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A Comparison of *Salmonella* Serotypes Isolated from New Zealand Sea Lions and Feral Pigs on the Auckland Islands by Pulsed-field Gel Electrophoresis

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ABSTRACT: The *Salmonella* serotypes *S.* Cerro and *S.* Newport were isolated from New Zealand sea lions (*Phocarctos hookeri*) and feral pigs on the Auckland Islands in the New Zealand subantarctic region. The isolates were typed by pulsed-field gel electrophoresis using *Xba*1 as the restriction enzyme. The isolates were indistinguishable, which suggests that *Salmonella* infection cycles between sea lions and pigs in this environment. Apart from a previous isolation from a single New Zealand fur seal (*Arctocephalus forsteri*), *S.* Newport has not been recorded in any animals from New Zealand, but it is associated with gastroenteritis in humans. Contamination of the marine environment by human waste is a possible source of infection for marine mammals and warrants further investigation.

Key words: Auckland Islands, feral pigs, New Zealand sea lion, *Salmonella*.

In January and February 1998, a mass mortality of New Zealand (Hooker's) sea lions (*Phocarctos hookeri*) occurred on the Auckland Islands (50°S, 166°E) and Campbell Island (52°S, 169°E) during the species' breeding season (Baker, 1999). Animals on the principal rookeries at Dundas Island, Enderby Island, and Figure of Eight Island within the Auckland Islands group were affected. During the course of an investigation into the cause of the mortality, a number of *Salmonella* serotypes and a novel species of *Campylobacter* were recovered from samples collected postmortem (Stratton et al., 2001). *Campylobacter* were consistently isolated from tissues collected from adult animals, but four *Salmonella* serotypes were also recovered from pup and adult tissues, including *S.* Cerro (from one Dundas Island pup and one Enderby Island adult), *S.* Newport (multiple isolates from one Dundas Island pup), *S.* Derby (from one Dundas Island pup and two Enderby Island pups), and three phage types (4, 8, and untypeable) of *S.* Enteritidis (from three Dundas Island pups and three Enderby Island adults). The *Salmonella* isolates were not thought to have been the cause of the mass mortality but may have been a result of opportunistic infections in debilitated animals.

One year after the outbreak, a group of 17 feral pigs was captured on the islands and transported to the South Island of New Zealand to enable the conservation of unique genetic material because the remainder of the population were due to be killed to protect endemic flora and fauna. These pigs had been isolated on the islands for many years, having been introduced from a visiting ship in 1807 as a source of food for whalers and shipwrecked sailors. They appeared to have thrived on the islands, being reported as "numerous" in 1840, when more pigs were released.

As part of New Zealand quarantine regulations, fecal samples were collected from the pigs, and three *Salmonella* serotypes, *S.* Typhimurium (two), *S.* Cerro (one), and *S.* Newport (one) were recovered from four of the 17 animals. Although *S.* Cerro and *S.* Newport had been recovered infrequently from cases of human gastroenteritis in New Zealand (five and 16 isolations, respectively, between 1997 and 1998) and from poultry feed (two isolations in 1997), they had not been isolated from domestic animals; as a result, a decision was made to keep the adult animals permanently in quarantine and to test the offspring before release. One year later, one of the piglets

tested positive for *S.* Newport (Stone, 2002). *Salmonella* Newport previously had been recovered postmortem from a captive New Zealand fur seal (*Arctocephalus forsteri*), the only nonhuman isolation reported in this country (Cordes and O'Hara, 1979).

To investigate a possible association between the pigs and the sea lions on the Auckland Islands, isolates of *S.* Cerro and *S.* Newport that had been recovered from the animals were analyzed by pulsed-field gel electrophoresis (PFGE). Macrorestriction profiling by PFGE with the restriction enzyme *Xba*I was performed using the method described by Alley et al. (2002). Genomic DNA was extracted according to the method of Böhm and Karch (1992), with some modifications. *Salmonella* isolates from sea lions and pigs were grown in 3 ml of brain-heart infusion broth (Difco, Becton Dickinson, Australia/New Zealand) overnight at 37 C. The optical density of the broth was measured and adjusted to 1.4 at 610 nm. A 200 μl aliquot of cells was pelleted by centrifugation at 13,000 × G for 5 min. Cells were washed once with 150 μ of Pett IV buffer (1 M NaCl, 10 mM Tris-HCl [pH 8.0], and 10 mM ethylenediaminetetraacetic acid [EDTA] [pH 8.0]) and were centrifuged and resuspended in 50μ of Pett IV buffer. The bacterial suspension was mixed with 100 μ of 1% low-melt preparative-grade agarose (Bio-Rad, Sydney, Australia) and was dispensed into plug molds. The agarose plugs were placed on ice for 1 hr to solidify. The plugs were lysed overnight at 56 C in a buffer solution (50 mM EDTA [pH 8.0], 50 mM Tris-HCl [pH 8.0], and 1% sodium lauroyl sarcosine) that contained 1 mg of proteinase K (Roche Diagnostics, Auckland, New Zealand) per milliliter. After lysis, the plugs were washed five times, for 1 hr each time, with 10 ml of TE buffer (10 mM Tris-HCl and 1 mM EDTA) on ice. A 3-mm slice of each plug was equilibrated in 100 μ of 1.2× restriction buffer (Roche Diagnostics) for 45 min on ice. The restriction buffer was removed and replaced with 100 μ of fresh 1× restriction buffer that contained 30 U of *Xba*I (Roche Diagnostics). The plug slices were held on ice for an additional 45 min before an overnight incubation at 37 C.

The restriction fragments were separated by PFGE using a CHEF-DRII system in a 1% agarose gel (Pulsed Field Certified Agarose; Bio-Rad) in 0.5× TBE buffer (45 mM Tris, 45 mM boric acid, and 1 mM EDTA [pH 8.0]) at 14 C. The gel was run at 6V per cm for 22 hr, with an initial pulse time of 5 sec and a final pulse time of 50 sec. Lambda Ladder PFG Marker and Low Range PFG Marker (New England Biolabs, Auckland, New Zealand) were included as molecular size standards. Gels were stained with ethidium bromide, and images were captured under ultraviolet illumination using the Gel Doc 2000 (Bio-Rad).

The results of PFGE showed that the isolates of *S.* Cerro and *S.* Newport from the sea lions and the pigs were indistinguishable (Fig. 1). According to criteria developed by Tenover et al. (1995), these results imply that the pig and sea lion isolates are clonal and derived from a common source.

On the basis of these results, it is probable that cross-infection with *Salmonella* serotypes occurred between sea lions and pigs on the Auckland Islands. Because pigs on the island are omnivorous and have been observed scavenging on sea lion carcasses (P. J. Duignan, pers. obs.), it is possible that they became infected by this route or that pig feces on the beach resulted in colonization of the sea lions and the subsequent opportunistic infections seen in 1998. Questions remain however, as to which animal species was the primary reservoir of infection, whether both were carriers, and how the *Salmonella* serotypes became introduced to these remote islands. The isolations of *Salmonella* from the pigs were made 1 yr after the sea lion mortality event, which suggests that they were either long-term carriers of the or-

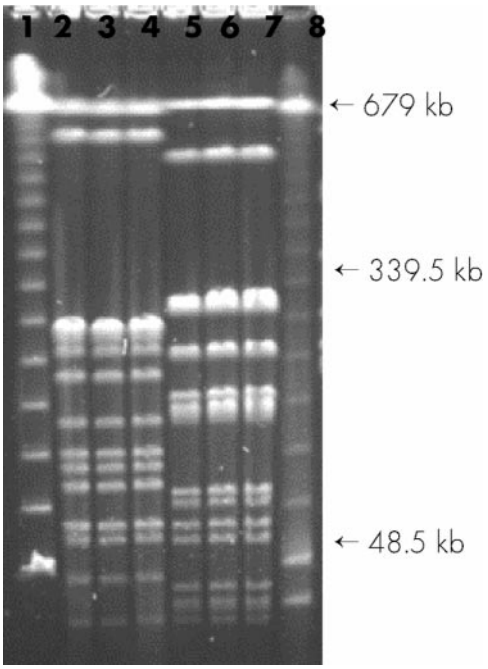

FIGURE 1. Pulsed-field gel elecrophoresis of *Salmonella* isolates from sea lions and pigs. Lanes 1 and 8–Lambda molecular weight and Low Range PFG markers; lanes 2 and 3–*S.* Cerro from sea lions; lane 4–*S.* Cerro from pig; lanes 5 and 6–*S.* Newport from sea lions; lane 7–*S.* Newport from pig.

ganism or were being constantly reinfected, and that they may have been the primary reservoir. This hypothesis is supported by the further recovery of *S.* Newport from a piglet born to one of the quarantined pigs 1 yr later, 2 yr after the sea lion mortalities. None of the original animals tested positive at this time, although, because they were still in quarantine, the strain would appear to have been maintained within the group. Nevertheless, it is possible that both species were naturally infected with the organism and that it cycled between them because of close contact on the islands.

Evidence to support the role of sea lions as a reservoir of infection is provided by research carried out in California (USA) and on other sub-Antarctic islands. One of the earliest studies recorded was from San Miguel Island (California) in 1979, where rectal swabs were obtained from 90 northern fur seal (*Callorhinus ursinus*) pups and 50 California sea lion (*Zalophus californianus*) pups. Three serotypes of *Salmonella* were recovered from 33% of fur seals and 40% of sea lions, including *S.* Newport (Gilmartin et al., 1979). In California, between 1994 and 1995, nine *Salmonella* serotypes were recovered from 49 stranded pinnipeds that had died during rehabilitation. Affected species included California sea lions, harbor seals (*Phoca vitulina richardsii*), and northern elephant seals (*Mirounga angustirostris*). *Salmonella* Newport was the most common serotype isolated from these species (Thornton et al., 1998). A further study in California during 1999–2000 resulted in recovery of seven serotypes of *Salmonella* from nine animal species at rehabilitation centers. Again, *S.* Newport was one of the serotypes isolated from California sea lions (Smith et al., 2002). Research into the occurrence of *Salmonella* serotypes in sub-Antarctic wildlife in the South Georgian Archipelago (UK) found an increase in carriage in Antarctic fur seals (*A. gazella*), from 5% in 1996 to 22% in 1998. *Salmonella* Typhimurium was only isolated from one fur seal in 1996, however, *S.* Newport was the most common serotype in 1998, and it was recovered from 23 fur seals and one black-browed albatross (*Diomedea melanophrys*; 52% of the total isolations). As in the current study, all *S.* Newport isolates had indistinguishable DNA macrorestriction profiles, and this was believed to have indicated transmission of the serotype among different species and reservoirs in the region (Palmgren et al., 2000). Although *S.* Newport appears to be endemic in pinnipeds from many different regions, *S.* Cerro has not been previously isolated from marine mammals.

The role of feral pigs as reservoirs of *Salmonella* is harder to elucidate, because no studies have been reported in the literature. Nevertheless, domestic pigs are known to be common carriers of a range of *Salmonella* serotypes in many countries (Barber et al., 2002), and there is no rea-

son to believe that feral pigs are different in this regard.

The initial source of *Salmonella* for the animals on the Auckland Islands is difficult to ascertain. Because of their apparent isolation, it is possible that human activities on the islands led to animal infections. The study reported from South Georgia correlated the serotypes recovered from marine mammals to those commonly reported from human infections and suggested that although only a very small number of people visited the island, the extensive foraging range of the mammals and birds that breed on those islands could predispose them to infection from a site some distance from the islands (Palmgren et al., 2000). The Auckland Islands are visited by up to 600 tourists annually on cruise vessels and by a variable number of scientists, and the surrounding waters are fished for squid and scampi by vessels from New Zealand and overseas. Any, or all, of these could act as a source for contamination of the islands or the marine environment.

In California, sewage effluent from a treatment plant was identified as the source of infection of an unusual serotype for commercial poultry, and the study recovered six *Salmonella* serotypes, including *S.* Cerro (Kinde et al., 1996). The issue of *Salmonella* survival in marine environments has been debated and investigations have shown that the organism can survive well in seawater with salinities as high as 3.5% (Minette, 1986). In a study conducted between 1992 and 1996, Spanish researchers recovered 42 serotypes of *Salmonella* from seawater, which indicates that pollution with human sewage poses a considerable risk of infection for marine mammals and has probably resulted in widespread intestinal colonization (Polo et al., 1999).

In conclusion, the weight of evidence suggests that the sea lions were infected with *Salmonella* before the mass mortality and that the infections were then maintained by transmission within the colony. Because the pigs had been isolated on the islands for over 100 yr, they may have become infected from the sea lions initially, or from other animals on the islands, and, as with other pigs, have been able to maintain the organism efficiently. Thus, a natural cycle probably exists between the two species on these remote islands.

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