Orphaned kangaroo and wallaby joeys as a potential zoonotic source of *Salmonella* spp.

ABSTRACT **Sixteen serotypes of *Salmonella* spp. were isolated from 37 (26.8%) of 138 orphaned kangaroo and wallaby pouch-young which were in the care of guardians in north Queensland. *Sal. lansing, Sal. virchow* and *Sal. wandsworth* were the most prevalent serotypes. Orphaned macropodid joeys are a potential source of zoonotic infection for *Salmonella* spp. and recommendations to reduce the risk of transmission to humans are presented.**
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The quokka, *Setonix brachyurus*, a macropodid marsupial, was implicated as the source of salmonellosis in an infant in Western Australia.[1] Subsequent investigations of the quokkas on Rottnest Island, Western Australia, showed a marked seasonal variation in the frequency of the isolation of *Salmonella* spp. from the wild population.[2] An increase in the prevalence of *Salmonella* spp. occurred rapidly over several weeks and was related to the disruption of the digestive physiology of the macropodids, which was caused by a rapid decline in the quality of the available feed in summer.

In more rural areas of Australia, orphaned kangaroo and wallaby pouch-young, which have been obtained from kangaroo-shooters or collected from wild macropodids that were killed on roads, sometimes are kept as pets. These joeys are subjected to severe stress, particularly of a nutritional nature, since the artificial diets that are fed commonly are deficient in comparison to the complex milk constitution of the mother. The prevalence of salmonellas in these orphaned joeys has not been determined previously.

This paper reports the results for the presence of *Salmonella* spp. from a bacteriological survey of orphaned macropodid young.

Methods

All orphaned macropodid young were presented for examination at the Graduate School of Tropical Veterinary Science, Townsville, north Queensland, from 1981 to 1985.

The kangaroos and wallabies that were examined were pouch-young, that had been orphaned by the death of their mothers and had been kept in captivity for periods ranging from one day to several months before presentation. Results from juvenile and adult macropodids are not included.

Forty-nine of the 138 joeys were dead on arrival or died soon after presentation and a post-mortem examination was carried out. In live joeys, faeces were collected directly from the cloaca into sterile bottles. In dead joeys, the rectum was opened and faeces were collected aseptically. Other regions of the gastrointestinal tract and internal organs were sampled as indicated by the gross pathological findings.

Faecal samples were inoculated onto brilliant-green agar (Oxoid Australia, Sydney, NSW) and into buffered peptone water (Oxoid Australia). Both were incubated at 37 °C for 18–24 h. Tissue samples first were macerated in buffered peptone water in a Coleman Stomacher blender (Seward Medical, London, UK) before being treated as for the faecal samples. One-millilitre aliquots of buffered peptone water then were inoculated into two tetrathionate brilliant-green bile broths, one of which was incubated at 37 °C and the other of which was incubated at 42 °C.

After 24 h, brilliant-green agar plates were streaked from these and were incubated at 37 °C for 24 h. Suspect colonies were picked off for biochemical and serological tests. Biochemical identification was carried out with the Microbact 24E system (Disposable Products, Adelaide, SA). Serological identification was carried out with polyvalent "O" and "H" *Salmonella*-specific antiserum (Wellcome Diagnostics, Dartford, UK). Salmonella cultures were forwarded to the Salmonella Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide, for serotyping.

Results

Samples were obtained from 103 joeys on one occasion — 65 joeys were examined live and 38 joeys after death. Thirty-five joeys were sampled on multiple occasions — 24 joeys were examined live and 11 joeys both live and after death. Details of the number of occasions that samples were taken are listed below.

Sampling occasions	*Number of joeys (number of samples)*
Single	103 (103)
Two	19 (38)
Three	10 (30)
Four	5 (20)
Five	1 (5)
Total	138 (196)

Thirty-seven (26.8%) of 138 orphaned joeys from 11 species of macropodid were found to be infected with *Salmonella* spp. Thirty (21.7%) of these joeys were excreting salmonellas in their faeces. *Salmonella* spp. were isolated on 42 (21%) of the 196 sampling occasions. Sixteen serotypes were isolated (Table). *Sal. lansing, Sal. virchow* and *Sal. wandsworth* were the most prevalent serotypes, although all isolates of *Sal. wandsworth* were from several joeys at the same location. Of the 35 joeys that were sampled more than once, five joeys were excreting *Salmonella* spp. on two occasions, but in three of these, different species of *Salmonella* were isolated at

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TABLE: *Salmonella* species isolated from 138 orphaned macropodid joeys

Salmonella species	Number of isolates ($n=42$)	Proportion of total
Sal. anatum	4	9.5%
Sal. bilthoven	3	7.1%
Sal. chester	1	2.4%
Sal. derby	1	2.4%
Sal. give	1	2.4%
Sal. lansing	7	16.7%
Sal. litchfield	2	4.8%
Sal. meleagridis	1	2.4%
Sal. muenchen	2	4.8%
Sal. newington	2	4.8%
Sal. orientalis	2	4.8%
Sal. rubislaw	1	2.4%
Sal. saintpaul	2	4.8%
Sal. thompson	1	2.4%
Sal. virchow	6	14.3%
Sal. wandsworth	5	11.9%
Not identified	1	2.4%

different times. Two other joeys were infected with two serotypes concurrently.

Salmonellas were isolated from 21/49 (43%) of the joeys that underwent a post-mortem examination and from 16/89 (18%) of the live joeys. These proportions were tested for equality by means of an uncorrected χ^2 test ($\chi^2=9.968$; $P=0.0016$). All isolates from live joeys came from faeces, while 14 (29%) of 49 dead joeys were excreting *Salmonella* in their faeces. In the remaining seven joeys, *Salmonella* spp. were isolated from internal organs only.

Salmonellas were not isolated from nine pouch-young that were examined directly after their removal from the pouches of the wild mothers.

Discussion

Orphaned macropodid pouch-young have a high prevalence of infection with salmonellas. This is probably due to the stress of orphaning and the subsequent inadequacies of artificial care, which allow low-level infections with *Salmonella* spp. to multiply. The promotion of infection by stress is well known in many animals, including humans[3] and macropodids.[2]

The techniques that were used in this survey are routine methods in a diagnostic laboratory. A higher rate of isolation would be expected if a specific search for *Salmonella* spp. had been carried out. Therefore, these results are not maximal infection rates. The detection rate of *Salmonella* in joeys that died was 2.4 times as great as that in live joeys owing to the isolation of *Salmonella* spp. from internal organs, particularly mesenteric lymph nodes. Although joeys that died had a prevalence of faecal excretion of salmonellas that was 1.6 times greater than that of live joeys, the difference was not statistically significant by the uncorrected χ^2 test ($\chi^2=2.085$; $P=0.1488$).

Macropodid pouch-young are unable to care for themselves and once separated from their mothers are dependent totally on human guardians for nutrition, hygiene and physical and psychological comfort.[4] Persons who care for macropodid joeys usually have close physical contact with their charges. Many of them also have children.

A concurrent study of the prevalence of salmonellal infections in the human guardians was not carried out, nor were enquiries made about the health of the guardians. However, a 31-year-old man had a severe, febrile gastroenteritic infection and *Sal. virchow* was isolated from his faeces. After his recovery, attempts to isolate *Salmonella* spp. from the faeces of the two joeys for which he was caring were unsuccessful. Gastroenteritic infection in a 20-year-old male guardian was associated with *Sal. litchfield*, but his joey at the time was infected with *Sal. lansing*. Two months previously, *Sal. newington* had been isolated from the same animal. Two dogs in close contact with joeys that were infected with *Sal. wandsworth* were found to be infected with the same species, while another dog–joey pair was infected with *Sal. lansing*. All the dogs were asymptomatic. These dogs had a tendency to lick the cloacas of the joeys after defaecation and probably acquired the infection by this means.

Sal. typhimurium, the species of *Salmonella* that is isolated most frequently from humans in Australia,[5] was not found in these orphaned joeys. However, 15 of the serotypes that were found in this study have been isolated from human hosts in Australia.[5] *Sal. bilthoven* has not been recorded in humans in Australia. It is an uncommon serotype with only five reports from Australia — four reports from joeys in this study and one report from water in Queensland (Chris Murray, Salmonella Reference Laboratory, Adelaide, personal communication). Reptiles, particularly pet tortoises, are a well-recognized source of human salmonellosis.[6] The prevalence of the faecal excretion of *Salmonella* spp. in tortoises is often 12% to 85%.[6]

Although the significance of orphaned macropodid pouch-young as a source of salmonellal zoonoses awaits investigation, the high prevalence of infection in joeys and the intimate and frequent contact between these animals and their surrogate "mothers" make orphaned macropodid joeys a potential source of salmonellal infection in an uncommon situation. A severely-ill joey may be more liable to be excreting *Salmonella* spp. and could be a greater risk than is a healthy joey.

The prevention of infection with *Salmonella* spp. from orphaned joeys should concentrate on three aspects: the level of faecal excretion; the degree of the contamination of the joey and its environment; and the transmission to handlers. The concentration of *Salmonella* spp. in the faeces of macropodids increases in response to nutritional stress.[2] Although quantitative studies were not performed on the joeys that are reported here, the excretion of increased numbers of *Salmonella* spp. would be predicted for joeys that are malnourished. A healthy joey, if it is excreting *Salmonella* spp., would be expected to have fewer organisms per gram of faeces than if it were malnourished, ill or otherwise stressed. Recommendations for the raising of orphaned joeys should be followed.[4]

Faecal contamination of the skin of the joey and its environment should be kept at a minimum. The daily washing of joeys to reduce skin contamination by bacteria that are derived from faeces, with additional washing if obvious faecal contamination occurs, has been recommended.[4] Artificial pouches must be replaced with clean ones immediately they become soiled and joeys should be stimulated mechanically to defaecate to enable the safe disposal of faeces.[4]

The dose of *Salmonella* organisms that is required to initiate an infection can be remarkably low.[7] Persons in contact with joeys must take precautions to prevent the direct ingestion of salmonellas or their transfer to food. Some joeys have a tendency to lick their minders. All joeys groom themselves by licking and possibly could contaminate their mouths with *Salmonella* spp. Mouth-to-mouth contact, kissing of the skin of the joey and the consumption of food while handling joeys are common practices among persons who care for orphaned joeys. These actions could result in the direct ingestion of *Salmonella* spp. and should be discouraged.

Salmonella spp. have been recovered from fingers three hours after their experimental contamination[8] but, after a soap-and-water hand-wash in convalescent carriers, salmonellas were not recovered from previously-contaminated fingers.[9] After handling orphaned joeys, a thorough hand-wash with soap and water and the washing of any other areas that have been contaminated by faeces should be sufficient to prevent skin carriage in minders. Clothing that has been soiled by faeces should be changed and laundered.

Any faeces that contaminate the environment should be removed and the contaminated area cleaned by washing with water and soap or with other cleaning agents. Dogs should not be allowed to lick the cloacas of joeys or to consume their excrement.

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