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Antibodies to the Ross River Virus in Captive Marsupials in Urban Areas of Eastern New South Wales, Australia

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ABSTRACT: Serum samples collected from 224 tammar wallabies (*Macropus eugenii*) in two captive populations in urban areas in eastern New South Wales Australia, between December 1999 and May 2004, were tested for antibodies to Ross River virus (RRV). In one population in northwest Sydney, 21 animals (11%) tested positive, and in another population in Newcastle, New South Wales, thirteen (33%) of the animals were positive. Antibodies were detected in four of 11 wallaroos (*Macropus robustus*) (36%) but not in parma wallabies (*Macropus parma*) ($n=5$), koalas (*Phascolarctos cinereus*) ($n=12$) and southern hairy-nosed wombats (*Lasiorhinus latifrons*) ($n=2$) from the Sydney area. These data support the possible role of marsupials as urban amplifying hosts for RRV.

Key words: Antibody, macropods, *Macropus eugenii,* marsupial, Ross River virus, serosurvey, tammar wallaby.

Ross River virus (RRV) is the most common and widespread arbovirus in Australia (Russell, 2002) and, in recent years, RRV infection has been increasingly reported close to metropolitan centers (Russell, 2002). In humans, RRV infection is characterized by arthritis, rash, fever, and fatigue and can vary in duration from a few weeks to several years. In Australia, RRV has been estimated to cost the community between $2.8 and $5.7 million annually based on an average of 4,745 cases per year (Harley et al., 2001). The first reported cases close to metropolitan Sydney occurred on the northwestern outskirts of the city in early 1997 (Amin et al., 1998). This was followed in 2000 by additional cases in the Werrington area, which is located approximately 30 km west of Sydney (Brokenshire et al., 2000). Both areas are a mix of urban and semirural environs inhabited by small remnant populations of native fauna along with a diversity of introduced species.

Domestic cats and dogs have been implicated as possible reservoirs of RRV, and antibodies have been detected using hemagglutinin inhibition and neutralization protocols (McManus and Marshall, 1986; Kay and Aaskov, 1989; Boyd and Kay, 2002). Similarly, other small mammals, such as New Holland mice (*Pseudomys novaehollandiae*; Gard et al., 1973), bush rats (*Rattus fuscipes*), swamp rats (*Rattus lutreolus*), and house mice (*Mus domesticus*) have tested positive for antibodies to RRV (Vale et al., 1991). Native mammals, including marsupials, have also been suggested as possible reservoirs, and antibodies to the virus have been detected in grey-headed flying fox (*Pteropus poliocephalus*; (Ryan et al., 1997), grey kangaroo (*Macropus giganteus*; Doherty et al., 1971), brushtail possum (*Trichosurus vulpecula*; Azuolas, 1997), Southern brown bandicoot (*Isoodon obesulus*), Eastern barred bandicoot (*Perameles gunnii*), long-nosed bandicoot (*Perameles nausata*), Northern brown bandicoot (*Isoodon macrourus*), brown antechinus (*Antechinus stuartii*), dusky antechinus (*Antechinus swainsonii*), and the white-footed dunnart (*Sminthopsis leucopus*) (Whitehead, 1969; Vale et al., 1991; Brokenshire et al., 2000). Experimental infection with RRV has been reported in the brushtail possum (Boyd et al., 2001) and, in this study, 30% of exposed possums developed high viremias that were capable of infecting 53% of vector mosquitoes. The isolation of RRV from serum of naturally infected agile wallabies (*Wallabia agilis*) also has been reported (Doherty et al., 1971).

The tammar wallaby, *Macropus eugenii,* is a small macropod marsupial that has been used as a model for marsupial research. It is easily maintained in captivity and, for this reason, is often found in zoo-

TABLE 1. Prevalence of Ross River virus antibodies in captive marsupials in Sydney and Newcastle enivirons.

Species	Negative	Low positive	High positive	Total
Wombats	2	0	0	2
Koalas	12	0	0	12
Parma wallabies	5	0	0	5
Wallaroos	7	1	3	11
Tammar wallabies				
Newcastle conlony	26	0	13	39
Macquarie colony	164	7	14	185
Total	190	7	27	224
All species total	216	8	30	254

logical collections for both research and display. To date, no serologic survey for RRV has been conducted on this species. In this study, serum samples were collected from May 2001 to May 2004 from 224 tammar wallabies in two captive populations in urban areas. Of these, 185 tammars were housed in a fauna park at Macquarie University, North Ryde, New South Wales (33°45′S, 151°07′E) and 39 animals were located in a colony at the University of Newcastle, Callaghan, New South Wales (32°53′S, 151°42′E). Fifty-five of these animals were tested more than once over the study period.

Serum samples also were collected from five female parma wallabies (*Macropus parma*) and nine wallaroos (*Macropus robustus*) from the Macquarie University site, and two wallaroos, 12 koalas (*Phascolarctos cinereus*) and two Southern hairy-nosed wombats (*Lasiorhinus latifrons*) from Taronga Zoological Gardens, Mosman, New South Wales (33°13′S 150°50′E). These samples were collected from December 1999 to March 2004. All samples were obtained using appropriate institutional animal ethics approvals.

Serum samples were tested for antibodies to RRV using a RRV total antibody DEB-ELISA kit (Biocene, Rozelle, Australia). This assay is an epitope-blocking enzyme immunoassay and is species independent. Positive samples were classified as those that had a percentage inhibition of greater than 40%, as per the manufacturers guidelines. Within this group, high positive samples had an inhibition greater than 60% while low positives were between 40% and 60%.

Of 254 marsupials tested, 216 were negative and 38 were positive (Table 1).

In the two tammar wallaby populations, 21 (11%) and 13 (33%) tested positive from the Macquarie University and Newcastle University sites, respectively (Table 1). Of the 21 positives from Macquarie colony, four were males (8% of total males) and 17 were females (13% of total females). This difference is not significant ($\chi^2 = 0.63$, df = 1, $P = 0.427$) and may reflect the greater numbers of females retained in the colony for breeding purposes. Tested tammar wallabies ranged in age from 18 mo to 9 yr, and all but one of the positive samples came from animals 4 yr or older. The exception was a 2-yr-old animal that had recently arrived from Wirrimbirra. Seroconversion was not observed in any of the 55 tammar wallabies tested more than once, suggesting that infection did not occur during the study period. No longitudinal samples were available from the Newcastle animal collection.

All koalas, wombats, and parma wallabies tested negative. Of the 11 wallaroos, four were positive (36%) and all were from the Macquarie University facility.

Of the animals housed at the Macquarie University Fauna Park, 82 of the tammar wallabies were bred at the Macquarie facility, 26 originated from Kangaroo Island, South Australia (35°45′S, 137°37′E), six were from Canberra, ACT (35°18′S,

149°08′E), three were from Wirrimbirra Sanctuary, Bargo, New South Wales (37°17′S, 150°35′E), and one was originally from the Newcastle colony. The origins of the remainder are unknown. Of these animals, five of 82 bred in the Macquarie Fauna Park (6%), seven of 26 from Kangaroo island (27%), one of six from Canberra (17%), one of three from Wirrimbirra sanctuary (33%), and the only one from Newcastle (100%) were positive for antibodies to RRV. All wallaroos were bred at Macquarie University.

Based on previously reported antibodies to RRV, macropods may be a reservoir for the virus (Vale et al., 1991). In our study, both macropod populations contained animals that were positive for antibodies to RRV. Although only 11% of the animals housed in the Northern suburbs of Sydney had antibodies to RRV, the detection of these antibodies in five tammar wallabies and four wallaroos born at the Macquarie University Fauna Park suggests that RRV infections occurred at this site.

Although both animal collections used in this study are in close proximity to possible mosquito-breeding sites, the study period coincided with a time of relatively low rainfall. This factor may have contributed to the higher prevalence of antibodies in older animals (>4 yr) and the failure to detect seroconversions. In addition, antibody prevalence estimates for the Macquarie population may have been biased by the numbers of animals that originated from other areas. The detection of antibodies in these relocated animals suggests a possibility of virus introduction through the movement of viremic animals and this should be considered in such translocations.

This study indicates that captive marsupials, in particular macropods, can be exposed to RRV in urban areas and may represent potential amplifying hosts for this virus. It also adds the tammar wallaby to the list of marsupials with demonstrated antibodies to RRV.

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