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▶ **To cite this version:**

Colleen Lau, Maite Aubry, Didier Musso, Anita Teissier, Sylvie Paulous, et al.. New evidence for endemic circulation of Ross River virus in the Pacific Islands and the potential for emergence. International Journal of Infectious Diseases, 2017, 57, pp.73-76. 10.1016/j.ijid.2017.01.041 . hal-01521265

HAL Id: hal-01521265
https://hal.science/hal-01521265

Submitted on 7 May 2018

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International Journal of Infectious Diseases 57 (2017) 73–76

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

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

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ARTICLE INFO

Article history:
Received 29 November 2016
Received in revised form 30 January 2017
Accepted 31 January 2017
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
River virus
Arboviruses
Emerging infectious diseases
Ecology
Pacific Islands
Epidemiology

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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http://dx.doi.org/10.1016/j.ijid.2017.01.041
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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

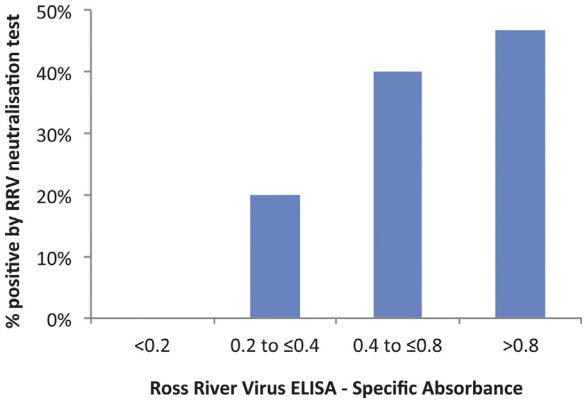

Figure 1. Ross River virus neutralization results for a subset of 60 samples: 15 samples each with negative (<0.2), weak (0.2 to ≤0.4), intermediate (0.4 to ≤0.8), and strong (>0.8) specific absorbance for RRV ELISA.

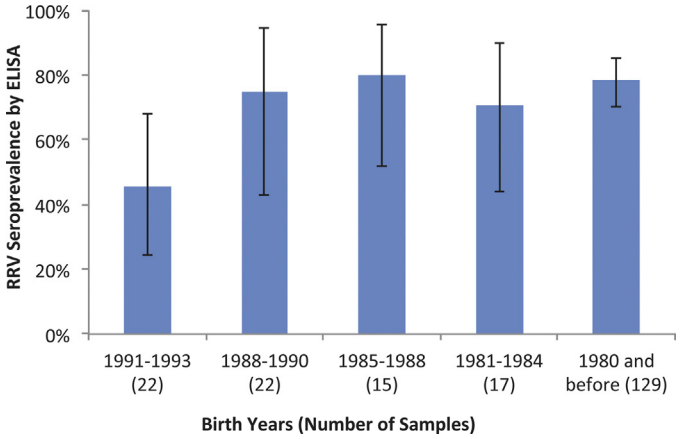

Figure 2. Ross River virus seroprevalence by birth year, and number of samples tested in each age group.

persons born before or during the 1979–1980 RRV outbreak (78.3%) compared to those born after 1980 (65.2%). RRV seroprevalence was 63.0% in those born after 1980 who had lived their entire lives in American Samoa. Figure 2 shows RRV ELISA IgG seroprevalence by birth year. Seroprevalence was 45% in people born between 1991 and 1993, 75% in people born between 1988 and 1990, and 80% in people born between 1985 and 1988, suggesting that RRV circulated in American Samoa long after the 1979–1980 outbreak.

Discussion

High RRV seroprevalence in persons born after 1980 who had only lived in American Samoa provides serological evidence that RRV circulation is likely to have occurred after 1980. All of the 60 samples subjected to seroneutralization were negative for CHIKV, confirming that the ELISA-positive results were not due to cross-reactions with this virus. No other alphaviruses are known to have circulated in American Samoa prior to 2010, when the serum samples were collected.

These findings are significant considering that there are no marsupials in American Samoa, implying that other animal species are capable of acting as reservoirs to sustain endemic circulation. This conclusion is consistent with serological evidence of RRV circulation in French Polynesia,[9] and infections in returned travellers from other PICTs,[7,8] where marsupials are also absent.

The information provided by the ELISA and neutralization tests differs, and the discrepancies between the results may be explained by the fact that, although detectable by ELISA, most of the anti-RRV IgG only had limited neutralizing capacity. This hypothesis is in accordance with the data presented in Figure 1

Table 1
Variables significantly associated with positive RRV ELISA IgG on univariate and multivariable logistic regression analysis.[a]

Variable	*n* (%)	RRV seroprevalence by ELISA IgG (%)	Univariate OR (95% CI)	*p*-Value	Adjusted OR (95% CI)	*p*-Value
Sex						
Female	99 (50.5)	66.7	1		1	
Male	97 (49.5)	81.4	2.2[b] (1.1–4.3)	0.020	2.5[b] (1.1–5.5)	0.026
Birth year						
1981–1993 (post-outbreak)	66 (33.7)	65.2	1		1	
1980 and before	129 (65.8)	78.3	1.9[b] (1.0–3.7)	0.050	2.1[b] (1.1–4.4)	0.045
Lived whole life in American Samoa						
Yes	139 (70.9)	69.8	1			
No	56 (28.6)	83.9	2.3[b] (1.0–5.0)	0.046		
Occupation						
Indoor	44 (22.4)	54.6	1		1	
Mixed indoor/outdoor	16 (8.2)	93.8	12.5[b] (1.5–103.0)	0.019	8.8[b] (1.0–75.6)	0.047
Outdoor	15 (7.7)	86.7	5.4[b] (1.1–26.9)	0.039	3.1 (0.6–17.1)	0.190
Cannery worker	21 (10.7)	90.5	7.9[b] (1.6–38.2)	0.010	5.9[b] (1.2–30.2)	0.032
Hiking						
Never	116 (59.2)	67.2	1			
Once a month or less	25 (12.8)	72.0	1.3 (0.5–3.3)	0.644		
More than once a month	54 (27.6)	88.9	3.9[b] (1.5–9.9)	0.004		
Gardening						
Never	114 (58.2)	71.1	1		1	
Once a month or less	20 (10.2)	55.0	0.5 (0.2–1.3)	0.159	1.1 (0.4–3.2)	0.907
More than once a month	62 (31.6)	85.5	2.4[b] (1.1–5.4)	0.035	3.0[b] (1.2–7.3)	0.015
Total	196 (100)	74.0				

RRV, Ross River virus; OR, odds ratio; CI, confidence interval.
[a] Seroprevalence in those born post-outbreak and who had lived their whole lives in American Samoa was 63.0%.
[b] Statistically significant odds ratio.

showing that the higher the specific absorbance by ELISA, the higher the percentage of RRV-positive sera by neutralization.

This study included only samples from adults, so it is not possible to know whether RRV has circulated recently in American Samoa. However, the detection of imported cases from Fiji in 2009 and RRV seropositive persons living in French Polynesia since 2006 suggests that RRV may be circulating undetected in several PICTs, possibly misdiagnosed as dengue virus (DENV), CHIKV, or Zika virus (ZIKV) infections.[15,16] Since RRV serology is not available in most of the PICTs and because symptoms are variable and overlap with other conditions, low-level RRV circulation could occur undetected.

These findings have important ecological, clinical, and public health implications, as they suggest that the geographic range of RRV is larger than previously documented and includes areas without marsupials. The animal species present in American Samoa that may potentially act as amplifying hosts are rodents, pigs, dogs, cats, and bats. There is insufficient evidence based on animal studies to implicate one or more of these as the specific non-marsupial host(s), and field-based animal studies are required to clarify this situation. Rodents have been suggested as potentially important in the transmission cycle based on epidemiological and modelling studies,[17,18] but pigs, dogs, cats, and bats were not considered in these studies. Importantly, all five potential animal reservoir hosts in American Samoa are pan-global in distribution, suggesting that RRV could therefore spread well beyond its currently known distribution.

Knowledge about the geographical range of pathogens is important for empirical clinical diagnosis in the PICTs where many infections have similar presentations, especially the arboviruses that have been responsible for large outbreaks recently (DENV, CHIKV, and ZIKV).[15] A regional approach to RRV surveillance is needed to improve understanding of disease ecology, identify emergence and outbreaks, and develop effective strategies to reduce the risk of global spread. The recent spread of ZIKV across the PICTs[19] and on to the Americas demonstrates that the potential of global spread of emerging arboviruses from the Pacific should not be underestimated.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. CL was supported by an Australian National Health and Medical Research Council Fellowship (grant number 1109035); the funder did not play any role in the study or decision to submit the manuscript for publication.

Ethical approval

Ethical approval was obtained from the American Samoa Institutional Review Board to perform RRV serology on serum bank samples collected for a leptospirosis study in 2010.

Conflict of interest

The authors do not have any conflicts of interest to declare.

Acknowledgements

We would like to thank the people of American Samoa for participating in the original field study, and the many staff members from the American Samoa Department of Health, LBJ Tropical Medical Center, and the American Samoa Community College who provided assistance with the data collection.

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