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Salmonella javiana infection in an infant associated with a marsupial, the quokka, *Setonix brachyurus*, in Western Australia

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SUMMARY

Salmonella javiana, a serotype rarely isolated in Australia, has been recovered from the faeces of a 14-month-old infant with symptoms of enteritis.

The child had been closely associated with a marsupial species, the quokka, during a vacation on Rottnest Island in Western Australia, and *S. javiana* was isolated from faecal pellets from adult quokkas, and also from a snake collected on the island.

Sampling revealed a high incidence of *Salmonella* infection in the quokkas. In all, 62 out of 87 animals (71 %) were found to be infected, and 17 *Salmonella* and 3 *Arizona* serotypes were identified from 100 isolations comprising 92 salmonellas and 8 arizonas. Multiple infections were frequently detected and up to four serotypes were recovered from individual animals. *S. javiana* was isolated from four quokkas.

A close parallel was observed between the serotypes isolated from quokkas and sea-gulls on the island, and abattoir effluents, lake waters, bird droppings and reptiles sampled on the adjacent mainland.

The epidemiological significance of *Salmonella* and *Arizona* infections in the quokka population and their possible association with the seasonal decline in condition and numbers of animals on Rottnest Island is discussed.

INTRODUCTION

Salmonella javiana was first isolated from the faeces of a child affected with gastro-enteritis on the island of Java, and from two symptomless carriers in Panama (Edwards & Bruner, 1942). In the same year the organism was responsible for a serious outbreak of infection in a group of Navajo Indians in New Mexico (Alley & Pijoan, 1942). A total of 15 patients were admitted to hospital and there were two deaths. The source of infection was traced to a locally prepared cottage cheese.

Over the years the majority of *S. javiana* infections in humans have occurred in

the United States of America, particularly in southern states bordering the Gulf of Mexico (Edwards, Bruner & Moran, 1948; Sanders *et al.* 1965; Martin & Ewing, 1969). Occasional human isolations have been reported from Canada and several European, Asian and South American countries (Kelterborn, 1967). *S. javiana* has also been isolated from symptomless children in rural areas of Ceylon (Gulasekharam & Velaudapillai, 1961) and from West Malaysian aborigines (Anandan, Lim & Haug, 1969). A single family outbreak has been reported from Australia (Atkinson, Carter, Wollaston & Wall, 1953). The serotype was isolated from three children with gastro-enteritis and from two healthy carriers.

In contrast to human isolations, only a few *S. javiana* strains have been recovered from animal sources; however, the serotype has been isolated from poultry, pigs, dogs and cats in America (Edwards *et al.* 1948; Bruner & Moran, 1949; Galton, Smith, McElrath & Hardy, 1954), a Canadian mink (Bynoe & Yurack, 1964), livestock in Holland (Kelterborn, 1967), and pigs in England and the Philippines (Taylor, 1969; Arambulo, Westerlund & Sarmiento, 1968). *S. javiana* has also been isolated from Chinese egg, desiccated coconut from Ceylon and Indian bone products, as well as human sources in Ceylon, England, Malaya, New Zealand and British Guiana (Semple, Graham & Dutton, 1961; Taylor *et al.* 1965).

The present case was diagnosed in a 14-month-old infant who had developed enteritis during a family holiday on Rottnest Island, a popular tourist resort 20 km. from the mainland close to Perth. *S. javiana* was isolated on two occasions from the child's faeces. In attempts to trace the source of the infection, it was noted from laboratory records that a single culture identified as a monophasic *S. javiana* strain had been isolated previously from a snake (*Demansia nuchalis affinis*) captured on the same island. There was no history of direct contact with reptiles; however, it was established that during the holiday period, and before the onset of symptoms, the child had been closely associated with the local quokka (*Setonix brachyurus*), a small marsupial wallaby present in large numbers on the island, and had frequently been observed playing with faecal pellets scattered throughout the settlement area.

A sampling programme was therefore initiated, and *Salmonella* investigations were undertaken, concentrating firstly upon faecal pellets scattered on the ground throughout the settlement area. The survey was then extended to include rectal swabs and faecal samples collected from adult quokkas from all parts of the island. The animals were swabbed immediately following capture and then held in cages until fresh faeces were voided. Finally, in an attempt to identify possible vectors for the *Salmonella*, many of which were serotypes commonly found in abattoir effluents from the mainland, cloacal samples were collected from sea-gulls (*Larus novaehollandiae*).

MATERIALS AND METHODS

Faecal samples from the infected infant and contacts of the case were collected and examined after the family had returned to the mainland. Animal droppings were collected on the island close to the holiday accommodation and throughout the settlement area.

Rectal swabs, previously moistened with buffered glycerol saline faeces transport medium (Sachs, 1939), were collected in duplicate from 87 quokkas and 83 silver gulls captured close to the settlement and from more remote areas of the island. Faecal samples were also examined from individual animals and birds held captive overnight. Samples of sea water were tested using Moore swabs immersed in the nearby harbour for 24 hr.

Faeces and swab samples were plated out direct on Oxoid deoxycholate-citrate (D.C.) agar and modified bismuth sulphite (B.S.) agar, and inoculated into strontium chloride B enrichment broth (Iveson, 1971) incubated at 43° C. and strontium selenite A broth (Iveson, 1973) incubated at 37° C. The enrichment media were plated out at 24 and 48 hr. intervals on D.C. and modified B.S. agar, and colonies were identified biochemically and serologically.

RESULTS

Direct culture of the child's faeces revealed a heavy growth of *S. javiana*. No other serotypes were detected by multiple-isolation techniques. *S. javiana* was again isolated a week later, but was not detected in subsequent faecal samples examined after 2 weeks, 6 weeks and 3 months.

Faecal samples from other family members, the domestic cat and persons who had shared or visited the holiday accommodation were all negative. The staff and children from a kindergarten attended by the elder sister were also examined with negative results. Foodstuffs consumed on the island were not available for examination; however, other children and adults who had attended the various meals had remained free of symptoms both before and during the vacation period. Moore swabs immersed in the settlement harbour were also negative.

Examination of quokka droppings collected throughout the settlement area showed that 50% (13) of the samples were infected. *S. anatum*, *S. chester* and *S. havana* were isolated close to the hut previously occupied by the family, and *S. javiana*, *S. decatur*, *S. muenchen*, *S. newington*, *S. typhimurium* and *Arizona* 26:26:25 were isolated from samples collected in the surrounding area. Multiple infections were detected in four samples and one sample yielded three serotypes.

A total of 100 isolations comprising 92 *Salmonella* and 8 *Arizona* species were recorded from 62 out of 87 adult quokkas (71%); 17 *Salmonella* and 3 *Arizona* serotypes were identified. Multiple infections were again frequently encountered and up to four serotypes were detected in individual animals. *S. javiana* was isolated from four individual animals. The distribution and relative frequency of *Salmonella* and *Arizona* serotypes in samples collected from individual quokkas, sea-gulls and their droppings are detailed in Table 1.

DISCUSSION

Salmonella organisms have been previously isolated from both healthy and diseased marsupials, and in some cases have been associated with fatal infections in captive animals (Atkinson *et al.* 1953; Lee & Mackerras, 1955; Winter & O'Connor, 1957; Yadav, Stanley & Waring, 1972). *Salmonella* infection in wild

Table 1. Salmonella *and* Arizona *serotypes from Rottnest Island and adjacent mainland sources*

	Rottnest Island			Mainland			
Serotype	Quokkas	Quokka droppings	Seagulls	Seagull droppings	Reptiles	Lake water	Abattoir effluents
S. adelaide	2	.	1	2	3	2	14
S. anatum	.	1	.	2	1	4	10
S. bootle	1	.	.	.	6	.	.
S. newbrunswick	1	.	.	.	.	.	.
S. bahrenfeld	9	.	.	.	2	.	5
S. chester	1	3	.	.	4	.	5
S. decatur	.	1	.	.	1	1	.
S. derby	.	.	1	8	.	14	44
*S. fremantle**	2	.	.	.	4	.	.
S. give	1	.	.	17	5	.	2
S. havana	1	6	.	2	1	7	24
S. javiana	4	2	.	.	.	.	.
S. muenchen	23	1	.	.	6	3	2
S. newington	17	2	.	2	1	2	9
S. oranienburg	3	.	.	.	6	2	5
S. orientalis	4	.	.	.	11	.	1
S. orion	5	.	.	1	.	.	3
S. typhimurium	8	1	2	4	9	5	13
*S. wandsbek**	9	.	.	.	8	.	3
S. waycross	1	.	.	.	1	.	.
A. 16:23:25	2	.	.	.	12	.	.
A. 26: 23:21	1	.	.	.	2	.	.
A. 26:26:25	5	1	.	.	23	.	.
E. tarda	.	.	.	.	6	10	.
Total isolations	100	18	4	38	112	50	140

* Salmonella subgenus II.

marsupials has not been previously reported from Australia; however, samples of kangaroo meat sold as pet food have been shown to be contaminated with many serotypes including *Arizona* species (Laurie & Kovacs, 1960; Anderson, Crowder & Woodruff, 1964; Vernon, 1966; Iveson & Mackay-Scollay, 1972).

Infections of marsupials with pathogenic Enterobacteriaceae have not been confined to Australian animals, and *Salmonella* and *Arizona* bacteria have been isolated from marsupials in the United States of America (Edwards, McWhorter & Fife, 1956; Schnurrenburger *et al.* 1968; Marx, 1969) and from South America (Lins, 1970).

The results of the present investigation have not only implicated the quokkas as the most likely source of *S. javiana* infection in the infant, but have also drawn attention to the need for further studies to evaluate the epidemiological significance of *Salmonella* infections within the quokka community.

The quokka (*Setonix brachyurus* (Quoy & Gaimard)) is a small nocturnal marsupial weighing approximately 3 kg and with a ruminant-like digestion (Moir,

Somers & Waring, 1956). The wallaby was once widespread on the mainland of Western Australia, being gazetted as a pest in the 1930s, but following an undocumented catastrophe the species is now restricted to two small offshore islands, Rottnest Island and Bald Island.

The total quokka population on Rottnest Island has been estimated at 5000 individuals or one per acre (Waring, 1956) and during the summer months, which extend from November to March, the animals undergo a marked decline in condition characterized by a loss of body weight, dehydration, a fall in body temperature and severe anaemia. It is during this period that mortality within this population is highest, affecting younger animals more than adults (Shield, 1959).

Over the past 15 years the quokka has been the centre of a number of investigations aimed at documenting changes in population size and elucidating possible causes of mortality, but seasonal debility has not been attributable to any single nutritional or physiological factor (Main, Shield & Waring, 1959; Storr, 1964*a*; Main 1970). Furthermore, the only pathogen isolated previously from morbid animals has been the hookworm *Austrostrongylus thylogale*, which was also present in large numbers in freshwater soaks frequented by the animals during the summer months (Waring, 1956). It was suggested that, while hookworm would certainly contribute to anaemia and loss of condition, protein deficiency was probably the major factor associated with seasonal loss of condition, which might be exacerbated in areas of the island lacking free water (Storr, 1964*a*).

The results of the present investigation have revealed a widespread and unexpected reservoir of infection in the quokka and this raises the question of whether the quokka (and other marsupial species) are natural carriers of *Salmonella* and *Arizona* bacteria or whether the high infection rate is related to the adverse conditions of late summer and perhaps contributes to the seasonal debility of animals in the field. More intensive sampling from animals in better condition on a seasonal basis will be needed before this question can be answered.

Salmonella and *Arizona* isolations from mainland marsupials have been largely from captive animals and, in general, isolation rates have been lower than those from quokkas (see Table 2). However, *Salmonella* organisms have been recovered from the majority of species tested, and high rates of infection have been detected, particularly in animals subjected to stress associated with transportation; for example, 70 % of droppings collected from a truck used to transport grey kangaroos (*Macropus fulginosus*) were infected with *Salmonella* or *Arizona* species. *S. jangwani* was also isolated from four short-eared wallabies (*Petrogale brachyotis*) with symptoms of acute enteritis, shortly after arrival in Perth from the Ord River area. On the other hand *Arizona* 26:26:25 was isolated from a healthy wild tammar (*Macropus eugenii*) immediately after capture on the Abrolhos islands.

The occurrence of anaemia in Rottnest quokkas has been observed to coincide with the decline of good-quality forage and with increased attendance at soaks, but was not associated in previous investigations with a raised leucocyte count or rise in the rate of sedimentation (Shield, 1958, 1959). Barret-Connor (1972) and Weinburg (1971) have recently reviewed the known associations of infection and anaemia, including haematological aspects of *Salmonella* infection and the role of

Table 2. Salmonella *and* Arizona *isolations from captive marsupials in Western Australia*

Species	Tests: Animals	Tests: Droppings	Total positive	Isolations
Macropus robustus (Euro)	18	—	2	*S. typhimurium* (1) *S. decatur* (1)
M. fulginosus (Grey kangaroo)	—	10	7	*S. adelaide* (1) *S. muenchen* (2) A. 26:26:25 (4)
M. eugenii (Tammar)	35	—	2	*S. newington* (1) A. 26:26:25 (1)
Megaleia rufa (Red kangaroo)	1	—	1	*S. wandsbek* (1)
Petrogale brachyotis (Short-eared wallaby)	4	—	4	*S. jangwani* (4)
Dasyurus geoffroii (Native cat)	4	—	4	*S. charity* (2) *S. chester* (1) *S. muenchen* (2)
Sminthopsis murina (Marsupial mouse)	1	—	1	*S. fremantle* (1)
Isoodon obesulus (Bandicoot)	1	—	1	*S. meleagridis* (1)
Tachyglossus aculeatus (Echidna)	1	—	1	*S. anatum* (1)
Totals	65	10	23	24

plasma iron in host–parasite interactions. Further studies are clearly required to assess the role of *Salmonella* infection in the quokka population, as the combination of a low-grade diet, protein-deficiency anaemia, dehydration, hookworm and *Salmonella* infection and pregnancy could be expected to reduce the viability of an animal population restricted to a small island, and collectively operate to the detriment of the host species.

The importance of the carrier animal as a source of *Salmonella* outbreaks in livestock, and the possible relation of infection to stress factors associated with inadequate diet and overstocking, has been reported by Josland (1953). It was also observed that the carrier state could be maintained for long periods in animals restricted to an infected environment. Enrichment culture was the only reliable method for the detection of carriers.

The significance of stress and poor physical condition on the incidence of salmonellas in animal populations harbouring a small number of carriers has been emphasized by Salisbury (1958).

Adverse factors which have influenced rates of *Salmonella* infection in wild animal populations have also been reported by Taylor (1968), who suggested that, while *Salmonella* were present in the healthy host in non-epidemic periods, adverse conditions might precipitate an active infection in one or several animals, thus initiating an outbreak. Contamination of the animal's environment would then be

much greater than that caused by the healthy carrier. Outbreaks of *S. typhimurium* infection in birds had been noted towards the end of adverse seasons when there was a scarcity of suitable food.

The multiplication of *Salmonella* organisms in the rumen of cattle, followed by intestinal infection and excretion of the organisms in faeces, have also been closely related to variations in the food intake (Brownlie & Grau, 1967).

Several factors point to the mainland as being a likely and possibly recent source for at least some of the *Salmonella* strains isolated from the adult quokkas. *S. adelaide*, *S. derby* and *S. typhimurium* were isolated from sea-gulls on the island and have been frequently detected in mainland abattoir effluents and nearby lakes on the mainland frequented by sea-gulls. Furthermore, 13 *Salmonella* serotypes isolated from the quokkas, which together made up 90% of the isolations, have also been recovered from a single mainland abattoir complex which discharges effluent into sea water only 25 km. from Rottnest Island. *S. adelaide*, *S. anatum*, *S. derby*, *S. give*, *S. havana*, *S. newington*, *S. orion* and *S. typhimurium* have also been isolated from bird droppings collected at a lakeside landfill site situated in close proximity to the abattoir complex.

Excretion of several *Salmonella* serotypes by wild gulls has been recorded previously by Windle Taylor (1967). Carriage of *Salmonella* by birds has been regarded as a transient feature, but the possible build-up of infection when large numbers of birds congregate together has been documented by Mitchell & Ridgwell (1971). Apart from humans and sea-gulls a further possible vector for *Salmonella* is wild ducks, which periodically migrate to Rottnest Island to breed, but sampling of 240 mainland black ducks (*Anas superciliosa*) yielded only five *E. tarda* strains and one isolation of *Salmonella wandsbek* – two species rarely associated with either urban or agricultural activity.

Apart from the quokkas, there are no other native mammals on the island; however, at varying intervals over the past 100 years, cattle, sheep, horses, dogs, cats, rabbits, foxes, rodents and several avian species, including peacocks and pheasants, were introduced. These have been restricted in more recent times to pheasants, peacocks, domestic mice, a few feral cats, and a small group of horses available to tourists. Animal feedingstuffs and organic fertilizers have also been used particularly in areas close to the settlement. The island is visited by several migratory bird species including the mutton bird (*Puffinus pacificus*), which nests on the island; however, predatory birds are few and lizards and snakes are abundant (Dunnet, 1962). Silver gulls are at all times plentiful and there is a regular interchange of birds between the island and mainland, particularly in the breeding season (Storr, 1964*b*).

The isolation of *S. javiana* from a snake, an infected child and from the faeces of four adult quokkas has drawn attention to the potential role of reptiles in the interchange of *Salmonella* and *Arizona* serotypes on the island. Reptilian species have been shown to be important natural reservoirs of infection in many parts of the world. *S. javiana*, for example, has been isolated from wild reptiles in Vietnam (Le Minor, 1964) and the Philippines (Westerlund, 1966), and from a captive snake in Germany (Schroder, 1970). Both captive and free-ranging reptiles in Western

Australia have been found to be frequently infected with *Salmonella*, *Arizona* and *Edwardsiella* organisms (Iveson, Mackay-Scollay & Bamford, 1969; Iveson, 1971).

With the exception of only *S. newbrunswick*, *S. javiana* and *S. orion* the serotypes recovered from quokkas have also been isolated from reptiles captured within 35 km. of Rottnest on coastal areas of the adjacent mainland. The importance of geographical isolation in speciation and subsequent adaptation and differentiation of organisms is well known, and although the isolation of the Rottnest fauna occurred before the more recent advent of European man and his domestic animals in the Australian region, it is not surprising, in view of the short distance involved, that there is a close relation between the serotypes occurring in the quokka population and strains recovered from the nearby mainland. Furthermore, the distribution patterns of serotypes will be derived from both the earlier presence on the mainland of reptilian and marsupial host species and, in more recent times, the introduction of organisms resulting from ecological changes brought about by the development of agriculture, domestication of animals and intensifying human settlement, on both the island and adjacent coast.

It is evident that epidemiological studies alone do not explain the extreme diversity of *Salmonella* and *Arizona* serotypes, and high rates of infection in the small colony of quokkas. Furthermore, it is surprising, in view of the large numbers of serotypes involved, that *S. javiana*, which was recovered from only four (4·6 %) animals, was the only strain associated with the human infection. Further seasonal studies of host–parasite relationships in selected mainland and island populations of native and introduced animals are required, to establish more precisely the origins and distribution patterns of individual serotypes, and their role as potential pathogens in the Rottnest quokka population.

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