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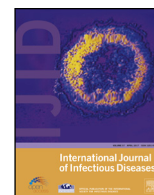

New evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence

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SUMMARY

Objectives: An epidemic of Ross River virus (RRV) occurred in the South Pacific in 1979–1980, but RRV has not been thought to occur endemically outside Australia and Papua New Guinea. A seroprevalence study was conducted to determine whether RRV has circulated in American Samoa since 1980.
Methods: RRV ELISA IgG was performed on 200 serum samples collected in American Samoa in 2010; seroneutralization tests were performed on 60 representative samples.
Results: Of 196 available ELISA IgG results, 145 (74%, 95% confidence interval 67–80%) were seropositive. Of the 60 samples subjected to seroneutralization testing, none of the 15 ELISA IgG-negative and 16 of the 45 ELISA IgG-positive samples neutralized RRV. ELISA IgG seroprevalence was higher in persons born before/during the 1979–1980 RRV outbreak (78.3%), but was also high (63.0%) in people born after the outbreak who had lived their entire lives in American Samoa.
Conclusions: This study provides serological evidence that RRV circulation is likely to have occurred in American Samoa after 1980. Considering there are no marsupials in American Samoa, this finding implies that other species are capable of acting as reservoir hosts and indicates the potential for RRV to circulate in a much wider area than those currently recognized.
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Introduction

Ross River virus (RRV) is an arthropod-borne virus (arbovirus) of the *Alphavirus* genus (*Togaviridae* family) endemic to Australia and Papua New Guinea. Macropod marsupials (kangaroos, wallabies) are the primary reservoir hosts and Aedes and Culex mosquitoes are the vectors.[1–3] In Australia, approximately 5000 infections are notified yearly.[2] Although 55–75% of cases are asymptomatic, RRV can cause debilitating joint pain lasting for months. Common symptoms include arthralgia, fever, fatigue, and a maculopapular rash.[2]

A large virgin soil epidemic occurred in 1979–1980 in the Pacific Island Countries and Territories (PICTs), with more than 500 000 cases reported across the region and dramatic attack rates in American Samoa (44%), Fiji (90%), the Cook Islands (69%), and New Caledonia (33%).[4–6] The outbreak was believed to have been initiated by a viraemic Australian who had travelled to Fiji.[4] During epidemics, human–mosquito–human transmission could occur, bypassing the reservoir hosts.[2] Soon after the outbreak, a study in American Samoa found serological evidence of infection in dogs, pigs, chickens, and rats.[6] This finding was consistent with knowledge that non-marsupials can become infected during epidemics and potentially act as short-term amplifying hosts, but most will be dead-end hosts that play no further role in transmission. Considering that marsupials, the only known reservoirs for RRV, are absent from the PICTs, it was assumed that RRV transmission in the region ceased soon after the outbreak.

Since 1980, no outbreaks of RRV have been recorded in the PICTs, but there have been ongoing concerns about low-level endemic transmission because of reports of RRV infections in

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returned travellers, most notably from Fiji. From 1997 to 2009, five New Zealanders were diagnosed with RRV infection after visiting Fiji. The patients had not travelled to other countries with RRV and there is no local transmission of any mosquito-borne diseases in New Zealand.[7,8] RRV infections were also reported in Canadians and a German after visiting the PICTs.[7] A recent seroprevalence study in French Polynesia found that 42.4% of 132 blood donors who had not travelled abroad were seropositive for RRV by ELISA IgG,[9] providing further evidence of endemic circulation of RRV in the PICTs.

Marsupials are absent in French Polynesia and Fiji, suggesting that non-marsupials can potentially act as reservoir hosts for RRV. It therefore appears that the known endemic area for RRV has undergone a significant range expansion, bringing RRV into the realm of an emerging infectious disease, which has global public health implications. This seroprevalence study was conducted in the islands of American Samoa to seek further evidence for RRV transmission in the PICTs after the 1979–1980 outbreak.

Methods

Study location and setting

American Samoa consists of five small remote islands in the South Pacific, with a highly stable human population of 56 000. The only endemic mammals are bats, and introduced feral mammals are limited to three species of rodent.[10] Also present are pigs, dogs, cats, and very few cows and horses. Mosquitoes are abundant in American Samoa, including *Aedes aegypti* and *Aedes polynesiensis* (both with vectorial capacity to transmit RRV),[1,11] as well as *Culex annulirostris*, an important RRV vector in Australia.[2,12]

Serum bank

With human research ethics approval from the American Samoa Institutional Review Board, RRV serology was performed on 200 serum bank samples collected for a leptospirosis study in 2010.[13,14] The community-based cross-sectional study included adults (aged 18 years and over) from all five inhabited islands of American Samoa. The study was designed to include a representative sample of the adult population in American Samoa, and consisted of random sampling on the main island of Tutuila (where >95% of the population reside) and the adjacent island of Aunu'u, and convenience sampling on the very small and remote Manu'a islands. Questionnaires were used to collect data on demographics, occupation, recreational activities, and household characteristics.

Serology

Serological analysis for RRV was conducted at the Institut Louis Malardé, French Polynesia. Immunoglobulin class G antibodies (IgG) to RRV were detected by indirect ELISA, using recombinant antigens and protocol as reported previously.[9] Briefly, sera were diluted 1:400 and added to wells of 96-well plates coated with RRV recombinant antigens (RR.sE2-SNAP). For each sample, specific absorbance was determined by deducting the absorbance value obtained with the control antigen (SNAP) from the absorbance value found with RR.sE2-SNAP recombinant antigens. Sera with specific absorbance values $\geq$0.2 were considered positive for the presence of IgG.

Seroneutralization tests

ELISA results were validated by testing a subset of the samples with RRV neutralization. As the ELISA protocol used in this study is based on the use of a recombinant antigen designed to be recognized as a target epitope by IgG antibodies that are very specific to RRV but that only represent a limited part of the whole population of anti-RRV antibodies, a subset of the initial samples were also submitted to neutralization assay to obtain additional information on the ability of the global population of anti-RRV antibodies to neutralize RRV. Seroneutralization assays were also conducted for chikungunya virus (CHIKV), another alphavirus, because it is antigenically similar to RRV and has circulated in the PICTs since 2011. Only one third of the samples were tested by neutralization because of limited resources. Fifteen samples with negative (specific absorbance <0.2), 15 with weak (specific absorbance 0.2 to $\leq$0.4), 15 with intermediate (specific absorbance between 0.4 to $\leq$0.8), and 15 with strong (specific absorbance >0.8) RRV ELISA results were selected randomly to provide a panel of sera with different IgG signal intensities. Neutralization tests were performed at Aix–Marseille University in France, in duplicate in a 96-well plate format using protocols and control sera from the French National Reference Laboratory for Arboviruses. Two-fold dilutions (1:20–1:160) were incubated (37 °C, 1 h) with 50 $TCID_{50}$ of RRV (strain 5281 v) or CHIKV (strain Haiti 5/2014), inoculated in duplicate onto monolayers of Vero cells (ATCC-CCL-81) and incubated at 37 °C for 5 days. Dilutions of viruses and sera alone were used as controls. Endpoints were dilutions that completely inhibited the cytopathic effects in the cell culture wells.

Statistical analyses

The Chi-square test or Fisher's exact test was used to identify significant associations between independent variables and the presence of RRV IgG antibodies. Independent variables examined included sex, birth year, living entire life in American Samoa, work location, and participation in outdoor activities (hiking, swimming, kayaking, and gardening). Birth year was classified into 1980 and before (born before or during the outbreak) and 1981–1993 (born after the outbreak). Work location was classified into indoor, outdoor, mixed indoor/outdoor, and tuna cannery (the major non-government employer in American Samoa). Variables with a *p*-value of <0.05 were selected for further analyses using univariate logistic regression, and statistically significant results are reported in Table 1. Independent variables associated with the outcome by a likelihood ratio test *p*-value of <0.1 were subjected to a stepwise backward elimination process ($p < 0.05$) to select the final variables for the multivariable model. Statistically significant odds ratios (OR) are indicated in the Table. Stata version 11.1 software (StataCorp, College Station, TX, USA) was used for the statistical analyses; *p*-values of <0.05 were considered statistically significant.

Results

RRV ELISA IgG results were available for 196 samples, of which 145 (74%, 95% confidence interval (CI) 67.2–80.0%) were seropositive (mean specific absorbance 0.75, range 0.21–2.61). All of the 15 samples with negative RRV ELISA IgG results (specific absorbance <0.2) were also negative by RRV neutralization test. Of the positive RRV ELISA IgG samples with specific absorbance of 0.2 to $\leq$0.4, 0.4 to $\leq$0.8, and >0.8, 20% (3/15), 40% (6/15), and 47% (7/15) neutralized RRV, respectively. The results of the neutralization tests for RRV are summarized in Figure 1. All 60 samples were negative on CHIKV neutralization tests.

Table 1 summarizes the study population and risk factors associated with the presence of RRV ELISA IgG. Seroprevalence was lower in females, indoor workers, and those who had never partaken in hiking or gardening. A higher seroprevalence was found in persons involved in outdoor activities, which is consistent with a mosquito-borne infection. Seroprevalence was higher in

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