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The Seroprevalence and Factors Associated with Ross River Virus Infection in Western Grey Kangaroos (*Macropus fuliginosus*) in Western Australia

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Abstract

A serosurvey was undertaken in 15 locations in the midwest to southwest of Western Australia (WA) to investigate the seroprevalence of Ross River virus (RRV) neutralizing antibodies and factors associated with infection in western grey kangaroos (*Macropus fuliginosus*). The estimated seroprevalence in 2632 kangaroo samples, using a serum neutralization test, was 43.9% (95% CI 42.0, 45.8). Location was significantly associated with seroprevalence ($p < 0.001$). There was a strong positive correlation between seroprevalence and the average log-transformed neutralizing antibody titer ($r = 0.98$, $p < 0.001$). The seroprevalence among adult kangaroos was significantly higher than in subadult kangaroos ($p < 0.05$). No significant association was observed between seroprevalence and the sex of kangaroos ($p > 0.05$). The results of this study indicate that kangaroos in WA are regularly infected with RRV and may be involved in the maintenance and transmission of RRV.

Key Words: Arbovirus(es)—Epidemiology—Mosquito(es)—Reservoir host—Vector borne.

Introduction

Ross River virus (RRV) (Togaviridae: Alphavirus) is the most common mosquito-borne pathogen in Western Australia (WA), causing fever, rash, arthralgia, and myalgia in clinically affected people (Lindsay et al. 1997, Russell 2002). The virus is predominantly transmitted between mosquito vectors and non-human animal hosts, yet there is a paucity of data on the identity of the vertebrate host species and the transmission dynamics of RRV amongst host species. Accumulating serological and laboratory evidence suggests that large macropods are likely to play a significant role in the transmission and maintenance of RRV (Kay et al. 1986, Kay and Aaskov 1989, Harley et al. 2001).

The putative reservoir host of RRV in the southwest of WA is the western grey kangaroo (WGK; *Macropus fuliginosus*). Unpublished serological evidence suggests that infection is common in this species (M. Lindsay 1995, unpublished data). Limited experimental infection studies have been undertaken in the eastern grey kangaroo (EGK; *Macropus giganteus*), agile wallaby (*Macropus agilis*), and tammar wallaby (*Macropus eugenii*). Inoculation of these species with the virus induced a viremia persisting for 6.0, 3.4, and 2–3 days, respectively (Marshall and Miles 1984, Kay et al. 1986, Kay and Aaskov 1989). Because the WGK is closely related to the EGK, it is likely that a similar response could be expected following infection with RRV. Ross River Virus has been recovered from two agile wallabies, one of the few vertebrate species from which the virus has been isolated (Doherty et al. 1971). This combined evidence has led to the belief that the WGK is a likely vertebrate host of RRV in WA.

This study was designed to further investigate the relationship between seroprevalence and age, sex, and geographic location of WGKs in the midwest to southwest of the state. It is hoped that a serosurvey of this nature will provide further evidence that mosquito vectors commonly feed on macropods, inducing a detectable antibody response in the animal. This information may provide additional data to support the notion that the WGK plays a significant role in the transmission of RRV in WA.

Materials and Methods

Sample collection

Whole blood samples were collected postmortem from commercially harvested, wild-caught WGKs, between May,

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2006, and May, 2009. Animals were sampled from 15 locations across the midwest to southwest of WA (Fig. 1). Serum was separated within 24 h of blood collection and stored at −20°C until tested. For each sample, the location and date of collection were recorded as well as the sex and approximate age cohort of the animal. Kangaroo shooters categorized kangaroos as pouch young (still in pouch), subadult (<3 years of age), or adult (≥3 years of age) on the basis of size and apparent sexual maturity.

Virus strains

RRV strain DC5692, representing a southwest genotype of the virus (collected from the Peel region, 75 km south of Perth, in 1999), was provided by the Arbovirus Surveillance and Research Laboratory (ASRL), The University of Western Australia (UWA). A virus stock was grown in 225-cm^2 cell culture flasks (Becton Dickinson, NJ) in M199 medium (Sigma-Aldrich Co., Saint Louis, MO), containing 2% fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA), 2 mM L-glutamine (Sigma-Aldrich Co., USA), 4.77 grams/L HEPES (Calbiochem, La Jolla, CA), 100 μg/mL benzyl penicillin (Commonwealth Serum Laboratories Ltd., Parkville, Australia), and 10 μg/mL gentamicin (Deltawest, Perth, Australia). Virus was harvested by centrifugation at 3000 rpm for 10 min at 4°C when 70–80% of the Vero cell monolayer inoculated with RRV had developed cytopathic effect (CPE). The final concentration of FBS was increased to 10% before 500 μL aliquots of supernatant were added to sterile vials and stored at −70°C (Johansen et al. 2005b).

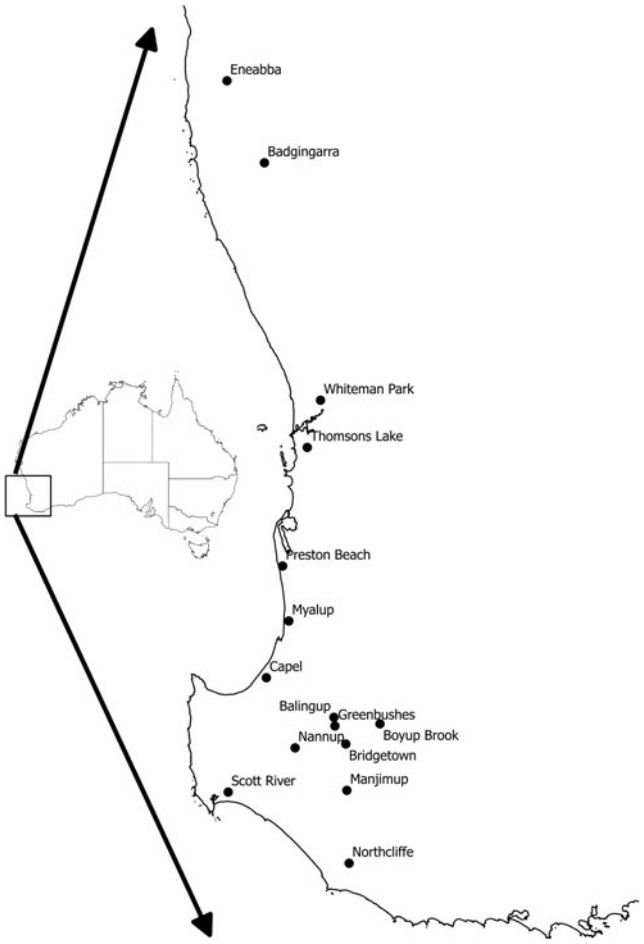

FIG. 1. Map displaying the 15 sample collection locations in Western Australia.

Neutralization test

The protocol for the neutralization test (NT) was adapted from Johansen et al. (2005b). Briefly, duplicate two-fold serum dilutions were mixed with 50–100 median tissue culture infective dose ($TCID_{50s}$) of virus in a 96-well Falcon tissue culture plate (Becton Dickinson, USA) and incubated at 37°C and 5% CO_2 for 1 h. Next, 100 μL of Vero cells (approximately 1.5×10^6 cells) were added to all wells. Plates were then incubated for 5 days and examined by microscopy for CPE. Neutralization titers were expressed as the reciprocal of the highest serum dilution where CPE did not occur. Neutralization titers ≥40 were considered positive. This cutoff was selected to minimize cross-reactivity with other closely related alphaviruses, such as Barmah Forest virus (Johansen et al. 2005b). A maximum neutralization titer of 640 signified a strongly positive sample. The assay was repeated if the infectious titer of virus used was less than or greater than 50–100 $TCID_{50s}$ per 25 μL. Samples were retested if the well containing the duplicate sample produced different results. Virus-control assays were performed in conjunction with each NT to ensure the virus titer used was accurate. Serum and cell controls were also used each time the assay was performed.

Data analysis

Data were analyzed by the Biometrics Group at the Department of Food and Agriculture, WA. A generalized linear model that assumed a binomial distribution (McCullagh and Nelder 1989) was used to determine whether RRV NT results (positive/negative) were associated with location, sex, or age using GenStat for Windows (v. 13; VSN International, Hemel Hempstead, UK). A similar, linear model with normally distributed residuals was fitted to the neutralization titer data in a comparable analysis. Neutralization titers were log transformed and represented in the following manner: Negative titer = 0; 40 = 1; 80 = 2; 160 = 3; 320 = 4; ≥640 = 5. Data from samples collected from pouch young were excluded from this analysis. The chi-squared test was used to determine whether differences between seroprevalences were significant (except where the Fisher exact text was indicated) using the Statistical Package for the Social Sciences (SPSS v. 17; SPSS Corporation, Chicago, IL). The *t*-test and one-way analysis of variance (ANOVA) were used to determine whether mean log-transformed neutralizing antibody titer values were significantly different from one another. The Pearson correlation coefficient was used to correlate seroprevalence and the average log-transformed neutralizing antibody titer.

Results

Sample collection

A total of 2632 WGK serum samples were collected across 15 different locations in this study. The sex of each kangaroo was determined and recorded for 2597 samples. The number

of males and females was well distributed across all sample locations except at Balingup and Manjimup, where significantly more males were sampled compared to females ($p < 0.05$). Age cohort was not consistently recorded for 267 animals sampled at Eneabba, Badgingarra, and Preston Beach. A further 19 samples from Thomsons Lake did not have age cohorts recorded due to the fast-paced nature of the cull. Of the 2346 samples with age cohorts recorded, 260 kangaroos were classified as subadults and a further 102 as pouch young.

Association between seroprevalence and location

The overall seroprevalence of RRV neutralizing antibodies from the 2632 samples was 43.9% (95% confidence interval [CI] 42.0, 45.8). Location was significantly associated with seroprevalence ($p < 0.001$). The seroprevalence was significantly higher in kangaroos harvested at Thomsons Lake compared to all other sites ($p < 0.05$) (Table 1). The seroprevalence in kangaroos harvested at Capel was significantly higher than the remaining 13 collection locations ($p < 0.05$). Seroprevalence was significantly lower at Badgingarra, Eneabba, and Northcliffe than all other collection locations ($p < 0.05$), except Nannup.

Association between seroprevalence, age cohort, and sex

The seroprevalence was significantly higher in adults than in subadults at 12 of the 15 locations in which samples were collected ($p < 0.05$). The remaining three locations were Northcliffe, Bridgetown, and Boyup Brook. At Northcliffe and Bridgetown, subadult samples were not collected, and therefore it was not possible to undertake an analysis of these two locations. At Boyup Brook, seroprevalence was higher in adult populations, but this was not found to be significant. No association was observed between seroprevalence and the sex of kangaroos.

Table 1. Estimated Seroprevalence (%) of Ross River Virus Neutralizing Antibodies and Average Log-Transformed Neutralizing Antibody Titer in Western Grey Kangaroos (Macropus fuliginosus) from Various Locations in Western Australia

Location	*Number Sampled*	*Seroprevalence (95% CI)*	*Antibody titer (95% CI)*
Eneabba	187	8.0 (5.7, 14,2)[a]	0.2 (0.1, 0.3)[a]
Badgingarra	348	9.1 (5.6, 11.4)[a]	0.2 (0.1, 0.3)[a]
Northcliffe	121	10.7 (6.3, 17.6)[a]	0.2 (0.1, 0.3)[a]
Nannup	59	11.9 (5.6, 22.8)[ab]	0.3 (0.1, 0.5)[ab]
Whiteman Park	29	24.1 (12.0, 42.4)[b]	0.8 (0.3, 1.4)[bcd]
Preston Beach	143	26.6 (20.0, 34.4)[b]	0.8 (0.5, 1.0)[bc]
Myalup	155	27.1 (20.7, 34.6)[b]	0.6 (0.4, 0.7)[b]
Boyup Brook	166	30.7 (24.2, 38.1)[b]	0.8 (0.6, 1.0)[bc]
Bridgetown	53	32.1 (21.0, 45.5)[b]	0.8 (0.4, 1.1)[bc]
Manjimup	215	32.2 (26.2, 38.6)[b]	0.9 (0.7, 1.1)[c]
Greenbushes	23	34.9 (18.7, 55.2)[b]	1.0 (0.3, 1.6)[bcd]
Balingup	16	37.5 (18.4, 61.5)[b]	0.8 (0.2, 1.3)[bc]
Scott River	116	42.2 (33.6, 51.3)[b]	1.4 (1.0, 1.7)[d]
Capel	677	74.7 (71.3, 77.9)[c]	2.5 (2.4, 2.7)[e]
Thomsons Lake	324	92.0 (88.5, 94.5)[d]	4.2 (4.0, 4.3)[f]

Different superscript letters represent a significant difference in seroprevalence/average log-transformed neutralizing antibody titer between locations ($p < 0.05$).
CI, confidence interval.

Correlation between seroprevalence and neutralizing antibody titer

There was a statistically significant, positive correlation between seroprevalence and the average log-transformed RRV neutralizing antibody titer of all samples ($r = 0.98$, $p < 0.001$). Western grey kangaroos from Thomsons Lake recorded the highest average log-transformed neutralizing antibody titer at 4.2 ($p < 0.001$) (Table 1). Kangaroos from Capel recorded the second highest average log-transformed neutralizing antibody titer at 2.5, compared to the remaining 13 sample collection sites ($p < 0.001$). The average log-transformed antibody titer at Northcliffe, Badgingarra, Eneabba, and Nannup was indicative of a negative result (when rounded to the nearest whole number), whereas kangaroos from all other collection locations recorded an average log transformed neutralization titer of 1. A similarly strong correlation was noted between seroprevalence and the neutralizing antibody titer of the positive samples only ($r = 0.83$, $p < 0.001$).

Maternal immunity

There was a statistically significant, moderate correlation between the serological status of 62 paired joey and doe (mother) serum samples ($r = 0.44$, $p < 0.001$). Of the 53 mothers testing seropositive, 78.5% of their joeys were also seropositive. Of the nine does testing seronegative, 77.8% of their pouch young were seronegative. One doe testing seronegative on the NT had a pouch young that was seropositive. When comparing the average log-transformed neutralizing antibody titers of the does and their pouch young, there was also a statistically significant, moderate correlation ($r = 0.43$, $p < 0.001$).

Discussion

Key steps to defining the vertebrate host(s) of RRV include determining if the animal is susceptible to infection and capable of developing a viremia of sufficient titer and duration to infect competent vectors (infection studies), determining if the animal is fed on by competent local vector mosquito species (vector blood meal analysis), and demonstrating that the animal is naturally infected in the wild (serosurveys). This study provides overwhelming serological evidence that WGKs are commonly infected with RRV in WA. The overall seroprevalence (43.9%) across the sample population supports unpublished data by M. Lindsay (1995), whose results indicated that 35% of all WGKs from a number of geographic regions in WA were seropositive for RRV neutralizing antibodies. The high seroprevalence within WGK populations indicates that mosquito vectors commonly feed on macropods and are able to induce a detectable antibody response. Blood meal analysis results from mosquitoes in WA have demonstrated that important RRV vectors, *Aedes camptorhynchus, Aedes vigilax*, and *Culex annulirostris*, do commonly feed on marsupials (Johansen et al. 2009).

Despite this evidence, the role of the WGK in the transmission of RRV remains unconfirmed because of the paucity of data on the magnitude and duration of viremia following RRV infection. Studies of this nature were undertaken in the 1960s but were limited to a small number of EGKs (*M. giganteus*), agile wallabies (*M. agilis*), and tammar wallabies (*M. eugenii*). Experimental infection induced a viremia that persisted for 6.0, 3.4, and 2–3 days in these species, respectively (Marshall and Miles 1984, Kay et al. 1986, Kay and Aaskov 1989). Given the close relationship with the EGK, it is expected that the WGK would experience a similar immune response following infection with RRV. The authors explored the possibility of undertaking experimental infection studies in WGKs, but they were met with a number of ethical considerations associated with regular blood sampling of kangaroos, including the risk of inducing capture myopathy (Chalmers and Barrett 1982). Given the serious public health implications of RRV, the logistical challenges associated with housing large-bodied macropods in a mosquito-proof enclosure were also significant.

RRV has only been isolated from non-human vertebrate hosts on a small number of occasions due to the short viremic period and lack of clinical signs in infected species. The virus was first isolated from the heart muscle of three birds at Mitchell River Mission in 1965 (Whitehead et al. 1968). Since that time, there has been no further evidence to suggest that birds play a significant role in transmission (Marshall and Miles 1984). The virus was later isolated from two agile wallabies in this same area, during which time 88% of the 147 wallabies tested for antibodies were also seropositive (Doherty et al. 1971). The virus was then isolated from the blood of an apparently healthy mare (Pascoe et al. 1978).

The significant difference in seroprevalence between collection locations in this study likely reflects the geographic variation of RRV activity in WA. Viral transmission is favored in regions where environmental conditions support breeding and survival of mosquito vector populations and reservoir host numbers are large. In this study, the two highest seroprevalence estimates were reported in kangaroos from Thomsons Lake and Capel. Capel is considered a RRV foci within the southwest of WA, on the basis of the number of human cases reportedly acquired from the region during major epidemics (Lindsay et al. 1997, Johansen et al. 2005a). Samples were collected shortly after an above-average period of viral activity over the 2005–2006 RRV season (Mosquito-Borne Disease Control [MBDC], WA Department of Health, 2014, unpublished data). The Local Government district of Cockburn, in which Thomsons Lake is situated, also reports high numbers of cases of RRV disease on a regular basis (MBDC, WA Department of Health, 2014, unpublished data).

The lowest seroprevalence estimates were noted in WGKs from Badgingarra and Eneabba. No human cases of RRV disease were reported at either location in the 5 years preceding sample collection (MBDC, WA Department of Health, 2014, unpublished data), suggesting that viral transmission in the area was either absent or occurring at a level that went undetected. The observed differences in the average seroprevalence amongst kangaroos in different geographical locations indicate that exposure to RRV varies from region to region and may provide an indicator of the level of background risk of RRV to people for any given location.

The significant difference in seroprevalence between regional locations may be partially attributed to variation in environmental conditions and mosquito abundance. Regular mosquito trapping in Capel indicates that mosquito populations are greatest between August and December (ASRL, UWA, 2014, unpublished data). It is not unusual for a trap to yield more than 1000 mosquitoes in a 24-h period during a month of peak activity (ASRL, UWA, 2014, unpublished data). *Aedes camptorhynchus* is the most widespread and abundant mosquito species in the southwest of WA, breeding in a variety of temporary and seasonal water bodies following rainfall, as well as brackish wetlands and tidal saltmarshes. Virus isolation, studies of mosquito fauna during outbreaks of RRV, and blood meal analyses indicate that *Ae. camptorhynchus* is likely to be a major vector of RRV in the Capel region (Lindsay et al. 1989, Lindsay et al. 1992, Johansen et al. 2009). The high seroprevalence in kangaroos in Capel reported in this study was likely due to the combined presence of virus among a large number of competent mosquito vectors that readily feed on marsupials.

Badgingarra and Eneabba are located in the midwestern region of WA, where few cases of locally acquired RRV have even been reported (MBDC, WA Dept of Health, 2014, unpublished data). Consequently, public health–driven mosquito surveillance is not undertaken in the area, making it difficult to accurately compare mosquito abundance. Limited historical trapping data from nearby towns indicate that mosquito populations are much smaller than at Capel (ASRL, UWA, 2014, unpublished data). Small numbers of a wide range of mosquito species from which RRV has been isolated were noted in traps, including *Cx annulirostris, Cx. quinquefasciatus*, and *Ae. bancroftianus* (ASRL, UWA, 2014, unpublished data, Lindsay et al. 1989). In particular, *Cx. annulirostris* is a well-established vector of the virus that feeds on marsupials and has been implicated in significant outbreaks of RRV in WA (Lindsay et al. 1989, Johansen et al. 2009). Despite the likelihood that competent vectors are present in the midwest, kangaroos remained largely seronegative for RRV antibodies in this study. Although it is difficult to speculate why this occurred without accurate mosquito data, it is likely that local environmental conditions do not generally support significant mosquito breeding. Given the largely naïve kangaroo population present in the region, a RRV epidemic may be supported in the event that unusually high rainfall or significant flooding occurred and there was substantial mosquito breeding. This pattern has been recently noted in Kalgoorlie following above-average rainfall and the substantial population increases of mosquitoes that follow (MBDC, WA Department of Health, 2014; unpublished data).

The strong positive correlation between seroprevalence and neutralizing antibody titers was a significant finding. Experimental infection studies in the EGK indicate that RRV antibody titers peak in macropods within 2–4 weeks of infection (Kay and Aaskov 1989). Therefore, high titers are suggestive of more recent infection whereas low titers suggest that some time has passed since RRV infection occurred. This pattern of antibody titers was evident in the results of this study. The highest average antibody titers of all collection locations were recorded at Capel and Thomsons Lake. Similarly, above-average numbers of human RRV cases were reported during the arboviral season immediately prior to sample collection at both locations. The low antibody titers in

the small number of kangaroos that were seropositive from Badgingarra and Eneabba suggest that infection at these two locations occurred some time ago. The absence of clinical cases at either location in the 5 years preceding sample collection further supports this interpretation. This association may offer a means of estimating how recently RRV was active within a region.

The differences in seroprevalence between adult and subadult kangaroos can only be explored at Thomsons Lake and Capel, where a larger proportion of subadult kangaroos were sampled. The observation that seroprevalence in adult kangaroos was significantly higher than in subadults is consistent with other infectious agents associated with kangaroos, such as macropod herpes virus, and is likely to be due to repeated exposure to the organism and the possibility that antibody levels remain high for a length of time following infection (Kerr et al. 1981).

Age-based selection bias was unavoidable at the majority of collection locations, with the exception of Thomsons Lake. Kangaroos were sourced through the commercial harvesting industry where shooters tend to take older animals because they are paid on a per kilogram basis. Given that older animals are more likely to be seropositive, it is possible that the overall seroprevalence reported in this study is an overestimation of the true seroprevalence among wild kangaroo populations. Despite this potential limitation, kangaroos from Thomsons Lake reported the highest seroprevalence of all collection locations. This is interesting given that the animals were taken as part of an organized cull by the (former) Department of Environment and Conservation (DEC) to reduce overpopulation of an enclosed nature reserve. Selection bias was unlikely because animals were taken regardless of size or maturity.

Sex was not significantly associated with seroprevalence, suggesting that both males and females are equally susceptible to being infected with RRV. This is consistent with other infectious agents in macropods, including macropod herpes virus (Kerr et al. 1981).

Evidence of maternal transfer of RRV neutralizing antibodies between does and their pouch young supports the work of M. Lindsay (1995, unpublished data), who detected two seropositive young that were 3 and 6 weeks old, respectively. A number of the seropositive young that were sampled in this study were naked, pink, and had not yet opened their eyes, suggesting that they were less than 120 days old (Dawson 2002). At this age, the pouch young were unlikely to have had exposure to mosquitoes because their heads do not emerge from the pouch until at least day 150 and their first exit is at 298 ± 34 days (Dawson 2002). Therefore, maternal transfer of immunity was the most likely explanation for this finding.

Western grey kangaroos are abundant throughout the midwest to southwest of WA, interacting with humans through the use of common resources. In the pastoral zones of the state, the provision of artificial watering points and irrigated pastures has created a niche habitat for the kangaroo. Therefore, it is logical that the WGK may play a significant role in the transmission of RRV in rural and semirural regions of WA. Over the past 5 years, more than 1500 serologically confirmed cases of RRV disease have been reportedly acquired from the Perth metropolitan region (MBDC, WA Department of Health, 2014, unpublished data). Whilst a small proportion of reported cases were diagnosed using a single immunoglobulin M (IgM) assay, the majority were confirmed with a second serological sample or followed up by MBDC to ensure that the onset of disease and nature of clinical signs were consistent with RRV infection. Given this substantial number of confirmed cases, further investigation needs to be undertaken to determine whether the WGK plays a role in RRV transmission in and around metropolitan Perth. As urban sprawl continues to encroach upon shrinking wildlife habitats, there is increased contact between humans, wild animal populations, and vector species, particularly in periurban areas. Removal of bushland, together with mining and urban development practices, all add to this pressure, and reduced living space naturally leads to an increased wildlife population density. Given that WGKs populate golf courses, parks, nature reserves, and bushland throughout Perth, it is still plausible that they may be the primary reservoir host population in this region.

A serosurvey of captive marsupials undertaken in urban New South Wales demonstrated that tammar wallabies (*M. eugenuii*) were commonly infected by RRV (Old and Deane 2005). These data support the possibility of marsupials serving as amplifying hosts for RRV in urban areas in Australia. Consideration also needs to be given to the possibility that other potential hosts, such as horses and the common brushtail possum (*Trichosurus vulpecular*), contribute to virus maintenance and transmission (Pascoe et al. 1978, Kay et al. 1987, Boyd et al. 2001). A more recent serosurvey in New South Wales indicated that common brushtail possum populations from urban and woodland habitats were negative for the presence of RRV antibodies (Hill et al. 2008). Further studies will need to be undertaken locally to determine whether these findings are representative of possum populations in urban regions of WA.

A limitation of using the NT in this study was that it was not possible to distinguish between a recent or previously acquired infection in individual kangaroos. Whilst correlation between seroprevalence and neutralizing antibody provides a means of estimating how recently RRV was active within a region, the development of an IgM assay may present a more reliable method of determining the proportion of animals recently infected with RRV. This additional dataset may assist in predicting RRV activity because it acknowledges the influence of vertebrate host factors on RRV epidemiology. The major challenge associated with developing an IgM assay in kangaroos lies in the paucity of data surrounding the antibody response in marsupials following infection.

As discussed earlier, there are many ethical and logistical challenges associated with experimentally infecting large-bodies macropods with an arbovirus of public health significance. If it is found that RRV IgM antibodies persist for long periods of time in the kangaroo, as has been described in people (Kuno 2001), the IgM assay may be of limited use. If deemed to provide a useful marker of recent infection, surveillance in kangaroos may assist in predicting viral activity. Ng et. al. (2014) recently demonstrated that the inclusion of reservoir host population data improves predictive model fit for RRV (Ng et al. 2014). The provision of data on herd immunity may further enhance this capability, as optimal weather conditions or a rise in kangaroo/mosquito populations is unlikely to support a RRV outbreak if overall herd immunity remains high. Given that the interepidemic period in the southwest is approximately 3–4 years, it is plausible

that it takes this period of time for the seroprevalence within local kangaroo populations to fall to a sufficiently low level to support another cycle of above-average viral activity.

Conclusions

This study provides overwhelming serological evidence that WGKs are commonly infected with RRV in WA. Given their abundance throughout the state, susceptibility to being bitten by competent mosquito vectors and close proximity to people in both regional and urban WA, it is highly likely that the WGK plays a significant role in the transmission of RRV. This involvement, however, cannot be confirmed until experimental infection studies are undertaken. It is necessary to determine both the magnitude and duration of viremia and the antibody response that develops in WGKs following RRV infection as well as the likelihood that mosquitoes feeding on viremic individuals can be reinfected.

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Author Disclosure Statement

No competing financial interests exist.

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