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PREVALENCE OF *SALMONELLA* IN FECAL SAMPLES OF WESTERN GREY KANGAROOS (*MACROPUS FULIGINOSUS*)

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ABSTRACT: This is the first extensive study of the prevalence of naturally acquired *Salmonella* infection in wild-caught kangaroos in Australia. Given the close association between kangaroos, livestock, and humans and the growing popularity of kangaroo meat, it is important to identify epidemiologic factors associated with infection in these marsupials in order to minimize the risk of *Salmonella* transmission. The overall prevalence of fecal *Salmonella* in 645 western grey kangaroos (*Macropus fuliginosus*) sampled across 10 locations in Western Australia was 3.6% (95% CI: 2.3–5.3). Seven *Salmonella* serovars were identified including *Salmonella enterica* serovar Muenchen, Kiambu, Rubislaw, Lindern, Champaign, Saintpaul and II 42:g,t:-. Prevalence was significantly associated with rainfall ($P<0.05$) and was highest in the April–June quarter ($P<0.05$). There was no association between age or sex and the prevalence of *Salmonella* in fecal samples. Our results suggest that, while kangaroos are infected with *Salmonella* in their natural habitat, infection is less common than in hand-reared joeys, pet kangaroos, and macropods raised in captivity. Care should be taken to maintain hygiene during the evisceration, processing, and handling of kangaroos and to adequately cook kangaroo meat prior to consumption to reduce the risk of salmonellosis.

Key words: Infectious disease, kangaroo, *Salmonella*, wildlife, zoonosis.

INTRODUCTION

Kangaroos were first suspected of being reservoirs of *Salmonella* in the 1950s and 1960s following reports of contamination of kangaroo meat for export and pet food preparation (Winter, 1957; Anderson et al., 1964; Suzuki et al., 1967). The investigation of a case of salmonellosis in an infant on Rottnest Island, Western Australia showed that quokkas (*Setonix brachyurus*) were commonly infected with *Salmonella* (Iveson and Bradshaw, 1973; Hart et al., 1985). *Salmonella* infection is primarily a problem in captive macropods and orphaned joeys, where it can result in gastroenteritis and septicemia (Speare and Thomas, 1988; Speare et al., 1989; Thomas et al., 2001). Contamination of kangaroo meat also suggests that infection occurs in free-ranging macropods (Bensink et al., 1991; Eglezos et al., 2007; Holds et al., 2008). We investigated the prevalence and epidemiologic factors associated with naturally acquired *Salmonella* infection in wild-caught kangaroos.

MATERIALS AND METHODS

Sample collection

Fecal samples were collected from 645 commercially harvested, wild-caught western grey kangaroos (*Macropus fuliginosus*) between May 2007 and November 2008. Animals were sampled from 10 sites in Western Australia including Badgingarra, Boyup Brook, Bridgetown, Capel, Eneabba, Manjimup, Nannup, Northcliffe, Preston Beach, and Whiteman Park (Table 1). With the abdominal organs externalized but still attached to the carcass, the distal colon was identified and a small number of fecal pellets were massaged caudally into the lower colon to ensure they remained following removal of the intestines. Evisceration was then completed and 10 cm of the caudal portion of gut containing the fecal sample was left in situ. The fecal pellets were then massaged from the intestines into a plastic specimen-storage bag without contact with the collector's hands. For each sample, the location and date of collection and the sex and approximate age of the animal were recorded. Shooters subjectively categorized kangaroos as subadult (<3yr) or adult (≥3yr) based on size and apparent sexual maturity. Fecal samples were stored at 4 C within 24 hr of collection and delivered to the Animal Health Laboratory, Department of Agriculture

TABLE 1. Estimate of *Salmonella* prevalence, number of specimens tested, and serovars isolated from fecal samples of wild-caught western grey kangaroos (*Macropus fuliginosus*) at 10 sample collection locations in Western Australia (2007–2008).

Location	Latitude, Longitude	No. tested	% Prevalence (95% confidence limits)[a]	Serotypes isolated (*n*)
Nannup	33°58′52′S, 115°45′53′E	65	0 (0, 4.8) A	
Manjimup	34°14′24′S, 116°8′42′E	48	0 (0, 6.4) A	
Bridgetown	33°57′27′S, 116°8′13′E	27	0 (0, 10.9) AB	
Northcliffe	34°40′56′S, 116 9′42′E	24	0 (0, 12.1) AB	
Capel	33°33′8′S, 115°33′14′E	202	3.0 (1.2, 6.5) A	Kiambu (6)
Boyup Brook	33°50′4′S, 116 23′18′E	30	3.3 (0, 18.1) AB	Muenchen (1)
Eneabba	29°48′57′S, 115°15′53′E	90	3.3 (0.7, 9.8) AB	Muenchen (1), Lindern (1), Champaign (1)
Whiteman Park	31°50′3′S, 115°57′5 E	30	3.3 (0, 18.1) AB	Muenchen (1)
Preston Beach	32°51′52′S, 115°40′23′E	27	7.4 (1.0, 24.5) AB	Saintpaul (1), II 42:g,t:- (1)
Badgingarra	30°20′17′S, 115°32′22′E	102	9.8 (5.3, 17.5) B	Muenchen (9), Rubislaw (1)

[a] Prevalences that share the same letter were not significantly different between locations ($P<0.05$).

and Food, Western Australia for culture and confirmation of *Salmonella* presence. Positive isolates were sent to Pathwest, Sir Charles Gairdner Hospital, Western Australia for further typing by slide agglutination (Oxoid Australia Ptd. Ltd., 2011). All positive results were reported to the National Enteric Pathogen Surveillance Scheme, Melbourne University.

Environmental data

Prevalence data were aggregated based on the accumulated rainfall that fell prior to sample collection. Daily rainfall data were obtained from the Bureau of Meteorology for weather stations closest to the sampling site. Rainfall in the preceding 30 days (RainCat30) and 60 days (RainCat60) were grouped into four categories: <25 mm, 25–49 mm, 50–99 mm, and ≥100 mm for RainCat30; and <50 mm, 50–99 mm, 100–199 mm, and ≥200 mm for RainCat60. Prevalence data were also aggregated based on the quarter of the year in which they were collected: January–March, April–June, July–September, October–December.

Data analysis

Data were analyzed by the Biometrics Group at the Department of Food and Agriculture, Western Australia. A generalized linear model, which assumed a binomial distribution for *Salmonella* shedding, was fitted to the data to determine whether there was an association with location, sex, age, quarter, or rainfall category (McCullagh and Nelder, 1989) using GenStat for Windows (v.13, VSN International, Hemel Hempstead, UK). Samples with unknown sex ($n=2$) or age ($n=202$) were excluded from this analysis. Whiteman Park was excluded from the rainfall analysis because accurate data could not be obtained. The model output was used to determine whether significant differences in prevalence existed among collection locations, sex, age, and rainfall categories. Nonparametric analysis of variance and chi-square tests were used to confirm these results using the Statistical Package for the Social Sciences (SPSS v.17, SPSS Corporation, Chicago, Illinois, USA). The prevalence at each collection site was compared to all other sites (Table 1). A separate analysis of the data from Capel, using a similar generalized linear model (Table 2), was carried out to determine whether there was an association between *Salmonella* shedding and location, sex, age, quarter, or rainfall category. This analysis was undertaken because Capel was the only location where a large number of samples were collected across all rainfall and quarter categories and across sex and age groups. The analysis of 'All Locations' in Table 2 includes the Capel data.

RESULTS

Analysis of all locations

The overall prevalence of *Salmonella* infection in 645 western grey kangaroos across 10 sample collection sites was 3.6% (95% CI: 2.3–5.3%). Seven serovars were identified (Table 1). *Salmonella enterica* serovar Muenchen (12/23) was the most common serotype isolated from kangaroos

TABLE 2. Association between the prevalence of fecal *Salmonella* in western grey kangaroos (*Macropus fuliginosus*) from Western Australia and quarter and accumulated rainfall category in which samples were collected. RainCat30 and RainCat60 represent accumulated rainfall over the 30 and 60 days preceding sample collection, respectively. Comparisons were made between variables within quarter and RainCat30 and RainCat60 categories separately for both location analyses.

	All locations			Capel only		
Category	No. positive	Prevalence (%)[a]	95% confidence limits	No. positive	Prevalence (%)[a]	95% confidence limits
Quarter						
January–March	1	2.0 A	0, 6.1	0	0 A	0, 7.4
April–June	17	5.9 B	3.5, 9.0	4	7.0 B	3.5, 9.0
July–September	3	1.2 B	0.4, 5.5	0	0 A	0.4, 5.5
October–December	2	1.8 B	0.1, 7.3	2	3.4 AB	0.1, 7.3
RainCat30						
<25 mm	3	1.9 A	0, 3.9	0	0 A	0
25–49 mm	10	11.5 B	3.3, 19.7	2	11.1 A	0, 25.6
50–99 mm	4	3.8 AB	0, 8.5	2	4.4 A	0, 10.5
>100 mm	5	2.1 A	0, 4.3	2	2.1 A	0, 5.0
RainCat60						
<50mm	9	4.7 A	1.0, 8.4	0	0.0 A	0
50–99 mm	7	5.9 A	1.4, 10.4	4	6.3 B	0.2, 12.4
100–199 mm	3	9.6 A	0, 21.4	2	6.7 B	0, 15.7
>200mm	3	1.0 A	0, 2.4	0	0 A	0

[a] Prevalences that share the same letter were significantly different between quarters or RainCat levels ($P<0.05$).

from Badgingarra (9), Eneabba (1), Boyup Brook (1), and Whiteman Park (1) followed by serovar Kiambu (6/23). The prevalence of *Salmonella* infection was significantly higher in kangaroos from Badgingarra compared to Capel ($P=0.01$), Manjimup ($P=0.031$), and Nannup ($P=0.007$; Table 1). There were no significant differences in prevalence among all other collection sites.

Fecal samples collected during May and June were brighter green, laden with intestinal worms, and unformed. This was particularly true for samples from Badgingarra, Preston Beach, and Boyup Brook. Consistency and color returned to normal by July.

There was a significant association between RainCat30 and the prevalence of fecal *Salmonella* across all collection locations ($P=0.014$). The prevalence of *Salmonella* in samples collected in the 25–49 mm RainCat30 category was significantly higher than in samples collected in the <25 mm rainfall category ($P<0.05$; Table 2). The effect of RainCat60 was not significant ($P=0.337$), although an increasing prevalence was observed between the <50 mm, 50–99 mm, and 100–199 mm RainCat60 categories. Shedding was significantly higher in April–June than in either the July–September or October–December quarters ($P<0.05$; Table 2). There was no significant association between age or sex and the prevalence of *Salmonella* when samples from all locations were combined.

Independent analysis at Capel

The prevalence of *Salmonella* infection in western grey kangaroos harvested in Capel was 3.0% (95% CI: 1.2–6.5%). RainCat60 was significantly associated with the prevalence of infection in kangaroos from this collection site ($P=0.023$). The prevalence of infection was significantly higher in the 50–99 mm (6.3%) and 100–199 mm (6.7%) ranges compared to all others, which had no samples containing *Salmonella* (Table 2). The prevalence of infection was significantly higher in

April–June (7.0%) than in either January–March or July–September, when there were no positive samples (Table 2). No significant association was observed between RainCat30 ($P=0.125$), age ($P=0.289$), or sex ($P=0.779$) and the prevalence of *Salmonella* isolation from feces collected from kangaroo at Capel.

DISCUSSION

The prevalence of *Salmonella* infection in free-living kangaroos was lower compared to previous studies on captive macropods and orphaned joeys (Speare and Thomas, 1988; Thomas et al., 2001). Speare and Thomas (1988) found that 26.8% of joeys were infected with *Salmonella* spp. and 21.7% were actively excreting the bacterium in feces. This observation was noted in a range of other macropod diseases including lumpy jaw and coccidiosis (Speare et al., 1989). Stressors associated with environmental and diet changes are likely responsible for elevated levels of infection reported in captive animals.

The prevalence of fecal *Salmonella* in this study is markedly lower than reported levels of infection in quokkas (*S. brachyurus*) on Rottnest Island, which peaks as high as 70–100% in summer (Iveson and Bradshaw, 1973; Hart et al., 1985). This finding can possibly be attributed to the increased exposure of quokkas to people and their waste products within a closed island population. During summer, when food availability is low and digestive physiology is disrupted, quokkas scavenge through food scraps left by tourists, increasing the risk of infection with *Salmonella* (Samuel, 1983; Hart et al., 1985).

In our study, kangaroos harboring *Salmonella* but not shedding the organism in their feces would not have been identified. The low prevalence of isolation of *Salmonella* in this study may, therefore, reflect the difficulties associated with detection of intermittent shedders and carrier animals. Intermittent shedding is described in other organisms residing in the gastrointestinal tract, including *Campylobacter* (Jones et al., 1999). Culturing of tissue samples in addition to feces, in particular mesenteric lymph nodes, can increase the likelihood of detection of *Salmonella* by 2.4 times (Speare and Thomas, 1988).

We isolated seven serovars including *Salmonella* serovars Muenchen, Rubislaw, Kiambu, Lindern, Champaign, Saintpaul, and II 42:g,t:-. Some of these serovars have been associated with salmonellosis in humans in Australia. Serovars Muenchen and Saintpaul were among the top 10 isolates from humans with salmonellosis in Australia between 1987 and 1992 (Murray, 1994), and serovars Saintpaul and Muenchen have been among the top five serovars isolated from humans in Western Australia (OzFoodNet Working Group, 2007). *Salmonella* serovar Kiambu has also been responsible for outbreaks of food-borne salmonellosis in Western Australia (OzFoodNet Working Group, 2006). Although there have been no reports of human salmonellosis associated with consumption of kangaroo meat, the carriage of *Salmonella* in the gastrointestinal tract increases the risk of carcass contamination. Therefore, consumption of poorly cooked kangaroo meat and inadequate hygiene following contact with kangaroos may provide a potential route for infection with *Salmonella*. In 1965 and 1966, 44.9% and 33.2% of kangaroo meat samples exported to Japan from Australia were contaminated with *Salmonella* (Suzuki et al., 1967). The higher proportion of contaminated carcasses at the processor compared to culture-positive fecal samples from wild kangaroos suggests that evisceration procedures and hygiene practices during processing of kangaroos has been inadequate. With more stringent industry regulations now in place, recent studies have reported lower rates of carcass and meat contamination (Eglezos et al., 2007).

In this study, the isolation of *Salmonella* serovars that are commonly detected in

livestock and their meat products suggests that transmission may occur between livestock and kangaroos. *Salmonella* serovars Muenchen, Kiambu, and Saintpaul have been isolated from cattle, sheep, horses, and other species in Australia (NEPSS, 2006, 2008). As cattle and sheep were the most abundant species at the livestock–wildlife interface in all study locations, cross-infection may have occurred with kangaroo populations.

The low estimated prevalence of *Salmonella* in the study population suggests that free-living kangaroos do not pose a greater risk of zoonotic transmission than do livestock. This comparison refers to rates of fecal shedding in the natural environment and not to carcass contamination at the processor, which has a different etiology. Prevalence of infection in livestock is generally higher at the slaughterhouse due to stress, high stocking rates, and carcass cross-contamination (D'Aoust, 1989). Poultry constitutes the most important animal reservoir of *Salmonella* (D'Aoust, 1989). Pigs are also significant reservoirs and are the focus of efforts to reduce herd infection, particularly in the Netherlands (van der Wolf et al., 2001). An on-farm examination of healthy, slaughter-age cattle and sheep in Australia demonstrated that dairy cattle were significantly more likely to shed *Salmonella* in feces than were pasture-fed beef cattle, mutton sheep, and prime lambs (Vanselow et al., 2007). Given that 4–9% of cattle and sheep shed *Salmonella* in their feces (Fegan et al., 2004; Edrington et al., 2009), it is unlikely that free-living kangaroos pose a greater risk of zoonotic transmission of *Salmonella* than do livestock.

The association between quarter, rainfall, and the prevalence of fecal *Salmonella* in kangaroos suggests there are seasonal fluctuations in shedding. Similar observations were made in the Kimberley region of Western Australia, where prevalence of *Salmonella* infection in mammals peaked in the wet season (How et al., 1983). In contrast, Eglezos et al. (2007) reported statistically significant higher rates of kangaroo carcass contamination at the processor in summer. The prevalence of fecal shedding in the quokka (*S. brachyurus*) also peaks during summer (Hart et al., 1985). It is difficult to extrapolate the results of these studies to free-living kangaroos because the studies do not take into account the epidemiologic factors directly influencing the route of infection in mainland kangaroos in their natural habitat.

We found fecal prevalence of *Salmonella* was highest in the April–June quarter following increased rainfall over the 30–60 days preceding sample collection. In the southwest of Western Australia, this represents mid-autumn through early winter, when the first significant rain falls. Vegetation growth lags rainfall by 30–60 days (Roderick, 1994; Chandrasekar et al., 2006); consequently, the abundance of green food is likely to have increased in April–June. In grazing animals, fecal consistency becomes less formed in winter due to a combination of a sudden change in diet and increased exposure to gastrointestinal parasites (Karlsson et al., 2004). Changes to the physical properties of food in pigs can also influence the survival of *Salmonella* in the gastrointestinal tract (Mikkelsen et al., 2004). A similar phenomenon may occur in kangaroos. Although not a classic example of diet-related stress, the sudden abundance of green food following higher rainfall may have temporarily disrupted digestive physiology and altered the intestinal flora such that the gut environment was more favorable for the multiplication of salmonellae. The unformed nature of feces noted in May and June may be evidence of this, contributing to increased fecal shedding of *Salmonella* at that time.

The association between domestic livestock and grazing kangaroos may also account for seasonal trends in fecal *Salmonella* in kangaroos. Jones et al. (1999) noted that shedding of *Campylobacter* in sheep increased during lambing,

weaning, and movement onto new pasture (Jones et al., 1999). Similarly, shedding of *Escherichia coli* in dairy cows increased in lactating and multiparous animals (Fitzgerald et al., 2003). Although farming practices vary from region to region, calving and lambing seasons are often timed to coincide with periods of increased food availability. It is possible that latent *Salmonella* infections in domestic livestock became active at this time and resulted in increased fecal shedding. Loose fecal consistency and the presence of surface ground water may have led to more widespread environmental contamination. Survival of pathogenic bacteria in the environment was likely prolonged due to the increased abundance of water from rainfall (Sinton et al., 2007). As the western grey kangaroo grazes between 5.9 and 9.8 hr/day (Priddel, 1986), the chance of ingesting *Salmonella* from heavily contaminated pasture is high. Hence, the significant association of prevalence with quarter and accumulated rainfall may be explained by the increased numbers of kangaroos cograzing with livestock at a time when environmental contamination with *Salmonella* was at a maximum.

The significantly higher prevalence of *Salmonella* in kangaroos at Badgingarra was surprising given that annual rainfall is lower than at Nannup, Manjimup, and Capel (Bureau of Meteorology, 2009). This finding may be attributed to the geographic variation between collection locations and to differences in climate, food composition, and the ecology of *Salmonella* spp. that are associated with this variation. With the exception of Eneabba, Badgingarra is the only collection site in the Wheatbelt region of Western Australia, north of Perth. The remaining sites are in, or south of, Perth. The sudden abundance of green food at Badgingarra in the April–June quarter following rainfall may have had a more profound effect on the digestive physiology of local kangaroos, as pastures are generally drier throughout the year. The change in fecal color and composition noted during May and June at this location may be evidence of this. In the southwest of the state, rainfall extends over a longer period and temperatures remain lower (Bureau of Meteorology, 2009). The effect of a sudden change in food composition may be diluted at Nannup, Manjimup, and Capel because kangaroos are more accustomed to lush pasture. Although not statistically significant, the highest prevalences were reported in the four most-northern collection sites (Table 2).

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