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HOST MICROBE INTERACTIONS

Genomic Epidemiology and Management of *Salmonella* in Island Ecosystems Used for Takahe Conservation

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Abstract Translocation and isolation of threatened wildlife in new environments may have unforeseen consequences on pathogen transmission and evolution in host populations. Disease threats associated with intensive conservation management of wildlife remain speculative without gaining an understanding of pathogen dynamics in meta-populations and how location attributes may determine pathogen prevalence. We determined the prevalence and population structure of an opportunistic pathogen, *Salmonella*, in geographically isolated translocated sub-populations of an endangered New Zealand flightless bird, the takahe (*Porphyrio hochstetteri*). Out of the nine sub-populations tested, *Salmonella* was only isolated from takahe living on one private island. The apparent prevalence of *Salmonella* in takahe on the private island was 32% (95% CI 13–57%), with two serotypes, *Salmonella* Mississippi and *Salmonella houtenae* 40:gt-, identified. Epidemiological investigation of reservoirs on the private island and another island occupied by takahe identified environmental and reptile sources of *S.* Mississippi and *S. houtenae* 40:gt- on the private island. Single nucleotide polymorphism analysis of core genomes revealed low-level diversity among isolates belonging to the same serotype and little differentiation according to host and environmental source. The pattern observed may be representative of transmission between sympatric hosts and environmental sources, the presence of a common unsampled source, and/or evidence of a recent introduction into the ecosystem. This study highlights how genomic epidemiology can be used to ascertain and understand disease dynamics to inform the management of disease threats in endangered wildlife populations.

Keywords Disease ecology · New Zealand · Whole genome sequencing · *Porphyrio hochstetteri* · Translocation

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Introduction

Disease control in threatened and endangered wildlife populations is integral to maintaining population viability [1]. However, management of pathogenic threats and conservation of threatened wildlife is frequently limited by a lack of informative data on the epidemiology and ecology of host-pathogen relationships in the species of concern and the environment they inhabit [2]. Epidemiological investigations facilitate conservation management and mitigation of disease in wildlife and inform risks of pathogen transmission within and between vulnerable populations.

Molecular characterization of pathogens derived from multiple sources provides insights into the epidemiology of infectious disease ecology. When combined with epidemiological data, genetic tools can be used to identify hosts, investigate

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pathogen adaptation, infer chains of transmission, and link heterogeneities in pathogen prevalence to host and environment associated factors [3]. Fine scale microbial genetics can be used to infer pathways of a transmission chain based on sequence profiling. Pathogen subtypes are assigned according to genetic similarity or differences. Therefore, if two individuals share a subtype, transmission may be inferred [4–6]. Although whole genome analysis has been extensively applied to understand the epidemiology of pathogens in human clinical research [7, 8], the application of these techniques is relatively new to field of wildlife disease [9], with few investigations in endangered translocated wildlife [10].

The takahe (*Porphyrio hochstetteri*) is an endemic New Zealand endangered flightless bird [11] with a population of approximately 230 individuals [12]. Intensive management of takahe, with up to 43 translocations per annum, has created a complex network of small fragmented sub-populations [13]. Reduced genetic variability associated with small populations may increase susceptibility of individuals to disease [14, 15]. Therefore, mitigation measures are in place to reduce the likelihood of transmission and exposure to potential pathogens, which may occur during the translocation process [16]. Each takahe translocation is subject to a disease-screening protocol including bacteriological testing for *Salmonella* [17]. *Salmonella* have caused mortality, enteritis and reproductive disease in native [18] and introduced birds [19] in New Zealand and around the world [20, 21]. Given the generalist nature of *Salmonella* and viability in the environment for long durations [22], infection and transmission of potentially pathogenic *Salmonella* to naive individuals could result in detrimental effects to endangered takahe populations, sympatric wildlife, or in-contact humans.

The first aim of this study was to determine the prevalence of *Salmonella* in takahe sub-populations. The discovery of *Salmonella* in a single population initiated an investigation to explore the hypothesis that location specific reservoirs of *Salmonella* were present on the offshore private island used for takahe conservation. We used genomic epidemiological tools, including single nucleotide polymorphism (SNP) analysis of *Salmonella* genomes to identify putative transmission pathways between reservoirs and hosts. Our findings provide evidence of localized inter-species transmission and inform practical measures to be implemented with the aim of reducing the prevalence of infection in takahe.

Methods

Takahe Prevalence Survey

Either cloacal (24 birds), fecal (15 birds), or both swab types (108 birds) were opportunistically collected from 147 takahe during pre-translocation disease screening, annual health checks, or observance of the birds. Samples were taken from nine populations/locations (Fig. 1) in New Zealand between November 2011 and April 2013. All swabs were transported and stored in Aimes Charcoal Transport media (Copan, USA) at 4 °C for 1 to 7 days prior to culture. Takahe were assigned

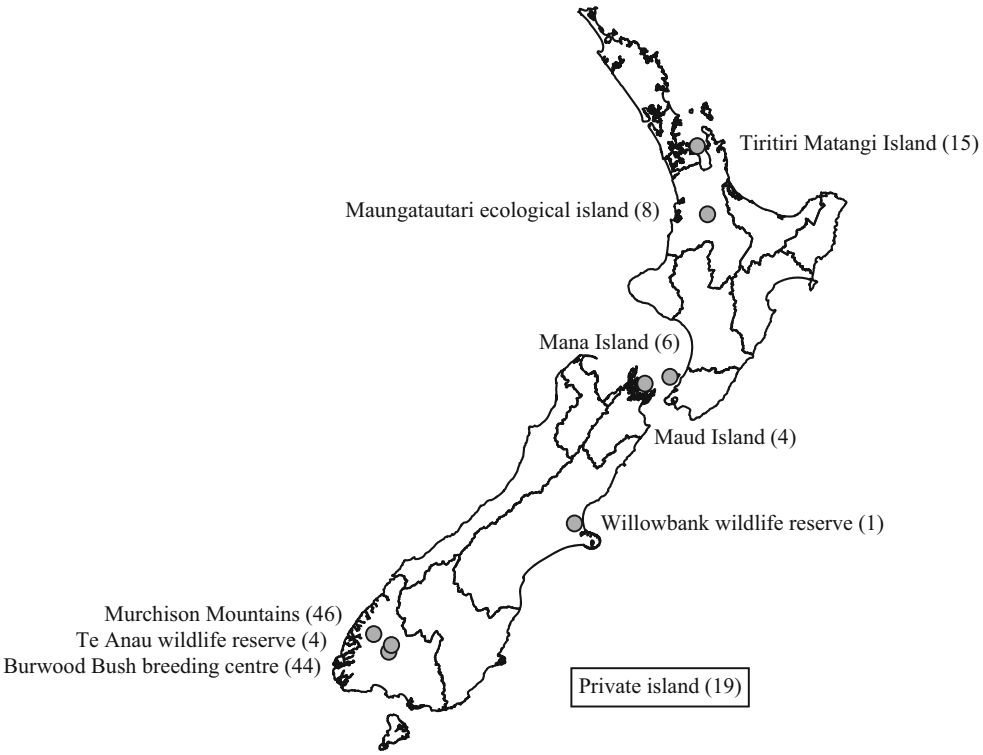

Fig. 1 Map of takahe (*Porphyrio hochstetteri*) study population locations in New Zealand, with sample sizes indicated in *brackets*. The location of the private island population is undisclosed due to confidentiality restrictions

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as test positive if *Salmonella* was isolated from either a fecal or cloacal swab.

Reptile and Environmental Prevalence Survey

Following the primary investigation of *Salmonella* in takahe sub-populations, a case-control study of two island sanctuaries, a private island (case), and Maud Island (control) (Fig. 1), was conducted to investigate potential reservoirs of *Salmonella* in the environment and reptiles.

The private island is an approximately 100-ha predator-free offshore island located in the South Island, New Zealand. For reasons of confidentiality and wildlife protection, the location is unable to be disclosed. The private island has a history of more than 100 years of intensive grazing by sheep and remained inhabited by humans until 1989. Vegetation cover is dominated by native and exotic grass pastures, and low growing shrubs. The island supports endemic wildlife, seabirds and introduced passerines. The private island takahe population was founded with six individuals translocated from a breeding center in 2001 and 2002, with few additional immigration and no emigration events prior to this study (*pers. comm.* P. Marsh). Twenty-two known takahe were resident on the island at the end of 2013 (*pers. comm.* P. Marsh). Maud Island is a 309-ha scientific reserve located in the Malborough Sounds, New Zealand. Seven known takahe were resident on Maud Island at the end of 2013 (*pers. comm.* P. Marsh). Eight accessible takahe territories were identified on the private island, and six on Maud Island. Territories were estimated based on takahe home range and sightings by island caretakers. Each territory contained an artificial feeder and either a natural or artificial water source intended for use by takahe (Fig. 2, and Supporting information).

Samples were collected from lizards caught in January 2014 on the private island and February 2014 on Maud Island. Pitfall traps were used to target the capture of lizards. Each trap contained a dampened sponge, apple puree (used as a lure), and was covered with an aerated lid approximately 3 to 5 cm above the trap. Within each takahe territory, three transect lines consisting of four pitfall traps each were set in random directions with a start point 10 m away from each territory's water source. In cases where selected pit fall placement sites were inaccessible, traps were placed in the nearest suitable area. Each trap, on a given transect line, was placed 4 m apart. Traps were checked once every 24 h for five consecutive days. Hand capture of lizards was conducted where pitfall capture rates were low (territories A, B, and D) on Maud Island (Supporting information). All lizards were released within the territory of capture.

Captured lizards were marked with a number on the ventral surface using a non-toxic, xylene-free pen. Cloacal swabs were collected from 6 to 7 lizards per takahe territory (n = 50) on the private island, and 3 to 11 lizards per takahe territory (n = 31) on Maud Island (Supporting information). Minitip swabs were transferred to Aimes transport media (Copan, USA) and stored at 4 °C for up to 6 days prior to processing.

Sterile containers were used to collect 500 mL of water from natural or artificial sources within a takahe territory (Fig. 2, Supporting information) and stored at 4 °C for up to 2 days prior to culture. Artificial water sources were rainwater-fed tank and trough systems. Streams or ponds were classified as natural water sources.

Soil samples were collected by rubbing a sterile gauze swab over the surface of a 50-cm^2 patch of soil directly underneath the takahe feeding station within each territory. Gauze swabs were transferred into 400 mL of sterile buffered peptone water and stored at 4 °C for up to 2 days prior to culture.

Microbiological Isolation of *Salmonella*

Cloacal swabs from takahe and lizards were suspended in 2 mL phosphate buffered saline (PBS) and agitated using a vortex mixer. Five hundred microliters of PBS was then inoculated into 20 mL buffered peptone water (BPW) (BioRad, New Zealand). Water samples were centrifuged and the resulting pellet re-suspended in 20 ml BPW. Soil swabs were divided into two and incubated in 200 ml of their existing BPW. All BPW samples were incubated at 37 °C for 24 h for pre-enrichment.

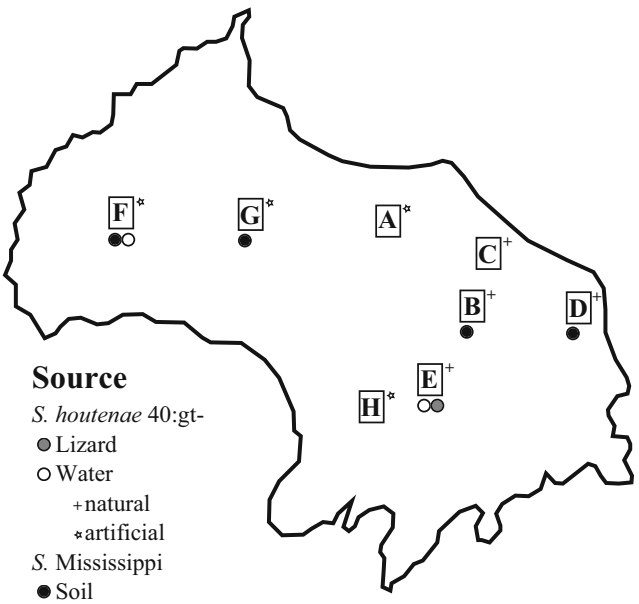

Fig. 2 Outline map of the private island (location undisclosed due to confidentiality restrictions). *Boxed letters* illustrate the sampling locations based around water sources within takahe (*Porphyrio hochstetteri*) territories. *Shaded circles* indicate the source and species of *Salmonella* isolated from within the territory

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Subsequently, 100 μl of BPW was inoculated into 10 ml Rappaport-Vassiliadis *Salmonella* (RVS) at 42 °C and 10 ml tetrathionate (TET) broth (Fort Richards, New Zealand) enriched with 100 μl iodine-iodide solution (0.25 g KI, 0.3 g I/ml H_2O). Inoculated RVS and TET were incubated for 24 ± 2 h at 42 °C and 37 °C, respectively. Incubated RVS and TET solutions were sub-cultured and streaked onto xylose lysine deoxycholate (XLD) and Brilliant Green Modified (BGM) agar (Fort Richards, New Zealand), and incubated at 37 °C for 18 to 24 h.

Two to four suspect *Salmonella* colonies of differing morphology were sub-cultured from each XLD and BGM plate onto MacConkey agar (Fort Richards, New Zealand) and incubated at 37 °C for 18 to 24 h. Non-lactose fermenting colonies identified on MacConkey agar were inoculated into triple sugar iron and lysine iron agar slopes (Fort Richards, New Zealand) and incubated at 37 °C for 18 to 20 h. Isolates with positive reactions on the slopes were further tested for oxidase reactivity and reaction to polyO and polyH antisera (Oxoid, New Zealand). Oxidase negative and polyO/polyH antisera positive isolates were sub-cultured and incubated at 37 °C on to Columbia Horse Blood Agar (Fort Richards, New Zealand) for 24 h prior to identification using a RapID One test (Oxoid, New Zealand) conducted according to the manufacturer's instructions. One isolate per sample identified as *Salmonella* species by RapID One was sub-cultured at 37 °C on to dorset egg agar slopes (Fort Richards, New Zealand) for 24 h and sent chilled to ESR, Wallaceville, New Zealand for serotyping.

Prevalence Estimates of *Salmonella*

Apparent prevalence calculations and 95% confidence intervals for takahe, lizards, soil, and water were conducted using the epiR package [23] within R [24]. Apparent prevalence estimates by population based on a total population estimate of 230 takahe. The influence of location on the prevalence of *Salmonella* in takahe was assessed using the Fisher's exact test in R [24].

Genomic Analysis of *Salmonella* Isolates

All isolates, one per source, identified as *Salmonella* by serotyping was subject to genomic DNA extraction and sequencing. Bacterial DNA was extracted using a Qiagen DNeasy blood and tissue kit (Bio-Strategy, New Zealand) according to the manufacturer's instructions for gram-negative bacteria, with the final elution step modified to 200 μl sterile MilliQ water (Merck, New Zealand). Qubit DNA, RNA, and protein assay kits (Life Technologies, New Zealand) were used to ensure DNA quality. DNA was stored at −20 °C prior to sequencing at New Zealand Genomics Ltd., New Zealand. Sequencing, genome assembly, curation, and annotation were conducted using previously described protocols [10].

Comparative Genomics of *Salmonella*

Extraction, alignment, and concatenation of 51 ribosomal multi-locus sequence typing (rMLST) genes was conducted within a local instance of BIGSdb [25]. Two genes, *rpm*E and *rpm*J, were not included in the analysis due to the presence of paralogs for these loci in *Salmonella*. NCBI *Salmonella* reference genomes, and assembled contiguous sequences of the private island and publically available pubMLST *Salmonella* Mississippi and *S. houtenae* (Supporting information) ribosomal gene sequences were aligned using Muscle [26] and checked for completeness using Geneious [27]. A median joining network of concatenated rMLST nucleotide differences between isolates was visualized in PopART (http://popart.otago.ac.nz).

Analysis of Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) within the core genome, a set of genes shared across all genomes of interest, were initially identified using kSNP v2 [28] without a reference genome to allow identification of core SNPs present among the study isolates. Isolate genomes were analyzed according to serotype designation. In other words, core SNPs were identified within all *S.* Mississippi, or *S. houtenae* 40:gt- genomes. SNPs were checked for read quality and read depth. Reads were aligned to each SNP using an in-house script. SNPs with <20 read coverage and ambiguous SNP calls, those that may be mapped to repeats or homologous regions, were removed from the analysis. A median joining network of SNP differences between isolates was visualized in PopART (http://popart.otago.ac.nz).

SNPs were visualized in Geneious [27] and categorized according to their translational effect on the amino acid sequence: synonymous (does not change the amino acid), non-synonymous (changes the amino acid sequence), or intergenic (in non-coding regions).

Results

Prevalence of *Salmonella* in Takahe

The apparent prevalence estimate of *Salmonella* in the takahe population was 4% (95% CI 1–9%) (6/147). Significant differences in *Salmonella* prevalence were found according to location (p value 0.002), with *Salmonella*-positive individuals, only detected in the private island population (Table 1). Two serotypes of *Salmonella* were isolated from takahe on the private island. Five takahe tested positive for *Salmonella*

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Table 1 Apparent prevalence of *Salmonella* estimates in takahe populations tested in this study

Location	No. positive/no. sampled	Apparent prevalence (95% CI)
Burwood Bush breeding center	0/44	0% (0–12%)
Mana Island	0/6	0% (0–58%)
Maud Island	0/4	0% (0–72%)
Maungatautari ecological island	0/8	0% (0–48%)
Murchison Mountains	0/46	0% (0–11%)
Private Island	6/19	32% (13–57%)
Te Anau wildlife reserve	0/4	0% (0–72%)
Tiritiri Matangi Island	0/15	0% (0–30%)
Willowbank wildlife reserve	0/1	0% (0–99%)

enterica subsp. *enterica* serotype Mississippi and one for *Salmonella enterica* subsp. *houtenae* serotype 40:gt-.

Prevalence of *Salmonella* in Reptile and Environmental Samples

Salmonella was not isolated from any soil (0/6), water (0/6), or lizard (0/31) samples on Maud Island. *Salmonella* was isolated from samples collected on the private island (Fig. 2); *S. houtenae* 40:gt- was isolated from a skink (*Oligosoma notosaurus*) in area E, apparent prevalence (AP) 2% (95%CI 0–11) (1/50); *S. houtenae* 40:gt- was detected in a natural water source in area E and an artificial water source in area F, AP 25% (95%CI 7–59) (2/8); *S.* Mississippi was isolated from four soil samples in areas B, D, F, and G, AP 50% (95% CI 22–79).

Genomic Analysis of *Salmonella* Isolates

Analysis of 51 rMLST gene sequences revealed strong differentiation between the two *Salmonella* subspecies, *enterica* and *houtenae* (Fig. 3, Supporting information). Sequences were relatively conserved between the private island isolates within a serotype, indicating clonal origins or evidence of the relatively recent common ancestry. Upon comparison of the study isolates to publically available reference and draft *Salmonella* genomes, *S. houtenae* 40:gt- study isolates clustered within *S. houtenae* serotypes and shared its closest common ancestor with an undescribed *S. houtenae* (SRR1427085) draft genome. Three ribosomal sequence types (rST) of *S.* Mississippi were identified from the private island takahe and soil isolates. The study isolates shared their closest common ancestor with *S.* Mississippi draft genome 2010K-1406. Within the pubMLST listed *S.* Mississippi draft genomes, a *S.* Mississippi rST was distantly related to the study isolates, instead clustering in *Salmonella* Weltevreden, *Salmonella* Typhi, and *Salmonella* Typhimurium.

Thirty-seven core SNPs were identified between 9 *S.* Mississippi isolates, of which 4 were intergenic, 12 were synonymous, and 21 non-synonymous (Fig. 4a, Supporting information). Eight SNPs were removed during filtering due to low read coverage or ambiguity of SNP calling. The closest related isolates differed by 2 SNPs, with the most distant containing a difference of 18 SNPs (Fig. 4a). There was little evidence of clustering according to the source, location, or time sampled between the *S.* Mississippi isolates, with the exception of two *S.* Mississippi takahe isolates sampled at the same time from different territories. Common ancestry in the bacterial phylogeny of *S.* Mississippi was inferred in the core SNP minimum spanning tree of isolates originating from both takahe and soil, in different areas of the island (Fig. 4a).

Five core SNPs were present between the five *S. houtenae* 40:gt- isolates (Fig. 4b, Supporting information). Of the five SNPs, four were non-synonymous and one was in an intergenic region between two coding sites (Supporting information). Three SNPs were removed during filtering due to low read coverage or ambiguity of SNP calling. Two water isolates, one natural and one artificial in different areas of the island, were clonal, with no core SNP differences (Fig. 4b). The lizard isolate from area E shared two SNPs with the water isolates (one from area E) and three SNPs with an isolate derived from a takahe occupying the same territory. There were no SNPs unique to the *S. houtenae* 40:gt- from a lizard (Fig. 4b).

Discussion

Prediction and prevention of threatening diseases in endangered wildlife requires epidemiological understanding of pathogen dynamics in the host ecosystem, particularly in those species, which are subject to population disturbance via translocation [2, 16, 29–31]. However, empirical characterization of wildlife host-pathogen relationships and the identification of reservoirs is a conceptual challenge due to the presence of complex ecological structures in natural systems [32, 33]. Here, we demonstrate how genetic epidemiology can be used to assess host-microbial relationships to identify sources and genetic linkages among reservoirs, lending insight into potential routes of transmission and guiding mitigation of pathogen infection in endangered species.

Prior to this study, there was a lack of baseline information on *Salmonella* prevalence in takahe, with anecdotal reports of *Salmonella* Brandenburg [34], *Salmonella* Typhimurium [35], and *Salmonella* Mana [36] isolated from a few individuals. Interestingly, we only detected *Salmonella* (*S.* Mississippi and *S. houtenae* 40:gt-) in one sub-population of takahe. Isolation of *Salmonella* from one population creates logistical difficulties when attempting to balance the disease risks associated with translocating members of this population into the

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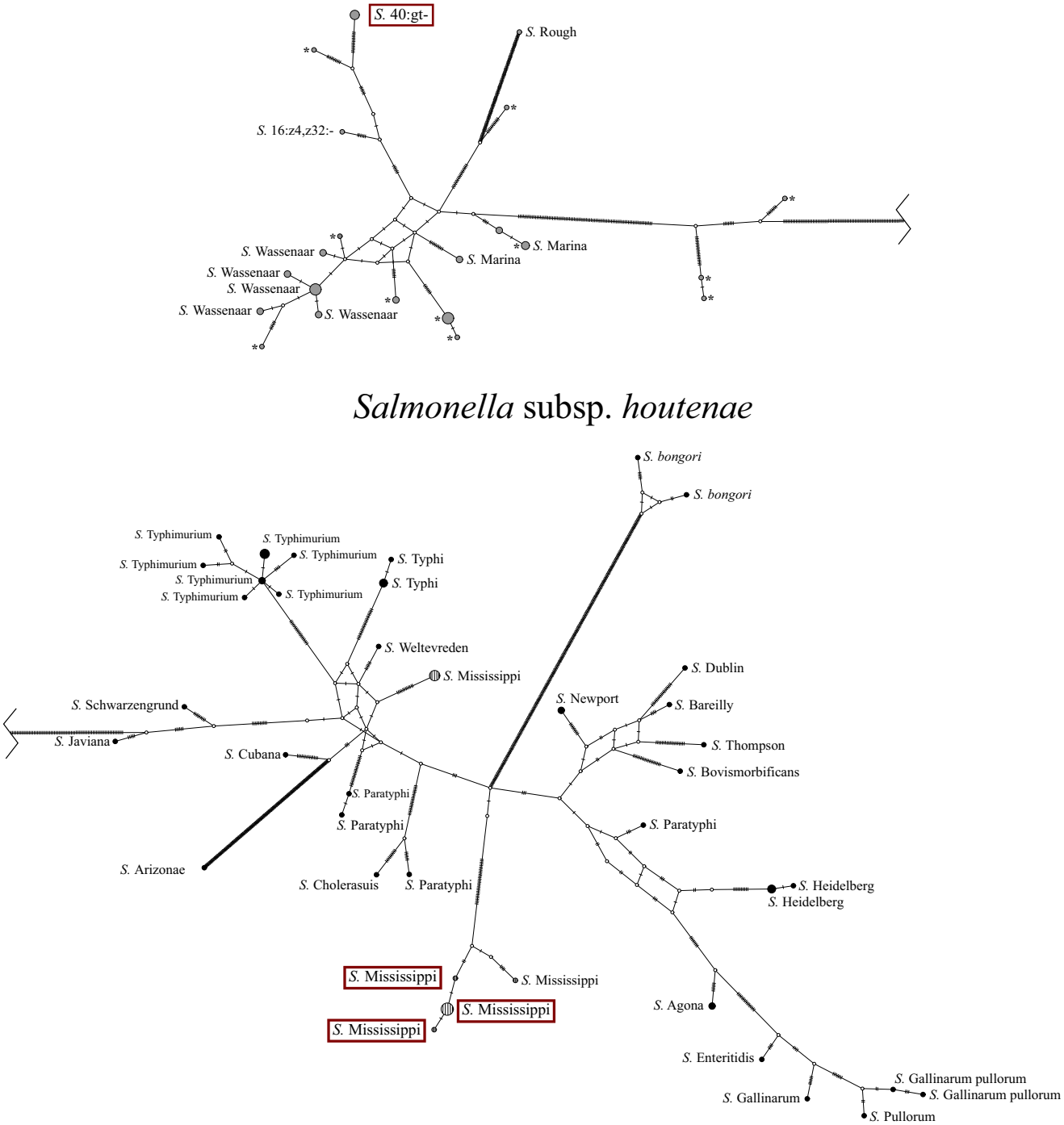

Fig. 3 Median joining network, split by zigzag line for ease of viewing, comparing concatenated 51 gene nucleotide rMLST sequences of *Salmonella* Mississippi (*striped circles*) and *Salmonella houtenae* 40:gt- (*gray circles*) from takahe (*Porphyrio hochstetteri*), a skink (*Oligosoma notosaurus*) and the environment (*red rectangles*) with publically available *S.* Mississippi (*striped circles*) and *S. houtenae* (*gray circles*) downloaded from the pubMLST database [60], and NCBI reference *Salmonella*. The *size of the circle* (*edge*) corresponds to the number of isolates with the same nucleotide profile. *Hatch lines* indicate the number of SNP differences between edges. *White circles* indicate inferred common ancestors

wider takahe conservation network [13], which is needed for intensive conservation management to minimize inbreeding [37]. Translocation restrictions imposed if an individual tests positive for *Salmonella* may have prevented the spread of

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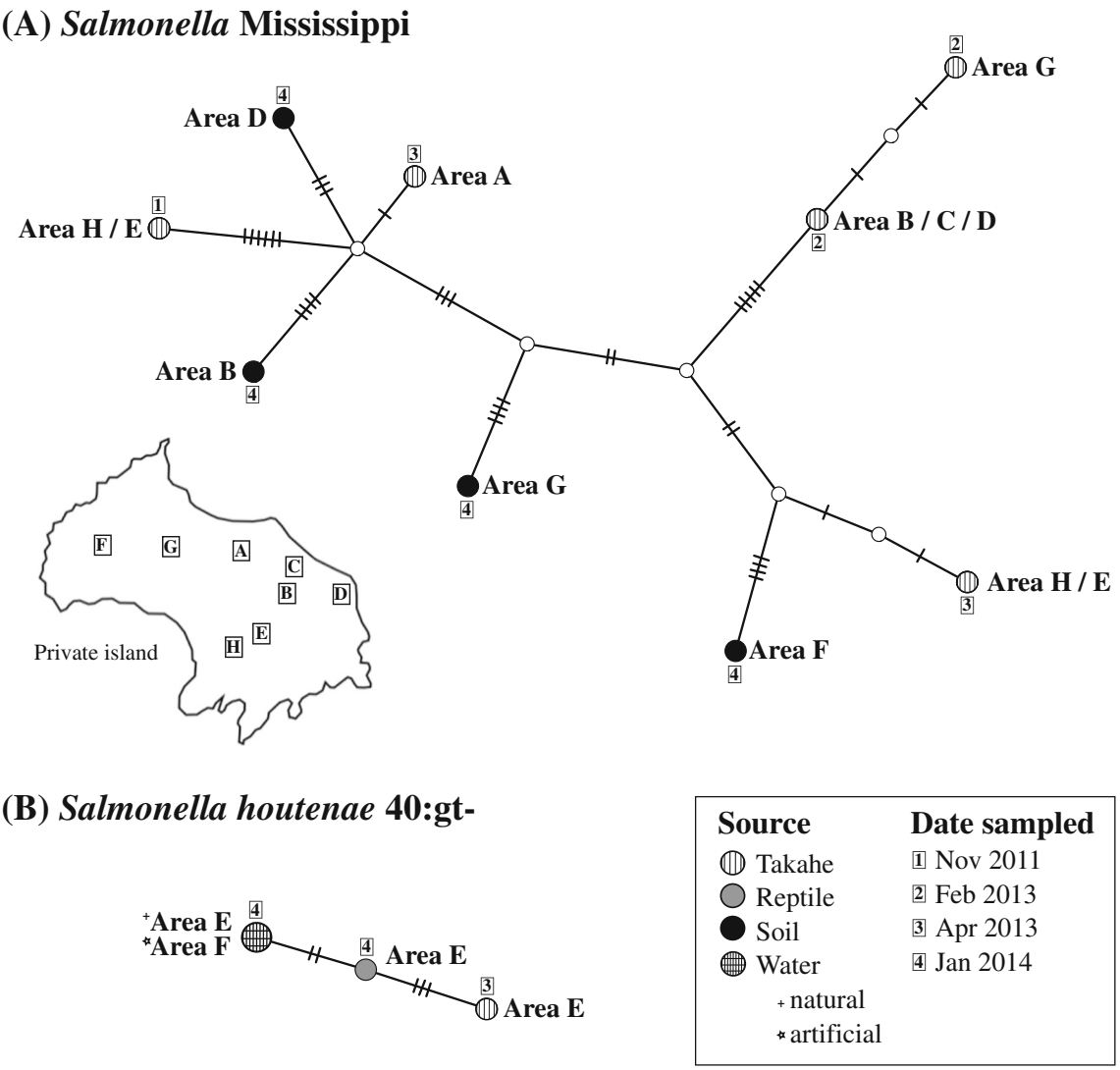

Fig. 4 Median joining networks of core single nucleotide polymorphisms (SNPs) of **a** *Salmonella* Mississippi and **b** *Salmonella houtenae* 40:gt- isolates from different sources on a private island in New Zealand. *Areas* correspond to territory the source/isolate occupies on the private island, see Fig. 2. The *size of the circle* (*edge*) corresponds to the number of isolates with the same SNP profile. *Hatch lines* indicate the number of SNP differences between edges. *White circles* indicate inferred common ancestors

Salmonella from an epidemiological "source" population throughout the takahe meta-population, thus explaining the low prevalence observed.

Epidemiological genomic investigation revealed potential reptile and environmental sources of *S.* Mississippi and *S. houtenae* 40:gt- on the private island and low diversity among *Salmonella* isolates belonging to the same serotype with little differentiation according to host and environmental source (Fig. 4). Lack of genetic variation could be representative of cross-species transmission between sympatric hosts and environmental sources, evidence of a recent introduction into the ecosystem or the presence of a common unsampled or under-sampled source. This is the first report of *S.* Mississippi and *S. houtenae* 40;gt in takahe; however, the *Salmonella* serotypes have previously been isolated from a range of taxa, including wildlife [38–40] and humans [41]. Interestingly, *Salmonella* was not isolated from reptile and environmental samples on Maud Island, a location where takahe have historically and in the present study, tested negative for *Salmonella*. However, S. Mississippi and *S. houtenae* have been detected in wildlife and environmental samples on another takahe island [42]. Therefore, external takahe populations may also be exposed to these serotypes and factors other than exposure are driving increased *Salmonella* prevalence and transmission risk on the private island.

For organisms transmitted via the fecal-oral route, intensified use of supplementary food and water sites may increase local densities of infectious stages of pathogens in the soil and thus host exposure [43, 44]. Elevated contact rates between song birds aggregating around anthropogenic resources have been attributed to outbreaks of *Mycoplasma gallisepticum* [45], *Trichomonas gallinae*

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[46], and *Salmonella* [42]. Takahe on the private island are free-ranging and mostly eat a natural diet; however, supplementary food and water is occasionally provided by island caretakers. The widespread distribution of *S.* Mississippi in soil surrounding feeders and the close relationship between soil and takahe *S.* Mississippi sequences (Fig. 4), indicates that fecal-oral exposure at feed stations may be an important route for transmission. Reduction in temporal and spatial overlap of hosts at focal points is a simple approach to limit intra- and inter-population transmission opportunities. This could be achieved through the use of multiple water troughs and feeders with regular repositioning of these within a territory. Isolation of *S. houtenae* 40:gt- from a water trough demonstrates the need for thorough decontamination of artificial containers. However, intensive maintenance may be logistically difficult to implement due to the remoteness islands.

Optimal management strategies for infectious organisms depend on the mode and route of transmission, and the role population density and contact plays in host-pathogen relationships [47]. Host density and connectivity have been highlighted as influential factors determining fecal-oral transmission of generalist bacteria such as *Escherichia coli* between wild ungulates in Kenya [4, 5]. The carrying capacity for takahe on the private island is calculated according to the proportion of suitable habitat and the estimated takahe home range [48]. All of the private island is deemed suitable for takahe with few environmental barriers delineating territories and is one of the most densely populated reserves used for takahe conservation (*pers. comm*. P Marsh). A higher than normal contact rate between individuals with overlapping home ranges may explain the high prevalence of *Salmonella* in private island takahe, not observed elsewhere, and could contribute to the widespread distribution of *S.* Mississippi on the island. Controlled translocation of takahe from the private island to an isolated interim quarantine facility on the mainland, coupled with long term microbial testing for *Salmonella* shedding, could be an effective way to reduce density dependent transmission in the source population, if density dependence is an important factor.

The sparsity of core SNPs between *S. houtenae* 40:gt- isolates a lizard, a takahe and a stream in one area of the island is suggestive of localized transmission (Figs. 3 and 4). However, sharing of a subtype may be indicative of exposure to a common source rather than direct transmission between two hosts. *Salmonella* are ubiquitous bacteria, and visiting and resident seabirds, including penguins and gulls, which have been described as carriers of *Salmonella* [38, 49], could be a cryptic unsampled reservoir of the *Salmonella* isolated in this study. Given that reduction in prevalence in the lizard population is not logistically feasible, simple mitigation measures could be enforced to reduce environmental transmission of *Salmonella* to takahe. Block perturbation of transmission routes [33], including erection of barrier fencing around natural streams and replacement with artificial water sources, are effective management strategies.

Although some samples were collected over a year apart, there was little evidence of recombination, and sequences were relatively conserved among *S.* Mississippi and *S. houtenae* 40:gt- genomes (Figs. 3 and 4). Novel mutations may be acquired over time frames of days to months [50]. However, the rates of recombination and mutation are thought to be highly variable depending on the S. *enterica* subsp. *enterica* lineage [51], and as with many *Salmonella* serotypes, those found in this investigation are yet to be characterized. The low number of core SNPs observed could be explained by a recent introduction of *Salmonella* onto the island and/or a selective bottleneck reducing genetic diversity and resulting in few polymorphic sites [52].

Although whole genome sequencing is rapidly advancing our epidemiological knowledge of host-microbe relationships, limitations still stand when attempting to identify the specific interactions in multi-host systems that lead to the pattern of genotypes observed. Prevalence estimates and genomic analysis of single *Salmonella* isolates from a given source are reliant on imperfect culture [53] and do not account for within-host bacterial variation [54]. Similarities and inference of cross-species transmission may be overestimated as a result of a small selection of isolates and low number of informative SNPs [55]. Additionally, in multi-host systems, the ability of genetic markers such as SNPs to differentiate transmission dynamics may be obscured by bidirectional flow of bacteria between the hosts and the environment [56]. Thus, uncertainty due to random selection, limited sample sizes and unaccounted for diversity within and between hosts is an inherent limitation in genomic epidemiological investigations until the cost of sequencing declines and more isolates can be analyzed. However, sequence analysis of only a few isolates in an ecological setting can provide a wealth of epidemiologically relevant information and allows quantification of differences between isolates which is unattainable through traditional *Salmonella* typing methods [57, 58]. The innovative approaches used in the current and previous investigations of host-microbe relationships in takahe [10, 59] are examples of how combining microbial genomics with epidemiological and ecological perspectives can be used to ascertain and understand disease dynamics and risks in endangered wildlife populations. The findings of this study raise important questions with regards to pathogen transmission dynamics on islands, the management of disease in endangered populations and the infectious disease risks associated translocation of infected wildlife to new environments.

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Supporting Information A map of pitfall locations on Maud Island (Appendix S1), the number of lizards captured and sampled (Appendix S2), the list of *Salmonella* genomes used for 51 gene ribosomal multilocus sequence comparison (Appendix S3), SNP information for *Salmonella* Mississippi (Appendix S4) and *S. houtenae* 40:gt- (Appendix S5) study isolates, and the alignment of rMLST gene sequences (Appendix S6) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than absence of the material) should be directed to the corresponding author.

Compliance with Ethical Standards All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. Reptile capture and sampling was conducted according to a Victoria University of Wellington animal ethics permit 2012R33 and the New Zealand Department of Conservation research permit 37543-FAU. Takahe samples were collected under a Massey University animal ethics permit MUAEC Protocol 11/95. This article does not contain any studies with human participants performed by any of the authors. This study was funded by the Allan Wilson Centre.

Conflict of Interest The authors declare that they have no conflict of interest.

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