Salmonella enterica serovars in lizards of New Zealand's offshore islands

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Published on-line: 27 January 2010

Abstract: The translocation of animals between populations is becoming a common conservation technique, which, in addition to the target species, unfortunately inevitably involves any parasitic organisms they host. Many of these organisms can become pathogenic in a new environment. We assessed the prevalence of intestinal carriage of *Salmonella*, a common disease organism in wildlife worldwide, in New Zealand native lizards on eight islands off the coast of New Zealand. Cloacal swabs were obtained from 703 lizards and cultured for *Salmonella*, using four aerobic enrichment and culture methods at two incubation temperatures. We recorded six environmental and physical variables that may affect *Salmonella* carriage. Logistic regression revealed that two variables were significantly correlated to the presence/absence of *Salmonella.* In general, *Salmonella* was found predominantly but not exclusively in six species of lizard and in beach habitats. *Salmonella* prevalence on the islands was not correlated to previous lizard translocations to the island. More research is required to determine the pathogenicity to New Zealand wildlife of the *Salmonella* serovars identified in this study. Pending further research, translocations of the six species highlighted in this study or to beach habitats should include disease screening in order to prevent the spread of this potential pathogen.

Keywords: *Cyclodina*, gecko, *Hoplodactylus*, *Naultinus*, *Oligosoma*, skink, translocation, zoonosis

Introduction

Species translocation is an increasingly common conservation practice. Translocations do not involve only a single species, but rather a wide collection of organisms including any bacteria, viruses, helminths or protozoa for which any single animal may be a host (Griffith et al. 1989). Stresses induced by captivity or transit can cause any of these organisms to become pathogenic, affecting not only the released animal but also other species present at the release site as well as the human handlers involved in the translocation (Griffith et al. 1989). Disease screening prior to translocation may prevent the spread of disease to naïve wild populations (Woodford & Rossiter 1994; Gartrell et al. 2006). Nevertheless, adequate disease screening is often not done because translocations are underfunded, diagnostic testing is not available, and species-specific biomedical knowledge is lacking (Woodford & Rossiter 1994; Gartrell et al. 2006).

Salmonellosis is the disease resulting from infection by the bacterium *Salmonella.* This is an important disease resulting in death in wild reptiles, birds and mammals throughout the world. The potential for cross-infection between mammals, birds and reptiles is high as *Salmonella* survives outside its hosts for extended periods and infects hosts across a broad phyletic spectrum (Otokunefor et al. 2003). Understanding *Salmonella* transmission is difficult because more than 2500 distinct serovars are currently described. The currently accepted system for naming *Salmonella* is the White–Kauffman–Le Minor scheme (http://www.pasteur.fr/sante/clre/cadrecnr/salmoms/WKLM_2007.pdf valid at 1 January 2007), in which *Salmonella enterica* serovars are given names alongside their antigenic formulae. Thus, for example, *Salmonella* ser. Enteritidis (alternatively written as *S. enterica* serovar Enteritidis) has the formula 1,9,12:g,m:– and that for *Salmonella* ser. Typhimurium is 1,4,[5],12:i:1,2. These naming forms supercede the earlier Kauffmann–White scheme described in Old (1992) in which serovars were accorded species status.

Herpetofauna have often been implicated as transmitters of *Salmonella* (Johnson-Delaney 1996; Mermin et al. 2004) and this transmission may be to other herpetofauna or to birds and mammals. *Salmonella* frequently results in invasive disease within the host, exhibited in symptoms such as septicaemia, pneumonia, coelomitis, abscessation, granulomatous inflammation and death (Johnson-Delaney 1996). Where invasive disease is not present in the reptilian hosts, the animals can act as a reservoir introducing the bacteria to indigenous fauna at the release site.

Salmonella serovars are frequently isolated from birds in New Zealand and overseas (Robinson & Daniel 1968; Twentyman 1999; Clark et al. 2002; Tizard 2004). *Salmonella* ser. Typhimurium DT160 resulted in the mortality of many sparrows (*Passer domesticus*) in New Zealand (Alley et al. 2002). Salmonellosis has also resulted in mortality of hihi (*Notiomystis cincta*) on Tiritiri Matangi Island. Although only nine hihi bodies were found, the mortality rate could have been as high as 30%. This outbreak was due to a novel strain of *Salmonella* ser. Typhimurium DT195 most likely introduced by tourists visiting the island (Ewen et al. 2007). The New Zealand (Hooker's) sea lion (*Phacarctos hookeri*) is also a carrier of *Salmonella* (Fenwick et al. 2004).

Approximately half of New Zealand's lizard species are registered as threatened or endangered (Daugherty et al. 1994). The New Zealand Department of Conservation's recovery plans for threatened lizards frequently involve translocating lizards to offshore islands, many of which already host some of New Zealand's rarest native fauna (Towns 1999). Because *Salmonella* is not host specific, any translocation to an island carries the risk of infecting a range of hosts.

This study aimed to (1) assess the prevalence of faecal excretion of *Salmonella* serovars by wild endemic lizards on offshore islands around the coast of New Zealand and (2) determine whether there is a geographical distribution of *Salmonella* serovars among New Zealand native lizards and whether this distribution is correlated with previous translocations.

Methods

In two Southern Hemisphere summers between March 2006 and March 2008 we sampled 703 lizards of 18 species from eight islands, assigned to four geographical locations (Northland, Auckland/Coromandel, Wellington, and Marlborough). Six variables were selected to describe the prevalence and distribution of *Salmonella* in New Zealand lizard species and recorded for each individual caught. In relation to island, geographical location, and history of lizard translocations, we recorded species, sex, and the habitat in which the lizard was found.

Study sites

The eight islands were (from north to south): Motuopao (34°59′ S,

174°03′ E) (Northland); Little Barrier (36°12′ S, 175°08′ E), Cuvier (36°26′ S, 172°38′ E) and Korapuki (36°38′ S, 175°52′ E) (Auckland/Coromandel); Mana (41°06′ S, 174°48′ E) and Matiu/Somes (41°16′ S, 174°52′ E) (Wellington); Maud (40°59′ S, 174°03′ E), and Stephens (40°35′ S, 175°55′ E) (Marlborough Sounds). Study sites were divided into two broad groups: islands with and without lizard translocations (Table 1).

Species and sampling

Species (n = 18) from the four genera of New Zealand lizards (Lacertilia) – geckos *Hoplodactylus* and *Naultinus* (family Diplodactylidae) and skinks *Cyclodina* and *Oligosoma* (Scincidae) (Towns et al. 2001) – were captured by hand or in pitfall traps. Following capture, we recorded species (according to Jewell 2008), mass (±0.2 g), and snout–vent length (SVL) (±0.5 mm) of each individual. Sex was only recorded when it could be assigned with confidence by cloacal examination during which the lizards were manually restrained for cloacal sampling. The Minitip cloacal swabs were put into individual polypropylene tubes containing Aimes agar gel with charcoal (Copan Diagnostics Inc., 2175 Sampson Ave, Suite 124, Corona, CA 92879, USA) and stored on ice for up to 5 h, followed by refrigeration on the island at 4–6°C for up to 13 days until transport to the laboratory where they were stored at 4°C until culturing.

Habitat preference

The habitat in which each species was found was noted (Table 2) and allocated to three preference categories: beach, bush and open terrain. Beach habitat was defined as anything within 5 m of the water's edge; bush was defined as having more than 50% canopy cover as estimated by eye; open terrain had less than 50% canopy cover. The distribution of lizards caught in each of these categories across the eight islands is shown in Appendix 1.

Laboratory isolation of *Salmonella*

Salmonella isolation was achieved by inoculating selenite F and tetrathionate enrichment broths (Fort Richard Laboratories, Otahuhu, Auckland, NZ). Duplicates were made of each broth and incubated at 35–37°C and 25–27°C for 24 h. The selenite broths were sub-cultured onto xylose lysine deoxycholate (XLD) plates and the tetrathionate broths onto MacConkey agar plates (Fort Richard Laboratories), which were incubated in normal atmosphere at 35–37ºC and 25–27°C for a further 24 h. Suspect colonies found with either method were used to inoculate triple sugar iron (TSI) agar slopes, urea slopes, and lysine decarboxylase tubes (Fort Richard Laboratories). These were incubated overnight at both 37ºC and 27°C. Colonies that resulted in glucose fermentation and hydrogen sulphide production on TSI slopes, acid (negative) urease tests, and positive lysine decarboxylase tests were considered to be consistent with *Salmonella*. Each organism suspected of being *Salmonella* was tested for fermentation of glucose, mannitol and xylose; hydrolysis of o-nitrophenyl-β-d-galactopyranoside (ONPG); indole and acetoin production; citrate utilisation; and production of indolepyruvate and ornithine decarboxylase (Quinn et al. 1994).

Samples positive for *Salmonella* were sent to Environmental Science and Research Services (ESR, Porirua, NZ) for confirmation and serotyping by antigenic determination, identifying the somatic 'O' and flagellate 'H' antigens present.

Data analyses

Body condition was calculated using the residuals from data fitted by a linear regression of log mass on log SVL. The prevalence rates of *Salmonella* were calculated using formulae described in Thrusfield (2005) and assuming that the *Salmonella* culture was 50% sensitive and 98% specific (Bager & Petersen 1991). True prevalence (P) was estimated from test prevalence (P^T) using the equation

$$P = (P^T + \text{specificity} - 1) / (\text{sensitivity} + \text{specificity} - 1).$$

Ninety-five percent confidence intervals (95% CI) are reported, where:

$$95\%\ CI = P \pm 1.96\sqrt{\text{variance}},$$

and

$$\text{Variance} = [P^T(1 - P^T)] / [n(\text{sensitivity} + \text{specificity} - 1)^2],$$

where n = sample size.

Table 1. Test (P^T) and true prevalence (P) of *Salmonella enterica* serovars (n = 11) found in lizards (Scincidae and Diplodactylidae) on New Zealand offshore islands. All are subsp. *enterica* (I) except for IV, which is subsp. *houtenae*.

Location Island	No. lizards sampled (n = 703)	P^T (%)	P (95% CI)	Serovars
Northland				
*Motuopao	12	0.0	0– 22.0	
Auckland/Coromandel				
Little Barrier	46	0.0	0–6.3	
Cuvier	101	6.9	0–20.6	Bousso[a]
*Korapuki	131	9.9	5.8–27.2	IV Houten Warragul Bousso Mississippi
Wellington				
*Mana	103	5.8	0–17.4	Mana[b] 6,7:z:– 48:k:–
*Matiu/Somes	111	2.7	0–7.7	Mana Infantis
Marlborough Sounds				
Stephens	100	4.0	0–12.2	Saintpaul Typhimurium phage type 135 4,12:–:1,2
*Maud	99	0.0	0–3.0	

*Islands with a history of lizard translocations. [a]First isolates of this serovar from New Zealand; [b]First isolated from a takahē on Mana Island in 1998;

The six variables affecting the presence/absence of *Salmonella* in New Zealand native lizard species on offshore islands were analysed using forward stepwise logistic regression in SPSS (2007). For islands where no *Salmonella* was detected, we calculated the 95% confidence limits for the maximum possible prevalence (Cameron & Baldock 1998), using the Detection of Disease component of Win EpiScope 2.0 (2000). For the purposes of this analysis, the lizard population (collectively) on Little Barrier and Maud islands was estimated at 10 000 individuals and that on Motuopao Island at 1000 individuals (estimates based on verbal information obtained from New Zealand Department of Conservation staff).

Results

Species (n = 18) of lizard caught in different terrain on each island varied (Appendix 1) and the test prevalences (P^T) of *Salmonella* on the eight islands ranged from 0 to 9.9% (Table 1). The mean test prevalence of faecal excretion of *Salmonella enterica* in the 703 lizards sampled was 4.7% (n = 33) and the true prevalence (P) was in the range 2.3–8.8% (95% CI). Faecal excretion of *Salmonella*, however, was not detected in all populations (Table 1). *Salmonella* serovars were found in 10 species (Table 2) and *Salmonella* prevalence differed interspecifically (Wald = 9.936, P = 0.002). Skinks had the highest prevalence of *Salmonella*; along with Duvaucel's geckos (*Hoplodactylus duvaucelii*) (2/21, P^T = 9.5%), which were caught on only one island (Korapuki).

Eleven serovars of *Salmonella enterica* were isolated from New Zealand endemic lizards (Table 1), and belong to one of two subspecies: 29 samples were subspecies I (subsp. *enterica*) and five were subspecies IV (subsp. *houtenae*). *Salmonella* was not detected in lizards from Motuopao, Little Barrier, and Maud islands. Based on these negative results, our sample sizes, and 95% confidence intervals, the maximum possible true prevalence of *Salmonella* that we failed to detect on these islands could be up to 6.3%, 3.0% and 22.0% respectively. The remaining five islands were the source of between one and three *Salmonella* serovars that were not found on any other island (Table 1). *Salmonella* ser. Bousso and *Salmonella* ser. Mana were the only two serovars found on more than one island. *Salmonella* ser. Bousso was in shore skinks (*Oligosoma smithi*) on both Cuvier and Korapuki islands. *Salmonella* ser. Mana was on Mana Island and Matiu/Somes Island. The test prevalence of serovars on

Table 2. Test prevalence (P^T) of *Salmonella* for adult New Zealand lizards and their habitat preferences on New Zealand offshore islands.

Species (P^T %)	Male		Female		Gender unknown		Total prevalence		
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Habitat preference
Skinks (Scincidae)									
Cyclodina aenea (2.6)	0	12	1	12	0	14	1	38	Forest. Coastal areas close to the high tide line[1,2]
C. alani (0.0)	0	8	0	7	0	7	0	22	Lowland, coastal forests[3]
C. macgregori (10.0)	2	10	0	8	0	0	2	18	Coastal forest and scrub[4]
C. oliveri (18.2)	0	2	2	5	0	2	2	9	Leaf litter under coastal forest and scrub[1]
C. whitakeri (0.0)	0	3	0	8	0	0	0	11	Coastal forest and scrub*1*
Oligosoma infrapunctatum (0.0)	0	0	0	1	0	0	0	1	Open forest, scrub and tussock[4]
O. moco (0.0)	0	2	0	3	0	2	0	7	Open scrub and grassland in coastal and lowland regions[4]
O. nigriplantare polychroma (17.2)	1	12	3	10	1	2	5	24	Dry, open areas with low vegetation[4]
O. smithi (16.7)	4	24	7	24	1	12	12	60	Coastal. Splash zone, open grass and shrubland[5]
O. striatum (2.0)	0	9	0	33	1	8	1	50	Rotting logs in native forest[4]
O. suteri (7.7)	3	14	0	15	0	7	3	36	Coastal. Boulder beaches and rocky platforms[5]
O. zelandicum (7.7)	1	5	0	4	0	3	1	12	Forest or moist shady areas[4]
Geckos (Diplodactylidae)									
Hoplodactylus chrysosireticus (0.0)	0	5	0	8	0	0	0	13	Forest, scrub and coastal vegetation[4]
H. duvaucelii (9.5)	1	6	1	9	0	4	2	19	Forest, scrub, coastal vegetation and cliffs[4]
H. maculatus (1.2)	3	129	1	108	0	92	4	329	Forest, scrub and grassland[4]
H. pacificus (0.0)	0	5	0	1	0	0	0	6	Many habitats close to splash zone in areas of scrub and forest[6]
H. stephensi (0.0)	0	1	0	0	0	0	0	1	Coastal forest and scrub[4]
Naultinus manukanus (0.0)	0	3	0	7	0	4	0	14	Forest and scrub[4]
Totals	15	250	15	263	3	157	33	669	
Percentage with *Salmonella*	6.0%		5.7%		1.9%		4.9%		

[1]Chapple et al. 2008a; [2]Hardy 1977; [3]Chapple et al. 2008b; [4]Jewell 2008; [5]Hare et al. 2008; [6]Parrish & Gill 2003.

islands ranged from 0.7% to 6.9% and the true prevalence from 0 to 20.5%. *Salmonella* ser. Bousso had the highest prevalence with 6.9% (7/101) of lizards caught on Cuvier Island testing positive for this serovar.

The prevalence of *Salmonella* on islands with a history of lizard translocations (P^T = 4.8% (22/456), P = 5.9%) was not significantly different from islands without a history of lizard translocations (P^T = 4.5% (11/247), P = 5.1%) (Wald = 2.427, n.s.). The number of serovars found on the islands was apparently not influenced by the number of translocations (Table 1).

The terrain in which lizards were caught was classified into three types: beach, bush, and open terrain (Appendix 1). The prevalence of *Salmonella* in lizards caught on the beach (17/148, P^T = 11.5%) was much higher than for those caught in the bush (9/276, P^T = 3.3%) or open terrain (7/279, P^T = 2.5%). However, the effect of habitat on *Salmonella* prevalence is confounded with species of lizard, because species tended to be habitat specific.

The effects of gender, island and terrain were analysed within species of lizard for which we obtained sufficient numbers. When compared within species, neither gender (Table 2), nor island nor terrain had a significant effect on *Salmonella* prevalence. The presence or absence of *Salmonella* had no significant effect on body condition of skinks (t = 0.977, n.s.) or geckos (t = 0.805, n.s.) in two-tailed *t*-tests.

Discussion

This study found test prevalence rates of *Salmonella* from skinks and geckos on offshore islands to be between 0 and 9.9% with a mean test prevalence of 4.7%. By comparison, Gartrell et al. (2006, 2007) found no *Salmonella* excretion by either captive or wild tuatara (*Sphenodon punctatus*). The prevalence of *Salmonella* excretion in New Zealand reptiles is much lower than that found in other parts of the world, where the prevalence of *Salmonella* in lizard populations may exceed 90% (Hoff & White 1977; Otokunefor et al. 2003; Chambers & Hulse 2006).

Salmonella enterica serovars were found in 10 of the 18 different lizard species we captured. Five of the six species with high prevalences of *Salmonella* belonged to the family Scincidae; Duvaucel's gecko (*Hoplodactylus duvaucelii;* Diplodactylidae) was the sixth species. Species differences between lizards in life history and diet are possible explanations for the differences seen in *Salmonella* prevalence. *Oligosoma smithi* and *O. suteri* both occur predominantly on boulder-beaches within the splash zone (Table 2) (Towns 1975; Parrish & Gill 2003). Although *O. smithi* occasionally occur in bare rocky areas several hundred metres inland (Parrish & Gill 2003), all *O. smithi* caught in this study were obtained from within the splash zone. Marine mammals and seabirds are carriers of a wide variety of *Salmonella* serovars (Gilmartin et al. 1979; Alley et al. 2002; Fenwick et al. 2004) so they could serve as vectors of this organism for lizards on islands. There has been debate about the survival of *Salmonella* in marine environments, but investigations have shown that the organism can survive well in seawater with salinities as high as 3.5% (Minette 1986). Between 1992 and 1996 42 serotypes of *Salmonella* were recovered from seawater sampled north-east of Spain (Polo et al. 1999). If, as this indicates, *Salmonella* is widespread in marine environments, it could lead to extensive intestinal colonisation of marine animals. It is interesting to note that in our study the majority of *Salmonella*-carrying species have coastal environments as their preferred habitat (Table 2). The exceptions were *O. nigriplantare polychroma* and *O. zelandicum*.

Our research found no evidence that *Salmonella* prevalence or serovar diversity was associated with lizard translocations to the islands. However, as there is no evidence of *Salmonella* serovars or prevalence in the endemic lizards prior to translocation, we cannot conclude that there has been no effect of translocations on the serovars present in the population; simply that a prior history of translocations has not increased the diversity or prevalence of *Salmonella* in comparison with islands without a history of translocation. Variance in prevalence of *Salmonella* found on islands was determined to be an effect of different combinations and proportions of lizard species caught on each island. Motuopao, Little Barrier, and Maud islands were all found to be free of *Salmonella* in the sampled lizards. Over 95% of lizards caught on Maud Island belonged to the species *Hoplodactylus maculatus,* which was found to have low prevalences of *Salmonella* on all islands in this study. Only small sample sizes were obtained from Motuopao (n = 10) and Little Barrier (n = 46) and due to the relatively low sensitivity of our test (50%) these data must be interpreted with care. The maximum possible true prevalence (Table 1) gives the upper limit of the true prevalence of *Salmonella* that might have been missed by our level of sampling. This calculation is a useful tool in disease surveys for evaluating the significance of negative results or the likelihood that a population is free from disease. The varying prevalence of *Salmonella* between islands could also be due to a variety of factors not explored in this study such as the density of seabird populations, frequency of marine mammal visitation, and an island's proximity to other land masses. It is likely that prevalence of *Salmonella* on islands is controlled by many different variables and cannot be pinned to one single cause.

For the islands we studied, the initial source of *Salmonella* in the lizards is still unknown. It is possible that human activities led to the spread of *Salmonella* serovars. Despite the current protection and isolation of most of our eight islands, many of them have a history of human habitation. Marine mammals and seabirds may also act as reservoirs of *Salmonella* and variation in *Salmonella* prevalence on islands may be a product of frequency of visitation by marine mammals or birds or densities of these species inhabiting the island. Our research shows that some species of lizard, particularly skinks, have a much higher prevalence of *Salmonella* than other species. At present we do not know the pathogenicity of these *Salmonella* serovars within New Zealand lizards. However, *Salmonella* has caused significant disease in other New Zealand wildlife. It would therefore be prudent to take a conservative approach until more information is available, and disease screening should remain an integral part of any translocation project but particularly those that involve the species highlighted in this study.

Acknowledgements

This project was funded by a Massey University Masterate Scholarship and several grants: from the New Zealand Federation of Graduate Women, a New Zealand Biosecurity Award, a J. P. Skipworth Scholarship and the Julie Alley Bursary. All procedures involving the use of animals were approved by the Massey University Animal Ethics Committee (MUAEC 07/31), Palmerston North, New Zealand. All islands in this study are Crown owned and access to the islands and procedures involving the use of lizards were approved by the Department of Conservation and the numerous tangata whenua who are kaitiaki of these islands. We thank Ngāti Hei, Ngāti Whānaunga, Te Āti-Awa, Ngāti Toa, Ngāti Koata and Ngāti Kuia for allowing us access to these islands and the lizards. Kate McInnes, Lyn Adams, Hugh Gardiner, Erica Van Reenen and Emma Crawford provided expert help in the field; Hamish Mack gave invaluable advice on the microbiological analysis of the samples.

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Editorial Board member: Phil Lester
Received 12 March 2009; accepted 24 October 2009

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Appendix 1. Species of *Cyclodina, Hoplodactylus, Naultinus and Oligosoma* lizards caught in different terrain on New Zealand islands (listed north to south)

Island	Beach	Bush	Open
Motuopao* (October 2007)		*C. alani*	
Little Barrier (November 2007)	*O. smithi* *H. maculatus*	*C. aenea* *C. oliveri* *O. moco* *O. striatum* *H. maculatus* *H. pacificus*	*C. oliveri* *H. maculatus* *H. pacificus*
Cuvier (October 2007)	*O. smithi* *O. suteri* *H. maculates*	*H. maculatus* *O. moco*	*H. maculatus*
Korapuki* (December 2007)	*O. smithi* *O. suteri* *H. duvaucelii* *H. maculatus*	*C. aenea* *C. alani* *C. oliveri* *C. whitakeri* *O. moco* *H. duvauelii* *H. maculates*	*C. aenea* *O. moco* *H. duvaucelii* *H. maculatus*
Mana* (March 2006)		*C. aenea* *H. chrysosireticus* *H. maculatus*	*C. aenea* *C. macgregori* *O. nigriplantare polychroma* *H. chrysosireticus* *H. maculates*
Matiu/Somes* (January 2008)		*C. aenea* *H. maculatus* *O. lineoocellatum* *O. nigriplantare polychroma*	*C. aenea* *H. maculatus* *O. lineoocellatum* *O. nigriplantare polychroma*
Stephens (February 2007)		*H. maculatus* *N. manukanus* *O. infrapunctatum* *O. zelandicum* *O. nigriplantare polychroma*	*H. maculatus* *H. stephensi* *N. manukanus* *O. infrapunctatum* *O. nigriplantare polychroma* *O. zelandicum*
Maud* (March 2008)		*H. maculatus* *O. zelandicum*	*C. aenea* *H. maculatus*

*Islands with a history of lizard translocations.