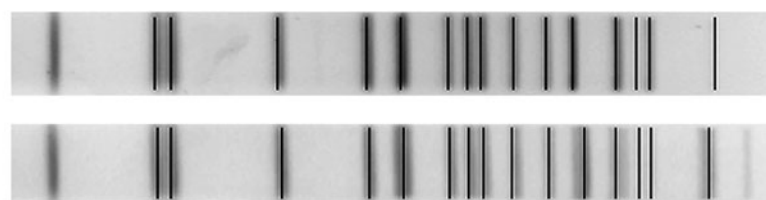


FIG. 1. Pulsed-field gel electrophoresis (PFGE) patterns showing the close genetic relationship of *Salmonella* Weltevreden isolated from the lower gut of Asian house geckos (*H. frenatus*) from two regions of Costa Rica.

TABLE 2. ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* ISOLATES RECOVERED FROM ASIAN HOUSE GECKOS

Isolate	Serovar	AMP	SXT	CEF	CHL	CIP	FOX	NAL	TET	STR	SUL	PIP	TAZ	NIT
We1	Weltevreden	23	28	26	27	38	29	25	22	16	6 <sup>R</sup>	22	24	18
We2	Weltevreden	24	26	28	26	32	32	24	25	17	19	25	27	20
G11	Glostrup	23	28	26	25	34	32	22	23	14 <sup>i</sup>	20	23	27	20
Ab1	Abaetetuba	24	27	28	24	32	31	23	21	15	9 <sup>R</sup>	25	25	19
Ab2	Abaetetuba	22	27	26	26	33	32	24	22	14 <sup>i</sup>	6 <sup>R</sup>	26	29	18
Ad1	Adelaide	23	27	27	27	32	31	24	20	15 <sup>i</sup>	23	24	26	19

Resistance testing followed the Kirby–Bauer method; numbers indicate the inhibition zone diameter in mm.

AMP, ampicillin; SXT, trimethoprim–sulfamethoxazole; CEF, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; FOX, cefotaxime; NAL, nalidixic acid; TET, tetracycline; STR, streptomycin; SUL, sulfonamides; PIP, piperacillin; TAZ, piperacillin–tazobactam; NIT, nitrofurantoin; R, resistant; i, intermediate.

et al. 2014). Geckos could be colonized by *Salmonella* through contact with animal reservoirs or via ingestion of contaminated food (*i.e.*, insects) or water (Smith et al. 2012). We suggest that cockroaches and house flies eaten by *H. frenatus* might be the main source of zoonotic isolates of *Salmonella* because these insects have high contact with human and animal excreta (Nwachukwu et al. 2014); however, further research is needed to elucidate the most important transfer mechanism. The low prevalence of *Salmonella* is similar to that determined for *H. frenatus* in Northern Australia (7%) (Callaway et al. 2011) and is much lower than the prevalence reported in *H. frenatus* from houses in Nigeria (25.7%) and in *Gekko gecko* from houses in Singapore (23%) (Oboegbulem and Iseghohimhen 1985, Murphy and Myers 1993, Nwachukwu et al. 2014).

All serovars detected in this study have been reported previously in reptiles and are of zoonotic concern (Oboegbulem and Iseghohimhen 1985, Murphy and Myers 1993). Despite the low prevalence of *Salmonella*, which is a positive insight for public health, the fact that all of the serovars detected are zoonotic pathogens is of concern because these geckos cohabit closely with humans. In Costa Rica, all of the serovars found in this study, with the exception of *Salmonella* Weltevreden, have been isolated sporadically from clinical human samples (Bolaños et al. 2014). *Salmonella* Glostrup is not commonly isolated in most countries and is considered to be a rare serovar. It was, however, reported from the feces of a child and from material in the tank housing the child's pet lizards (*Mabuya quinquetaeniata*); because both isolates were genetically related, the pets are thought to be the most likely source of infection (Old et al. 1999). To our knowledge, the current study provides the first report of *Salmonella* Glostrup isolated from a gecko. *Salmonella* Adelaide is another rare serovar that has been cultured from reptiles, including turtles and snakes (De Jong et al. 2005, Chen et al. 2010). From 1983 to 1984, the reported human isolates of *Salmonella* Adelaide increased from 45 to 78 in the United States; Illinois, New York, and Virginia accounted for 51% of this serovar (Hargrett-Bean 1988). *Salmonella* Abaetetuba has been detected in wild iguanas in Ecuador, and in Canada it was reported causing salmonellosis in humans (Woodward et al. 1997, Franco et al. 2011). *Salmonella* Weltevreden has been reported as one of the most common serovars and an increasing cause of human infection in Southeast Asia, including in Thailand, Malaysia, and Vietnam (Yasin et al. 1995, Bangtrakulnonth et al. 2004). It is not very common in the Americas, Caribbean, Europe, or Africa (Bangtrakulnonth et al. 2004). This serovar has been isolated previously from geckos in Asia, Australia, and Nigeria

(Oboegbulem and Iseghohimhen 1985, Murphy and Myers 1993, Callaway et al. 2011), and also from well water (Thong et al. 2002) and from animal and vegetable products (Heinitz et al. 2000, Vo et al. 2006, Barua et al. 2014). Also, introduced house geckos in Hawaii have been reported to harbor *Salmonella* Weltevreden and they appear to be the major source of *Salmonella* infection in that area (Steele 2008). In Costa Rica, this serovar has recently become a problem for human health.

The National Bacteriology Referral Center (NBRC) reported an increased frequency of cases of human salmonellosis by *Salmonella* Weltevreden from 2010 ( $n=8$ ) to 2012 ( $n=21$ ), and it is one of the five most frequent serovars identified in human samples from Costa Rica (Bolaños et al. 2014). This pathogen is currently distributed in all Costa Rican provinces and, to date, the vehicle(s) of infection that favored its dispersion is unknown (Bolaños et al. 2012). Furthermore, NBRC reported that 11 isolates of this serovar, from six of the seven provinces of the country, showed indistinguishable PFGE patterns, suggesting that it is a clone with wide distribution in Costa Rica (Bolaños et al. 2012). Interestingly, the pulsotype from our two isolates of *Salmonella* Weltevreden (CRINJQPX01.0002; Fig. 1) was identical to the one from these 11 isolates previously reported from human samples by Bolaños et al. (2012).

The wide distribution of *Salmonella* Weltevreden in humans, along with the close genetic relationship between isolates from humans and *H. frenatus*, suggests that the Asian house geckos harbor a serovar of public health importance in Costa Rica. Therefore, this gecko might be a possible source of this serovar for human infections via feces in houses. Further research is required to better understand the role of *H. frenatus* in the dynamics of this relevant zoonotic serovar in Costa Rica.

Worldwide, the prevalence of antibiotic resistance in *Salmonella* has increased, which is becoming a serious problem for treatment of this infection (Chen et al. 2013). Nonetheless, the *Salmonella* isolates from geckos in this study expressed no resistance to the antibiotics most frequently used in clinical treatment (Table 2), suggesting that people infected with these clones can be treated effectively with a wide range of antibiotics. This result also suggests that the geckos have had little exposure to antibiotics or antimicrobial-resistant bacteria from domestic animals or humans. Streptomycin was the only antibiotic to which one isolate of *Salmonella* Weltevreden, and both isolates of *Salmonella* Abaetetuba, presented antibiotic resistance, and streptomycin-resistant *Salmonella* is also known from other studies of reptiles (Corrente et al. 2004, Chen et al. 2010).

Our study shows that a widespread, introduced, and peridomestic species has a low prevalence of *Salmonella* and harbors isolates of *Salmonella* associated with human salmonellosis that are sensitive to the antibiotics most commonly used in clinical treatment. These results are highly relevant for health authorities attempting to prevent and control *Salmonella* infections in humans. The major finding of this work is a PFGE pattern for *Salmonella* Weltevreden that is indistinguishable between *H. frenatus* and human isolates with wide distribution in the country, suggesting that this gecko may serve as a vector of a serotype of public health importance in Costa Rica.

Furthermore, we highlight that the relationship between humans and geckos in houses identified in this study might have zoonotic implications because we found zoonotic *Salmonella* serovars, and in particular isolates of *Salmonella* Weltevreden, in both regions. The droppings from this gecko likely contaminate the human environment with zoonotic enteropathogens, including nontyphoidal salmonellae (Callaway et al. 2011, Singh et al. 2013, Nwachukwu et al. 2014). Future studies must determine to what extent or how often pathogens from infected gecko feces enter human bodies and affect people. For now, we recommend that people should be cautious and clean up areas in their houses where gecko feces are found, because these feces are considered a relevant route of direct and indirect contamination and transmission to humans. This study identifies a potential risk for salmonellosis in two regions of Costa Rica, but we propose that more detailed epidemiological studies are needed to understand better the role of the Asian house gecko with human salmonellosis, especially that caused by *Salmonella* Weltevreden, and to accurately quantify its risk in Costa Rica.

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### Author Disclosure Statement

No competing financial interests exist.

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