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Seroprevalence of chikungunya virus among military personnel in Papua New Guinea, 2019

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ABSTRACT

Objectives: The first outbreak of chikungunya virus (CHIKV) was reported in West Sepik, Papua New Guinea (PNG) in June 2012, and spread rapidly throughout PNG. CHIKV imported from PNG to Queensland has been reported occasionally, but transmission of CHIKV in PNG remains unclear due to the lack of testing capability. This study investigated the degree of CHIKV exposure among PNG military personnel (PNGMP) in 2019, 7 years after its first emergence.

Methods: Sera of 204 PNGMP recruited in April 2019 was tested for the presence of anti-CHIKV immunoglobulin G (IgG) antibodies using a commercially available IgG detection kit, and anti-CHIKV neutralizing antibodies against a CHIKV Reunion strain using a neutralizing assay.

Results: Anti-CHIKV seropositivity of the sera was 47% and 35%, respectively, using the enzyme-linked immunosorbent assay (ELISA) and neutralizing assay. Five percent (n=11) of samples were found to be IgG negative or borderline, but neutralizing antibody positive.

Conclusions: The prevalence of anti-CHIKV neutralizing antibody of 35% suggests that CHIKV infection has become endemic among PNGMP. Current commercially available CHIKV ELISA detection kits may not be suitable for diagnostic purposes in multiple alphavirus endemic areas such as PNG, due to serological cross-reactivity among alphaviruses. Re-emergence of CHIKV in PNGMP is possible.

Introduction

Chikungunya virus (CHIKV) is a mosquito-borne, single-stranded, positive-sense RNA virus that belongs to the *Alphavirus* genus of the family *Togaviridae* (Harapan et al., 2019). CHIKV infection causes an acute febrile illness, commonly with polyarthralgia, fever, maculopapular rash, headache, fatigue and myalgia, that is indistinguishable from dengue, Ross River virus (RRV) and Barmah Forest virus (BFV). The first outbreak of CHIKV in PNG was reported in June 2012 (Horwood et al., 2013), and it spread rapidly throughout PNG. There have been reports of CHIKV imported from PNG to Queensland (Huang et al., 2019), but the transmission in PNG remains unclear due to lack of testing capability. Based on research by Indonesian scientists, CHIKV is still circulating in PNG (Sari et al., 2017). Currently, laboratory diagnosis of CHIKV infection is based on the detection of CHIKV-specific immunoglobulin M (IgM) antibody, which normally appears in serum collected 5–7 days after onset of illness. In this study, a population-based CHIKV seroprevalence survey was conducted on sera obtained from PNG military personnel (PNGMP) in April 2019, using a commercial enzyme-linked immunosorbent assay (ELISA) IgG kit and a neutralization assay (Reunion strain).

Methods

This study was part of an infectious disease surveillance conducted by the Australian Defence Force in conjunction with the Papua New Guinea Defence Force. In total, 76 PNGMP from Manus Island, the largest of the Admiralty Islands, and 132 PNGMP from Wewak, located on the northern coast of the main island of PNG, consented voluntarily to participate in this survey conducted in April 2019. Four samples collected from PNGMP from Wewak were excluded due to insufficient sera.

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Table 1
Prevalence of anti-chikungunya virus antibody observations for 204 Papua New Guinea military personnel participating in this study, 2019

Military participants	Manus Island	Wewak	Total
Number of participants	76	128	204
Percentage	36.4	63.6	100%
Male/female	76/0	127/1	203/1
Age range (years)[a]	23–62	21–59	21–62
Mean	35.2	39.2	37.5
Median	29	41.5	34
ELISA IgG+	39.5% (30/76)	51.5% (66/128)	47.1% (96/204)
ELISA IgG±	13.1% (10/76)	10.2% (13/128)	11.2% (23/204)
ELISA IgG-	47.4% (36/76)	38.3% (49/128)	41.6% (85/204)
Neutralizing assay+	21.1% (16/76)	43% (55/128)	34.8% (71/204)
Neutralizing assay-	78.9% (60/76)	57% (73/128)	65% (133/204)
ELISA+, neutralizing assay+	18.4% (14/76)	35.9% (46/128)	29.4% (60/204)
ELISA±, neutralizing assay+	0% (0/76)	4.7% (6/128)	2.9% (6/204)
ELISA-, neutralizing assay+	2.6% (2/76)	2.3% (3/128)	2.5% (5/204)
Age, years, no. neutralizing assay positive/no. tested[b]			
Group 1, age 20–35 years	26% (13/50)	22.7% (29/128)	38.9% (42/108)
Group 2, age 36–50 years	7.1% (1/14)	14.1% (18/128)	28.4% (19/67)
Group 3, age 51–62 years	16.7% (2/12)	6.3% (8/128)	34.5% (10/29)

+, positive; -, negative; ±, borderline; ELISA, enzyme-linked immunosorbent assay.
[a] Age = bleeding date - date of birth.
[b] No significant differences were found between the three age groups.

Anti-CHIKV structural-protein-specific IgG was detected using a commercial ELISA kit (Euroimmun; https://www.euroimmun.com), and a neutralizing assay for anti-CHIKV neutralizing antibodies (NAb) against a CHIKV Reunion strain was performed as described in Appendix 1 (see online supplementary material).

Chi-squared test and *t*-test were employed for statistical analysis.

Results

The prevalence rates of anti-CHIKV IgG and NAb against the Reunion strain from 204 PNGMP samples were 47% (96/204) and 35% (71/204), respectively (Table 1). Five and six samples that tested negative and borderline on ELISA, respectively, were NAb positive. The prevalence of anti-CHIKV NAb (χ^2=10.1, P=0.0015) and NAb titre (unpaired *t*-test, P<0.0001, Figure 1) were significantly higher in the PNGMP from Wewak compared with those from Manus Island. The NAb seropositivity rate did not differ between age groups (20–35, 36–50 and 51–62 years) (Table 1).

One of nine known prior RRV control sera was also CHIKV IgG positive on ELISA, and four were borderline. Nine RRV- and five BFV-positive human sera controls neutralized RRV and BFV, but did not neutralize CHIKV. Five Australian Defence Force sera controls were CHIKV negative on both ELISA and the neutralizing assay.

Discussion

Previous CHIKV serosurvey results conducted in countries on different continents reported seroprevalence rates ranging from 10.2% to 75% depending on the subpopulation studied, the timing of the study, and the intensity of virus circulation (Dias et al., 2018). These studies applied either indirect immunofluorescence IgG/IgM or Euroimmun IgG/IgM ELISA kits for detection. It has been suggested that anti-CHIKV NAb correlates with immune protection in humans (Yoon et al., 2020). NAb cross-reactivity among antigenically related CHIKV, RRV and BFV remains unclear. The present results indicated that anti-RRV and anti-BFV human serum does not cross-neutralize CHIKV. Unfortunately, it was not possible to obtain sera that was anti-CHIKV alone, as RRV and BFV (but not CHIKV) are endemic in Australia. Nevertheless, the prevalence of CHIKV NAb of 35% amongst PNGMP implies that CHIKV has been circulating amongst PNGMP since its first outbreak in 2012.

The finding of higher anti-CHIKV NAb titres in PNGMP in Wewak may suggest increased risk of transmission on the main PNG island compared with Manus Island, which is more remote, as the first confirmed CHIKV outbreak occurred in Vanimo, a PNGMP outpost of Wewak. The higher population density in Wewak could increase human–mosquito–human transmission, and other related environmental factors such as a wider variety or different mosquito species on the main island compared with the remote island may also have contributed to differing exposure levels.

The proportion of samples showing anti-CHIKV IgG positivity on ELISA was higher than the proportion exhibiting anti-CHIKV NAb positivity, which could be due to the endemicity of other alphaviruses that are antigenically closely related to CHIKV; for example, 94% of the samples tested were also RRV IgG positive on ELISA (data not shown). Other alphaviruses, such as Getah virus, BFV and Sindbis virus, were also identified in PNG (Goi et al., 2022). The present finding that one of nine RRV-positive controls also tested positive for CHIKV IgG on ELISA implies that the current commercially available ELISA detection kits for CHIKV may not be suitable for diagnostic or seroprevalence survey purposes in areas endemic for multiple alphaviruses, such as PNG, due to possible serological cross-reactivity among alphaviruses (Martins et al., 2019). All samples that tested positive for CHIKV IgG on ELISA should be confirmed by neutralizing assay for diagnostic purposes. The positive neutralizing results of 11 ELISA-negative or -borderline samples could be due to antigenic changes in the PNG CHIKV lineage (Langsjoen et al., 2018), or ELISA assay sensitivity as that of the Euroimmun ELISA was only 88% (Prat et al., 2014).

This preliminary finding requires further support from additional investigations as the present study had a small sample size and participants were limited to PNGMP alone. However, the results provide a representative view of the entire PNG population as PNGMP are recruited from the general population. The authors intend to expand their arbovirus surveillance programme in PNG to include identification of dominant circulating strains, mosquito population behaviours, arbovirus transmission and antibody prevalence in the entire population.

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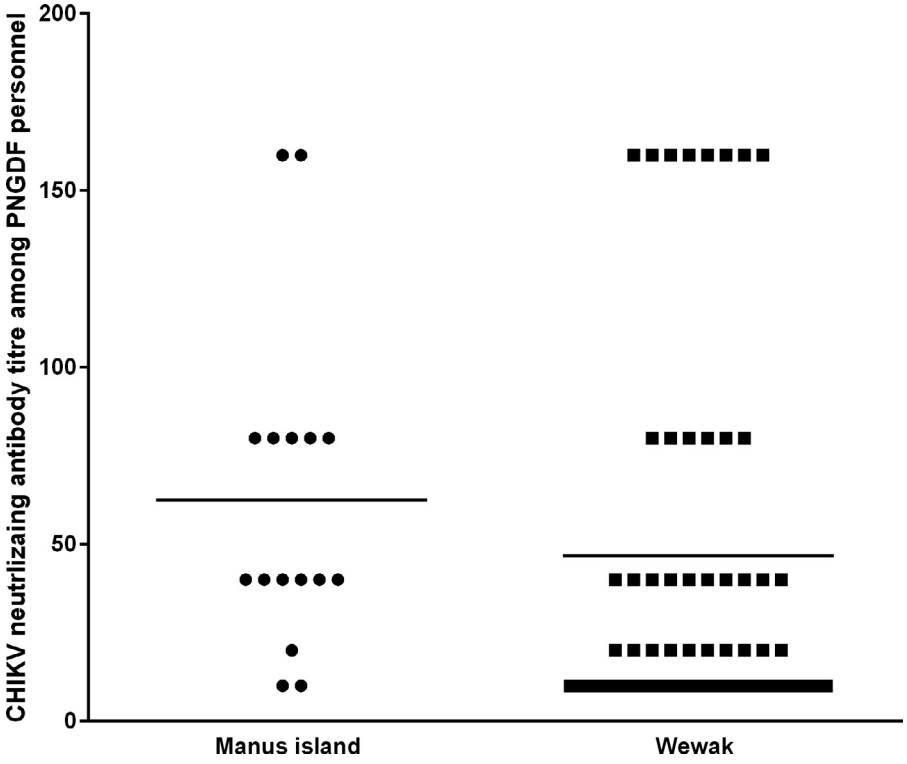

Figure 1. Micro-neutralization titres against chikungunya virus (CHIKV) (Reunion strain) among Papua New Guinea Defence Force (PNGDF) personnel located in Wewak and Manus Island, 2019. Bars represent means.

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Author contributions

LT Melissa Graham completed the CHIKV micro-neutralization tests. CAPT Joanne Kizu completed the ELISA tests. Associate Professor Greg Devine designed and supervised the neutralization assay. Fiona McCallum was involved in organizing PNG deployment, ethical application and blood collection. LTCOL Brady McPherson, LTCOL Alyson Auliff and Dr. Peter Kaminiel conceived and organized the Papua New Guinea Defence Force infectious disease surveillance programme. Dr. Wenjun Liu was responsible for designing the experiment, data collection and analysis, and drafting the manuscript. All other authors edited and approved the manuscript.

Conflict of interest statement

None declared. The opinions expressed by the authors do not necessarily reflect the opinions of the institutions with which the authors are affiliated.

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Ethical approval

The study was approved by the PNG Medical Research Advisory Committee (MRAC No. 18-21) and the Department of Defence and Veteran Affairs Human Research Ethics Committee (DDVA HREC No. 084-18 and DDVA HREC 157-19). Written formal consent was obtained from all participants.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijregi.2022.02.009.

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