

West Nile virus seroprevalence for blood donors in the Wellington region

(2007 – 10606)

MAF Biosecurity New Zealand Technical Paper No: 2010/17

Prepared for MAFBNZ Operational Research

By Sue Huang, Helen Brady, Ange Bissielo, Institute of Environmental
Science and Research, National Centre for Biosecurity and Infectious
Disease

Dorothy Dinesh, Wellington Branch of New Zealand Blood Service
(NZBS)

ISBN 978-0-478-37075-1 (print)

ISBN 978-0-478-37076-8 (online)

ISSN 1179-5832 (paper)

ISSN 1179-5840 (online)

June 2010



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Requests for further copies should be directed to:

Strategic Science Team
Policy and Risk Directorate
MAF Biosecurity New Zealand
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

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1. Introduction

West Nile virus (WNV) is a mosquito-borne flavivirus that is transmitted primarily among birds¹. Birds are the natural reservoir (amplifying) hosts, and WNV is maintained in nature in a mosquito-bird-mosquito transmission cycle primarily involving *Culex* species mosquitoes. The virus is indigenous to Africa, Asia, Europe, and Australia and has recently caused large epidemics in Romania, Russia, and Israel. Humans serve as incidental hosts. Most of human infections in temperate and subtropical zones occur in summer or early autumn. In the tropics, the incidence is greatest during the rainy season when mosquitoes are most abundant. Although WNV was first isolated in 1937 from a human patient in Uganda, it was not seen in the Western hemisphere until 1999, when 62 cases of WNV encephalitis were reported in USA¹. It is an unsettling reminder of the ability of WNV to jump continents and hemispheres. By the end of 2002, WNV activity had been identified in 44 US states and the District of Columbia. The 2002 WNV epidemic and epizootic resulted in reports of 4156 reported human cases of WNV diseases (including 2942 meningoencephalitis cases and 284 deaths), 16741 dead birds, 6604 infected mosquito pools and 14571 equine cases (<http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnvguidelines2003.pdf>). During 1999-2005, the virus spread throughout the USA and into Canada, Mexico, and the Caribbean Basin. With global warming under the influence of increasing temperatures and rainfall through warming of the oceans, and alteration of the natural cycles that stabilise climate, West Nile virus will continue to emerge in new regions².

Most WNV infections (~80 percent) in humans are asymptomatic. The incubation period is approximately 2-14 days for symptomatic infections overall, but 2-6 days is typical in WNV fever cases. Clinical symptoms range from fever, headache, myalgia, gastrointestinal symptoms, maculopapular rash, meningitis, encephalitis, acute flaccid paralysis, respiratory failure and death¹. Serology continues to have a dominant role in the laboratory diagnosis of West Nile viral infections in humans. A recent West Nile viral infection can be inferred by the detection of IgM in serum or CSF and antibody-capture enzyme immunoassays (EIA) are optimal for this purpose. Serum IgM can persist for extended periods in some WN encephalitis cases (up to 12 to 16 months post-onset)¹. Therefore, in a patient with acute meningoencephalitis, WNV-specific IgM detected in serum could theoretically be unrelated to the current illness. The detection of WNV-specific IgG in serum indicates post exposure.

Although mosquito-borne transmission of WNV to human is by far the predominant mode, another 5 routes of WNV transmission to humans have been documented (<http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnvguidelines2003.pdf>): 1) blood transfusion, 2) organ transplantation, 3) transplacental transfer, 4) breast-feeding, and 5) laboratory-acquired infections via percutaneous inoculation or the airborne route. About 23 transfusion-transmitted WNV infections have been documented in 2002³. In response to this, a combination of mini-pool Nucleic Acid Testing (NAT) during the “non-season” coupled with the conversion to the more sensitive individual NAT in epidemic locations during epidemic times has been successful in detecting approximately 1500 infected blood donors in the USA during 2003-2005. Assuming that each donation was infectious and manufactured into 1.45 blood components, testing has therefore prevented close to 2200 recipient infections and potential clinical diseases⁴.

The prevalence of immunity to WNV depends on geography and the human population studied. Comparing the results of different serosurveys is difficult because methods have varied. At one extreme are some endemic areas of Africa, where background immunity to WNV has been observed in approximately 50 percent of children and 90 percent of adults⁵. By contrast, general background immunity to WNV in Europe is probably low, being 2-4

percent in Burcharest, Roumania in 1966. Moreover, the low post-epidemic seroprevalence estimated among residents of Queens, New York, in 1999 (3 percent) 7, and Staten Island and Suffolk County, New York, and Fairfield County, Connecticut in 2000 (0-1 percent) 8, suggest that no significant levels of background immunity resulted from recent epidemics in those areas.

There has been no documented West Nile Virus disease in New Zealand. However, WNV may enter New Zealand through viraemic mosquitoes, viraemic migratory birds and viraemic human travellers. For example, since 1998, there have been 35 interceptions of exotic mosquitoes involving 18 species. Four exotic species of mosquito (including the Southern Saltmarsh Mosquito) have established at some time in New Zealand, complementing 12 endemic species. Each of these cases represents an opportunity for entry of vector-borne diseases. To better understand the ecology and epidemiology of vectors and vector-borne diseases and improve New Zealand's capabilities to new and emerging threats, the Ministry of Agriculture and Forestry (MAF) led a multi-disciplinary research project with the active participation of the Ministry of Health and the Department of Conservation and the expertise of the MAF Investigation Diagnostic Centre (IDC), ESR, LandCare, AgResearch, Auckland Zoo, Auckland District Health Board (DHB) and Westmead Hospital (NSW, Australia).

As part of this multi-disciplinary research, the National Arbovirus Reference Laboratory at ESR focused on understanding of the seroprevalence of WNV in New Zealand blood donors. In particular, we were interested in understanding whether there was a risk of exposure to WNV for blood donors who had travelled to WNV endemic areas prior to blood donation. This allows assessment of the risk of WNV entry into New Zealand through the entry of viraemic individuals from WNV endemic areas. It thus provides evidence-based research to guide an effective strategy in reducing risks of transfusion-transmitted WNV infections in New Zealand. The diagnostic capability developed in this study can then be applied in future human surveillance for vector-borne diseases in New Zealand which will directly improve our capability and preparedness in the event of an incursion of WNV or its vectors. The knowledge generated from this study will contribute to our understanding of the ecology and epidemiology of vectors and vector-borne diseases.

2. Methods

2.1. SETTING

Blood donors at the Wellington Branch of New Zealand Blood Service (NZBS) were recruited in the study.

2.2. SUBJECT RECRUITMENT

All blood donors at the NZBS Wellington Branch were approached for recruitment into the study during May 2009-May 2010. Ethics approval (CEN/09/02/06) was given by the Central Regional Ethics Committee. An information sheet was given to the participants. Participants gave their written informed consent. Information on the health and overseas travel history of participants was collected through a questionnaire.

2.3. SAMPLE COLLECTION

A once only blood sample (~5 ml) was collected by a trained nurse at NZBS Wellington Branch.

2.4. LABORATORY TESTING

The commercially available serological ELISA-based assay (*DxSelectTM - Focus Diagnostics*) for West Nile virus IgG antibodies is an FDA-approved assay. The National Arbovirus Reference Laboratory at ESR validated the test and the validation report was been submitted to MAF as Milestone 6 report. The Focus IgG test kit was used to detect the immunity against West Nile virus in NZ blood donors.

2.5. SAMPLE SIZE CALCULATION AND JUSTIFICATION

No previous studies have determined WN virus antibody seroprevalence in New Zealand. Overseas studies on WNV seroprevalence vary. The prevalence of immunity to WN virus depends on geography and the human population studied. Comparing the results of different serosurveys is difficult because methods have varied. We realise there are limitations when extrapolating overseas prevalence and other data findings to New Zealand as ecology vary from country to country. Consequently, such overseas prevalence determinations may not be directly applicable to the New Zealand situation. We assumed that the frequency of cases who had seroconverted against WN virus was likely to be small (nearly zero) for blood donors who had never travelled to the WNV endemic areas. In addition, we assumed the frequency of seroconversion in blood donors who had travelled to WNV endemic areas would be higher. Thus, we tested the null hypothesis that the proportion of sero-positive cases would be very small (0.001 percent) against the one sided alternative hypothesis that this would be greater than 0.001 percent. We used a one-sided Fisher's Exact test to statistically test this hypothesis. We calculated the sample sizes estimates required to detect an effect size (i.e. a difference in proportion between the two samples) as statistically significant at an alpha (significance) level of 0.05. We used a ratio of one NZ blood donor who had travelled to WNV endemic countries (travellers) to three NZ blood donors who had never travelled to those countries (never travellers). Calculations suggested that a sample size of 300 travellers to 900 never travellers (considering each individual sample as an independent observation) would provide 80 percent power to detect an effect size of at least 0.015 (1.5 percent difference in proportion).

3. Results

3.1. DIAGNOSTIC EVALUATION

The WNV IgG ELISA tests (*DxSelectTM - Focus Diagnostics*) were performed at ESR for all sera samples. WNV IgG ELISA assay is a primary diagnostic screening assay. This assay does not distinguish between reactive antibodies for WNV and for other antigenically closely related flaviviruses such as Murray Valley Encephalitis, Japanese Encephalitis, Dengue virus and Kunjin. In addition, it has also been shown that this assay may also detect cross-reactive antibodies to cytomegalovirus (CMV) and LaCross virus (a bunyavirus).

A total of 1208 blood donor sera were collected from blood donors at the Wellington Branch of New Zealand Blood Service (NZBS) between 30 April 2009 and 10 March 2010. Of 1208 samples tested at ESR, 25 were IgG positive.

All 25 IgG positive samples were forwarded to the Institute of Clinical Pathology and Medical Research (ICPMR), an Arbovirus Reference Laboratory in Australia. ESR received results from ICPMR for 24 samples. ICPMR used a range of tests: Generic Flavivirus IgG and IgM, WNV IgG and IgM, Kunjin total antibodies, Dengue total antibodies and Dengue IgM. However, ICPMR did not perform plaque reduction neutralisation assay which is the gold standard assay to differentiate cross-reactive antibodies between flaviviruses such as WNV and Dengue. Comparison between ESR and ICPMR results are shown in Table 1.

Among 24 samples tested at ICPMR, 20 (20/24, 83.3 percent) were confirmed to be positive to WNV only (4/24, 16.7 percent), WNV and Dengue (7/24, 29.2 percent), Dengue (8/24, 33.3 percent), or a flavivirus not otherwise specified (1/24, 4.2 percent). The remaining four sera (4/24, 16.7 percent) were negative for WNV, Dengue or flavivirus.

All the WNV IgG positive samples (11) were negative for WNV IgM ELISA. This indicated that none of these WNV IgG positive blood donors had a recent WNV infection. WNV IgG positivity may be due to a past exposure.

Table 1: Comparison of ESR and ICPMR results

	A	B	C	D	E	F	G	H	I	J
1	WNV	ESR result	Institute of Clinical Pathology & Medical Research							
2	Number	WNV IgG	Flavivirus IgG	Flavivirus IgM	WNV IgG (IF)	WNV IgM (IF)	WNV(Kunjinn) Total Ab	Dengue Total Ab	Dengue IgM	Conclusion
3	WNV09005	positive	positive	Negative	Negative	Negative	Negative	negative	not done	past flavivirus exposure
4	WNV09014	positive	Negative	Negative	Negative	Negative	Negative	negative	not done	negative
5	WNV09110	positive	positive	Negative	Negative	Negative	Negative	positive	Negative	Past Dengue exposure
6	WNV09152	positive	positive	Negative	Negative	Negative	Negative	positive	Negative	Past Dengue exposure
7	WNV09185	positive	positive	Negative	positive	Negative	positive	positive	not done	past WNV & Dengue exposure
8	WNV09186	positive	positive	Negative	positive	Negative	low positive	positive	not done	past WNV & Dengue exposure
9	WNV09236	positive	Negative	Negative	Negative	Negative	not done	Negative	not done	negative
10	WNV09283	positive	positive	Negative	positive	Negative	low positive	Negative	not done	past WNV exposure
11	WNV09342	positive	positive	Negative	positive	Negative	strong positive	Negative	not done	past WNV exposure
12	WNV09358	positive	positive	Negative	positive	Negative	strong positive	positive	not done	past WNV & Dengue exposure
13	WNV09472	positive	positive	Negative	positive	Negative	strong positive	positive	not done	past WNV & Dengue exposure
14	WNV09482	positive	positive	Negative	positive	Negative	strong positive	positive	not done	past WNV & Dengue exposure
15	WNV09583	positive	positive	Negative	positive	Negative	moderate positive	positive	not done	past WNV & Dengue exposure
16	WNV09653	positive	positive	Negative	positive	Negative	moderate positive	Negative	not done	past WNV exposure
17	WNV09663	positive	positive	Negative	positive	Negative	moderate positive	Negative	not done	past WNV exposure
18	WNV09766	positive	not done	not done	Negative	Negative	not done	positive	negative	Past Dengue exposure
19	WNV09821	positive	not done	not done	positive	Negative	strong positive	positive	negative	past WNV & Dengue exposure
20	WNV09839	positive	not done	not done	Negative	Negative	not done	negative	not done	negative
21	WNV09841	positive	not done	not done	positive	Negative	strong positive	positive	negative	past WNV & Dengue exposure
22	WNV09846	positive	not done	not done	Negative	Negative	not done	positive	Negative	Past Dengue exposure
23	WNV09981	positive	negative	negative	negative	negative	negative	negative	not done	negative
24	WNV09999	positive	not done	not done	negative	negative	not done	positive	negative	Past Dengue exposure
25	WNV091015	positive	not done	not done	negative	Negative	not done	positive	negative	Past Dengue exposure
26	WNV1022	positive	not done	not done	not done	not done	not done	not done	not done	waiting for ICPMR result
27	WNV1064	positive	not done	not done	negative	negative	not done	positive	negative	Past Dengue exposure

3.2. DESCRIPTIVE ANALYSIS

Table 2 shows a summary of the results for the 1208 serum samples tested for WNV by ESR and ICPMR. Eleven WNV IgG positive cases were detected by both ESR and ICPMR and one was detected only by ESR. This gave an overall seroprevalence of 0.99 percent (CI:0.43-1.55). There is no significant difference in seroprevalence between male (0.88 percent;CI:0.17-1.58) and female (1.18 percent;CI: 0.24 - 2.12).

Table 2 Distribution of seropositive individuals to WNV by travel history and demography

	No. samples	Positives	Proportion* (%)	95% CI*
Overall	1208	12	0.99	0.43 - 1.55
Travelled¹				
Yes	617	8	1.3	0.40 - 2.19
No	576	4	0.69	0.01 - 1.37
Ethnic group				
Maori	23	0	-	-
Pacific	9	1	11.11	0.00 - 31.67
Asian ²	48	5	10.42	1.76 – 19.07
European	1052	6	0.57	0.11 – 1.03
Age group				
4 to 27 yrs	305	3	0.98	
28 to 42	283	1	0.35	
43 to 53	295	4	1.35	
54 and over	288	4	1.38	
Sex				
Female	508	6	1.18	0.24 - 2.12
Male	679	6	0.88	0.17 - 1.58

¹Had travelled to an endemic country within the 12 months prior to sampling. No. samples included only data where travel history was recorded.

²Asian ethnic group included ethnicity recorded as Asian, Chinese, Indian, and Malaysian. Ethnicity was not indicated in 76 samples and was not included in the ethnic-specific seroprevalence analysis.

*Results (%) and confidence intervals should be interpreted with care especially for small numbers < 5.

3.3. TRAVEL HISTORY

To understand whether there is a risk of exposure to WN virus for New Zealand’s blood donors who have travelled to WNV endemic countries, information on travel history in the past year prior to blood donation was recorded. Results from the descriptive analysis indicated higher seroprevalence among participants (1.3 percent; CI:0.40-2.19) who had travelled to endemic countries compared with those (0.69 percent; CI:0.01-1.37) who had not (Figure 1, Table 2). However, there was no significant difference in seroprevalence between the two groups (Figure 1). Four WNV IgG-positive cases who had not travelled prior to blood donation might have travelled previously and being exposed to a WNV-like virus.

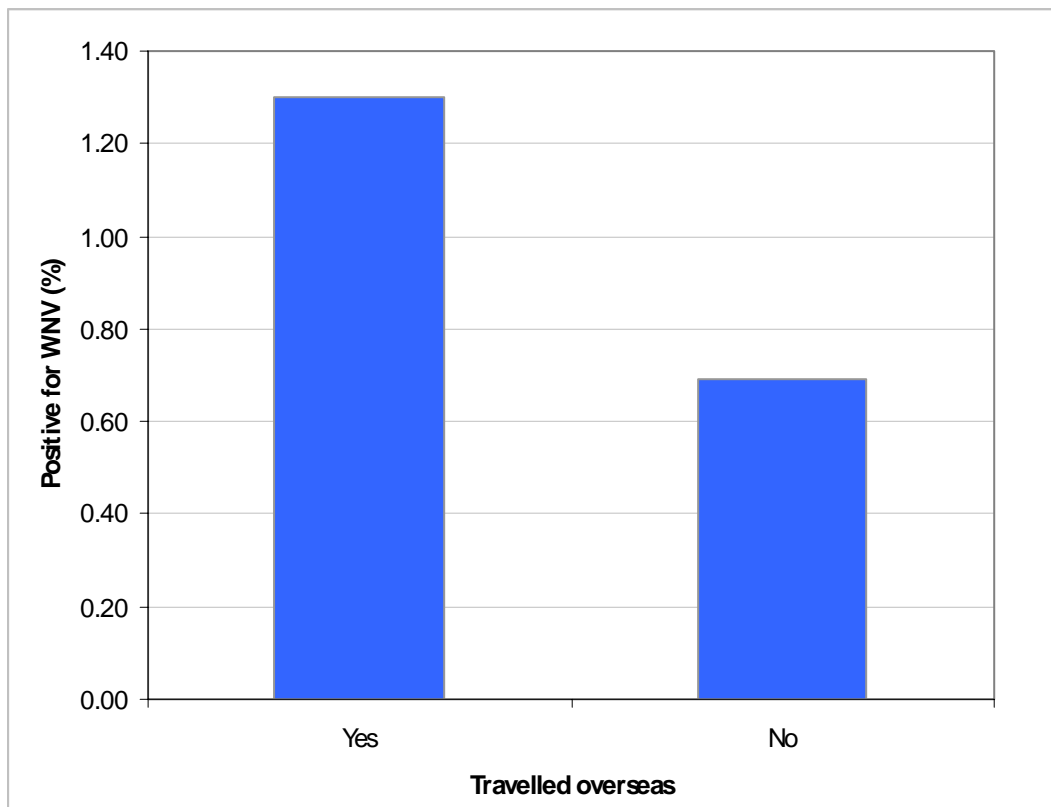


Figure 1 Proportion of seropositive individuals to WNV by overseas travel history

3.4. ETHNICITY

Ethnic-specific seroprevalence was calculated for the 1132 participants with known ethnicity (Table 2). The seroprevalence was 0.57 percent (CI:0.11–1.03) among Europeans. One of the nine (11.11 percent) Pacific people had seropositive results. None of the 23 Maori participants had IgG antibodies against WNV. Of the 48 Asian participants, five (10.42 percent; CI: 1.76–19.07) had WNV IgG antibodies. Among these five Asian cases, four (WNV09185, WNV09186, WNV09583, WNV09841 in Table 1) had both WNV and Dengue IgG antibodies. WNV and Dengue are antigenically closely related flaviviruses. The assays performed at ICPMR are not able to differentiate cross-reactivity between WNV and Dengue. It is possible that these four Asian participants were exposed to Dengue-like virus which has high prevalence in the Asian region. In order to obtain a final definitive diagnosis, it would be valuable to refer these samples to a reference laboratory that performs a plaque reduction neutralisation assay for both WNV and Dengue which is the gold standard confirmatory test with the highest specificity.

3.5. AGE

Age was categorised using quartiles: 4-27, 28-42, 43-53 and ≥ 54 . The median age for the participants was 42 years. The seroprevalence ranged from 0.35 percent in participants aged 28-42 years to 1.38 percent in the age group 54+ years. The seroprevalence was 0.98 percent and 1.35 percent in age groups 4-27 years and 43-53 years, respectively. Because the number of positives was small, the confidence intervals were not stable and therefore are not indicated here.

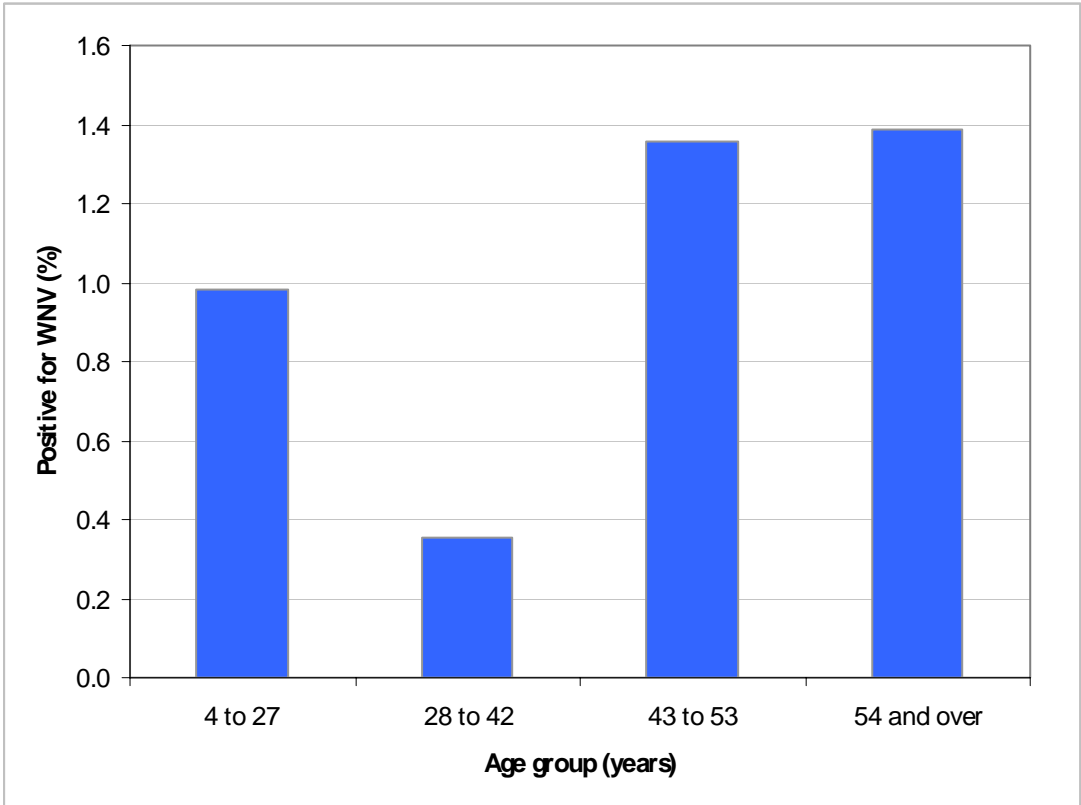


Figure 2 Proportion of seropositive individuals to WNV by age group

4. Discussion

The human immune response to WNV is essentially an antibody-mediated response. The antibody response can be directed against flavivirus cross-reactive epitopes which is the basis for the inherent serological cross-reactivity between WNV and other flaviviruses. The antibody response can also be directed against virus-specific epitopes. IgM antibodies (Ab) are detected 2-8 days after the onset of illness, peak at 2 weeks, and then rapidly decrease over several weeks or months. In some cases, serum IgM can persist up to 12-16 months. On the other hand, IgG antibody appears a few days after the appearance of IgM related Abs, generally 12 days after the onset of symptoms, and may persist for years

Serological testing remains the primary method of diagnosing WNV infection. The IgM and IgG ELISA assays are rapid, reproducible and less expensive, suitable for primary testing and large-scale screening. The Focus IgM and IgG ELISAs employed at ESR use a prM/E recombinant protein. Compared with the CDC IgG/IgM ELISA, the Focus IgG ELISA has a sensitivity and specificity of, respectively, 97.6 and 92.1 percent and the Focus IgM ELISA has a sensitivity and specificity of 99.3 percent⁹. ICPMR enhanced diagnosis by using a battery of tests including: indirect immunofluorescence assay (IFA) for WNV IgG and IgM, generic flavivirus IgM and IgG, Kunjin Total Ab assay, Dengue IgM and IgG ELISA. However, the limitation of ELISA-based assays is their inability to specifically differentiate WNV from other flaviviruses, particularly from viruses of the same serocomplex. For this reason, confirmation by Plaque Reduction Neutralisation Test (PRNT) is required. ESR is in the process of referring 7 sera with both WNV and Dengue IgG positive results to a northern hemisphere arbovirus reference laboratory that performs PRNT. As a future development for ESR's arbovirus reference capability, the establishment of PRNT is highly recommended.

In our milestone 6 report to MAF, we detailed verification of the Focus IgM and IgG ELISA tests with 30 positive sera from proficiency panels referred from CDC and Winnipeg Arbovirus Reference laboratories and 450 presumptive negative sera from ESR. ESR's test results indicated 100 percent agreement with the two reference laboratories. This validates the test conditions, procedures and equipment used in ESR's Arbovirus Reference Laboratory.

Diagnostic evaluation was conducted using 1208 blood donor samples. All WNV IgG positive samples were forwarded to ICPMR for parallel testing with a confirmation rate of 83.3 percent (20/24). Focus IgG and IgM ELISA can serve only as initial primary screening tests. ESR intends to develop a battery of ELISA or IFA based tests as does ICPMR, coupled with PRNT in order to provide a definitive diagnosis for WNV. Before this new capability is established at ESR, we will continue to utilise overseas arbovirus reference laboratories.

To our knowledge, this is the first study that has been undertaken to examine the presence of WNV antibodies in New Zealand blood donors. This increases New Zealand's capability in response to WNV importation. The validation of these assays allows ESR to offer these tests to the hospital and community laboratories.

There was no significant difference in seroprevalence between participants (1.3 percent; CI:0.4-2.2) who had travelled within 12 months prior to blood donation and participants (0.69 percent; CI:0.01-1.4) who had not travelled. All WNV IgG positive blood donors were IgM negative. This suggests that these blood donors had been exposed to a WNV-like virus in the past, that they had had no recent infection and were not viraemic. There appears to be a low risk of WNV entry through travellers from WNV endemic areas who donate blood in New Zealand.

The current procedures employed by the NZ Blood Service appear to be effective in reducing the risk of transfusion-transmitted WNV infection in New Zealand.

The seroprevalence among different age groups of blood donors was very similar. This finding was similar to the results of a household-based seroepidemiological survey in New York⁷ although hospital-based surveillance for severe complications indicated a higher WNV incidence in people aged 65 years or older than in other age groups. For example, one meningoencephalitis case occurred per 50 infections among people aged 65 years or more compared with about one per 300 among those younger than 65 years.

Asian and Pacific peoples appeared to have a higher seroprevalence than Europeans and Maori, although there were only a small number of Asian and Pacific participants in this study which may reduce the statistical power. This finding may also be explained by diagnostic test characteristics. Among these five positive Asian cases, four had both WNV and Dengue IgG antibodies. WNV and Dengue are antigenically closely related flaviviruses, the assays performed at ICPMR are not able to differentiate between WNV and Dengue. It is possible that these four Asian participants were exposed to Dengue-like virus with a high prevalence in the Asian region. In order to obtain a final definitive diagnosis, these samples will be referred to a reference laboratory that performs the Plaque Reduction Neutralisation Assay for both WNV and Dengue, the gold standard confirmatory test with the highest specificity.

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