Salmonella Infections in a Marsupial, the Quokka (Setonix brachyurus), in Relation to Seasonal Changes in Condition and Environmental Stress

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An unusual abundance of Salmonella infections was studied in an island population of a wild marsupial, the quokka (Setonix brachyurus), which experiences starvation in summer associated with significant mortality. The frequency of infections was found to vary seasonally over most parts of the island, with high infection rates (70 to 100%) in summer and low infection rates (0 to 30%) in winter. In some samples, there was an average of as many as two isolations per animal, and up to five isolations were made from a single animal. By the end of summer, virtually all animals excreted Salmonella spp., with a median rate of excretion of approximately 3,000 Salmonella organisms per g of feces. The seasonal changes occurred over intervals of only weeks. The infections are believed to be associated with disruption of the digestive physiology of the animals caused by the poor quality of feed available in summer. This conclusion was supported by a quantitative study of the infections and by a field manipulation experiment which delayed the initiation of the infections as long as a food supplement was available. The proliferation of Salmonella spp. is discussed in terms of the ecology of the quokka and of the use of Salmonella spp. as indicators of environmental stress acting on the animals.

Materials and Methods

Sampling, isolation, and identification of Salmonella spp. Rectal swabs and fecal samples were used. The Salmonella spp. were isolated and identified by the scheme of Iveson (11, 12), with enrichment in strontium selenite A and strontium chloride B broths, plating onto deoxycholate citrate and modified bismuth sulfite agars, and biochemical and serological identification to the serotype level. The use of this system has been studied in detail and reported previously (9). Samples were divided into the two enrichment media, and each enrichment was subcultured three times, once onto deoxycholate citrate agar and twice onto modified bismuth sulfite agar. This procedure was found to give better than 80% recovery of the serotypes from samples containing many Salmonella organisms. Fecal samples were found to be more effective than rectal swabs in detecting isolations in which small numbers of organisms were involved. However, the use of fecal samples was not as convenient in the field because the animals had to be held in cages to obtain a sample, and it was difficult to obtain samples without contamination. For these reasons, duplicate rectal swabs were used in field surveys.

Salmonella counts were made by a conventional dilution and enrichment technique by using strontium chloride B broth with three replicates of 10-fold dilutions. The results were expressed as the most probable number of Salmonella spp. For feces, 1 ml of a 10-g/100 ml dilution was used to begin each replicate, and for samples of stomach contents, 1 ml of undiluted stomach fluid was used.

In one study, animals were used for an intensive investigation of the site and timing of infections within the animals. The animals were killed with an overdose of pentobarbitone sodium and dissected immediately. Samples of stomach contents, feces, heart, lung, spleen, lymph nodes, and gallbladder were obtained by sterile dissection and tested for the presence of Salmonella spp.

Well-being of animals. The well-being of the animals was measured by a condition index (1). The index is measured by deviations from a regression of the cube root of body weight against a convenient section of the tibia-calcaneum (called the short leg). The index is designed as an age- and size-independent measure of condition (as measured by body weight) and expressed on an arbitrary scale with each unit of change equal to about 10% of body weight. Males and females cannot be compared directly because of morphometric differences, and values from males are regularly 0.5 to 1.0 unit higher than those from females in the same sample. Although the condition index is undoubtedly an approximate measure of physiological condition, it has been shown to be a reliable index of weight loss affecting the Rottnest quokka.

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Study sites and animal trapping. The study sites were selected to represent the diversity of situations seen on the island. Over most of the island, the vegetation has been severely altered by burning and overgrazing by the quokkas. The natural woodland and shrubland has been replaced by an open steppe dominated by winter-growing exotic species and native species which are less palatable to the quokka (22). There are freshwater soaks on the island, but the majority of the quokkas have no access to free water. In three areas (know as the Settlement, Golf Course, and Tip Site), the animals have access to a food supply of handouts and garbage from human residents and visitors.

The quokkas were caught with long-handled nets or traps. With trapping, it was possible to recapture the same animals repeatedly, but this was rarely possible with hand catching.

RESULTS

Relationship of Salmonella infection and animal well-being. A survey of the extent of Salmonella infections was combined with a test of the hypothesis that the number of Salmonella organisms was related to the summer starvation by examining the infection rate and well-being in a range of populations.

Numerous serotypes of Salmonella spp. were encountered in this study (see reference 9 for an indication of the serotypes). The highest isolation rate recorded from a sample of animals was 24 isolations from 12 animals, and up to five serotypes were isolated from one animal at a time. The animals were scored as positive if one or more serotypes were isolated, and the infection rate was taken as the percentage of positive animals. Scoring of the infection rate as the mean number of isolations per animal simply exaggerates the trends seen here. Analysis of the distribution of multiple isolations showed that the isolations were randomly distributed among animals (R. P. Hart, Ph.D. dissertation, University of Western Australia, Perth, 1980). Infection is used here to indicate an isolation of Salmonella spp. from an animal, but the term carries no implication of a disease state in the host.

In all areas except the Settlement, Golf Course, and Tip Site, there were large seasonal differences in condition and infection rate. A typical example is given in Fig. 1. In the Settlement, Golf Course, and Tip Site, there were small seasonal differences, but the animals did not show very high rates of infection and were not in very poor condition in summer. These three areas were not studied further.

The peaks of infection rate were remarkably abrupt, with a change from most animals being negative to most animals being positive over an interval of probably only weeks and an equally abrupt reversal at the end of summer. The isolation rate rose to its maximum value very early in the summer and before the animals began to lose weight. It was apparent that while the two events are related in that both or neither occurred in any one area, they are not simply and causally related. The condition index measures the progressive effect of starvation, and a more reasonable conclusion is that the increase in the number of Salmonella organisms is triggered by some aspect of the starvation but not by its effect on the animals.

The hypothesis advanced to account for the abrupt proliferation of Salmonella organisms was that very early in the summer (in fact, as soon as the rains end), changes take place in the vegetation which disturb the digestive physiology of the animals and permit the opportunistic Salmonella organisms to multiply. This hypothesis was then tested.

Tests of the hypothesis. (i) The site, timing, and course of the infections. From the hypothesis advanced to account for the proliferation of infections, it was predicted that Salmonella organisms would appear in the intestine in substantial numbers and virtually simultaneously in all animals at the beginning of summer. This was tested by studying the seasonal course of infections in a field population.

Initially, one group of animals was killed at the critical change of season, and another was killed just after the change: the number of Salmonella organisms was counted in the stomach contents and feces, and various internal organs were tested for the presence of Salmonella spp. The number and distribution of Salmonella spp. are given in Table 1.

There was good agreement among the serotypes present in different parts of the animal, and there was a linear correlation between the numbers of Salmonella organisms in the stomach and feces \( r^2 = 0.31, P < 0.05 \); isolations below the minimum countable value were omitted), although it is difficult to postulate a simple linear correlation. Observation of the carcasses showed that all of the animals had extensive fat deposits and were clearly in good condition by this criterion. This supports the conclusion from the condition index that even in December the animals had not begun to lose weight as the result of starvation.

The quantitative results with the stomach contents were sufficient to illustrate the processes involved, and in subsequent studies, only fecal excretion rates of Salmonella organisms were used. The seasonal pattern of Salmonella excretion rates is shown in Fig. 2.
(ii) Experimentally deferring the initiation of the infections. In a field manipulation experiment, one half of a well-studied population was given a food supplement to see whether the initiation of the infections could be deferred by eliminating the postulated cause of the infections. The population was divided by a fence 1 month before the experiment to give the animals time to readjust their social structure, and one half was given a complete supplement of food and water. The other half served as the control. The food supplement was a commercial mix specifically formulated for quokkas. Captive breeding colonies have been maintained in good health on this diet for many years. The food and water were available to the animals continually after a preseason sampling was made. By using a population which had been trapped for some time, it was often possible to capture the same individuals repeatedly. The animals were only held long enough to obtain a fecal sample.

The fecal excretion rates are shown in Fig. 3. It can be seen that the supplementary feeding almost entirely deferred the initiation of the infections for the duration of the feeding. At the end of the feeding interval, the two groups had significantly different rates of Salmonella excretion ($P < 0.01$, Wilcoxon rank sum test), and the test group gave values not significantly above those recorded before the change of season ($P > 0.1$). Only 3 weeks after the withdrawal of the food supply, the two groups were not statistically different in their rates of Salmonella excretion ($P > 0.1$), and after 3 months, they were indistinguishable. The condition index values of both the test and control groups increased during the feeding interval and had not begun to

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**TABLE 1. Distribution and number of Salmonella organisms isolated from quokkas**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Rectal swab</th>
<th>Appendix</th>
<th>Gallbladder</th>
<th>Stomach</th>
<th>Count</th>
<th>Salmonella spp. isolated from</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. muenchen</td>
<td>S. muenchen</td>
<td>S. muenchen</td>
<td>110</td>
<td>S. muenchen</td>
<td>2,400</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S. adelaida</td>
<td>S. adelaida</td>
<td>S. adelaida</td>
<td>110</td>
<td>S. adelaida</td>
<td>1,100</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>S. muenchen</td>
<td>S. muenchen</td>
<td>S. muenchen</td>
<td>0.9</td>
<td>S. muenchen</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>S. adelaida</td>
<td>S. adelaida</td>
<td>S. adelaida</td>
<td>0.36</td>
<td>S. adelaida</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>S. wandsbek</td>
<td>S. wandsbek</td>
<td>S. wandsbek</td>
<td>110</td>
<td>S. wandsbek</td>
<td>24,000</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>S. newington</td>
<td>S. newington</td>
<td>S. newington</td>
<td>24</td>
<td>S. newington</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>S. singapore</td>
<td>S. singapore</td>
<td>S. singapore</td>
<td>9.4</td>
<td>S. singapore</td>
<td>23</td>
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<tr>
<td>8</td>
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<td>S. newington</td>
<td>1,100</td>
<td>S. newington</td>
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</tr>
<tr>
<td>9</td>
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<td>S. newington</td>
<td>S. oranienburg</td>
<td>2.3</td>
<td>S. oranienburg</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>S. muenchen</td>
<td>S. orientalis</td>
<td>S. orientalis</td>
<td>2.3</td>
<td>S. orientalis</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>S. waycross</td>
<td>S. newington</td>
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<td></td>
</tr>
<tr>
<td>12</td>
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<td>S. newington</td>
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<td>240</td>
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<td>&lt;0.36</td>
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<td>23</td>
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</tr>
<tr>
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<td>3.6</td>
<td></td>
</tr>
<tr>
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<td>S. newington</td>
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<td>S. newington</td>
<td>240,000</td>
<td></td>
</tr>
<tr>
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<td>S. muenchen</td>
<td>S. muenchen</td>
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<td>S. oranienburg</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>S. typhimurium</td>
<td>S. adelaida</td>
<td>S. typhimurium</td>
<td>46</td>
<td>S. orientalis</td>
<td>460</td>
<td></td>
</tr>
</tbody>
</table>

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* All samples of heart, lung, spleen, kidney, and lymph nodes were negative for *Salmonella* spp., and all samples of gallbladder from animals killed 5 December 1977 were negative.
* Animals 1 through 10 were killed 28 October 1977, and animals 11 through 18 were killed 5 December 1977.
* Count is the most probable number.

**FIG. 2.** Seasonal variation in *Salmonella* fecal excretion rates from a population of quokkas. MPN, Most probable number. Symbols: △, median rate of excretion; ○, maximum rate of excretion.
decline by December. The actual food intake of the animals was not measured, but observation of the animals showed that they consumed the food supplement readily and that the food supply was sufficient to ensure that all animals had access to the food.

**DISCUSSION**

On Rottnest Island, the quokka is obviously a major source of *Salmonella* organisms, with a median rate of excretion of 3,000 *Salmonella* organisms per g of feces at the end of summer. These results account for the extensive contamination of the island by *Salmonella* spp. described previously (13).

The quokka has been studied extensively with the aim of describing the summer stresses acting on the animals. Studies have included water availability (3), nitrogen availability (25), trace elements (2), vitamin E deficiency (15), volatile fatty acid production (B. A. Ramsay, M.S. thesis, University of Western Australia, Perth, 1966), heat stress (16), and adrenocortical function (21). These studies have usually been directed at a comparison between the winter and summer patterns. Despite these studies, there is no general agreement as to the precise nature of the summer stresses experienced by the Rottnest quokka or even to the relative importance of these possible contributing factors.

The results reported here describe a dramatic seasonal cycle of *Salmonella* infections. The dominant features of the seasonal cycle are the speed of the changes and the early rise in the number of *Salmonella* organisms at the very beginning of summer. There is a clear separation of *Salmonella* proliferation and weight loss (Fig. 1). The most likely change which could permit such an abrupt increase in the number of *Salmonella* organisms is a breakdown of the normal defenses against an opportunistic invader such as *Salmonella* spp. A typical change would be the effect of a sudden shortage of readily usable carbohydrate, leading to a drop in volatile fatty acid production and a rise in the pH of the stomach contents. The low pH of the stomach contents in ruminants is well known as a defensive mechanism against potential invaders such as *Salmonella typhimurium* (5), and a similar mechanism in the quokka is likely. This effect can be marked. Grau et al. (8) have shown that the infective dose of *Salmonella* organisms can be reduced from $10^7$ to $10^3$ cells to as little as 400 cells in fasting sheep.

Physiological parameters were not measured here because the significance of any one factor is not known, but the results in Table 1 support the hypothesis that some change occurred in the stomach contents and that populations of *Salmonella* spp. were established there. The largest number of *Salmonella* organisms counted in the stomach contents was greater than $10^3$ organisms per ml of fluid, and this suggests that the bacteria were present as an actively multiplying population and not as incidental contamination. This value is an average for the entire stomach contents, and if multiplication of the bacteria occurs, then quite high values may occur in the posterior portions of the large and sacculcar stomach. Similarly, the levels of fecal excretion of *Salmonella* spp. are consistent with actively multiplying populations of bacteria but not necessarily with a disease state. The median value of fecal excretion of *Salmonella* spp. rose to a maximum of approximately 3,000 *Salmonella* organisms per g of feces, but this represents an increasing incidence of values in the range of $10^3$ to $10^6$ and not an increase in the magnitude of the highest values (Fig. 2). The maximum value from each sample is virtually constant during the summer at about $10^5$. The maximum value ever recorded was less than $10^6$ *Salmonella* organisms per g of feces. Symptomless human carriers of *Salmonella* spp. have been recorded to excrete $10^6$ to $10^8$ *Salmonella* organisms per g of feces (23). The median value of excretion was only approximately 100 organisms per g of feces in the December sample (Table 1).

The very abrupt decline in the infections (Fig. 2) at the end of summer also gives support to the hypothesis that the infections are related to food intake rather than to the condition of the animals. The animals are in very poor condition at that time of the year and only regain weight slowly. The change may be caused by something as simple as a sudden increase in suitable feed.

The source of the infections is not known. Even in winter, the infection rate is rarely observed to be zero by using rectal swabs (Fig. 1), and it must be assumed that there would be other low-level infections not detected by rectal swabs. Low-level symptomless *Salmonella* infections are well known in domestic animals, and a similar situation in the quokka is likely. The two isolations from gallbladders shown in Table 1 may represent latent sites of infections in those animals.

In the quokka, new infections resulting from reduced host defenses and proliferation of existing infections probably both occur. The promotion of infection by stress is well known in many animals, including numerous diseases in humans (23), food and water deprivation in mice (26), food deprivation in cattle and sheep (7, 8), disease and environmental stress in cattle (6), and handling stress in pigs (28).

It is not possible to distinguish the possible components of the seasonal stress acting on the quokka. However, the quality of diet appears to be the dominant stress because in the supplementary feeding experiment it was shown that the infections did not proliferate if adequate food and water were available. In the field situation, the possible components of the stress (shelter, water, food quality and quantity, and social stress) are probably interrelated.

There is no evidence from this study that a disease form of infection was ever observed in the field even though virtually all animals were positive throughout the summer. Shield (24) has described how mortality in the Rottnest quokka occurs primarily in late summer. On hematological criteria, Shield found that disease was not a major factor in the summer decline. The levels of *Salmonella* excretion reported here are not necessarily high enough to indicate disease, although they are high enough to suggest that infections are maintained by multiplication of the bacteria within the intestinal tract. The study reported here was not designed to investigate the disease form of infection, and experience with *Salmonella* spp. in other hosts suggests that a disease form of infection is almost certain to occur under some conditions. From the present study, it is not clear whether the *Salmonella* organisms contribute in any way to the summer difficulties of the Rottnest quokka.

The results reported here are consistent with what is known of the nutrition of the quokka on Rottnest. The climate is Mediterranean, with hot dry summers alternating sharply with mild wet winters. The summer is a very stressful season, with a dramatic decline in the nutritive quality of the vegetation (25). At the end of winter, there are dramatic changes in the vegetation which are most obvious in the annual plants. Similarly, at the abrupt end of the summer period there are equally obvious changes in the vegetation. By using the quantitative data of *Salmonella* excretion, there is a strong negative correlation between the number of *Salmonella* organisms excreted by quokkas and...
FIG. 3. *Salmonella* fecal excretion rates from quokkas given a food supplement and from control animals. The feeding interval is indicated by the bar.

the rainfall of the previous 30 days (correlation coefficient, $r^2 = 0.84; P < 0.001$).

Although *Salmonella* isolations from wild animals are widely reported (27), there are few ecological studies concerned with *Salmonella* spp. in wild mammals. Kourany and his colleagues (17-20) have studied *Salmonella* infections in wild animals in Panama. They found a significant reservoir of infections in these animals, including serotypes associated locally with human disease. Among the mammals, isolations were most commonly made from marsupials (opossums) and in the Panamanian dry season. They comment on the fact that the opossums are scavengers with a propensity for human-disturbed areas and that they are most active during the dry season, searching for food. They believed that these animals could serve as sources of infection for humans and domestic animals, and this was particularly so in developing areas where sanitation was poor and contact with wild animals was very close.

How et al. (10) have described an ecological study of *Salmonella* spp. in wild animals in the tropical north region of Australia. Interestingly, they also reported that isolations were more common from marsupials than from eutherians, but unlike Kourany et al., they found the highest incidence in the wet season. The results of How et al. have not been related to the biology of the animals.

*Salmonella* spp. appear to be very useful indicators of the environmental stress acting on the Rottnest quokka in summer. The infections appear at the beginning and disappear at the end of the stressful period; they are not clearly related to the effect of the stress on the animals, but they do appear to be related to the primary cause of the stress. The application of this information to management has been discussed elsewhere (14).

ACKNOWLEDGMENTS

The senior author (R.P.H.) held an Australian Government Commonwealth Postgraduate Research Award and a research grant from the University of Western Australia during the course of this study. We thank the many people who assisted in field work and particularly Joy Carter for help in the laboratory. We also thank the Director of Fisheries and Wildlife for permission to collect quokkas and J. C. McNulty (commissioner of Public Health and Medical Services) and V. Blackman (director of the State Health Laboratories) for their support in this study.

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