



Supporting Information

Supplementary methods and results

**This appendix was part of the submitted manuscript and has been peer reviewed.
It is posted as supplied by the authors.**

Appendix to: Baldwin Z, Hueston L, Roberts-Witteveen A, et al. The seroprevalence of antibodies to Japanese encephalitis virus in five New South Wales towns at high risk of infection, 2022: a cross-sectional serosurvey. *Med J Aust* 2024; doi: 10.5694/mja2.52320.

1. Consent form and questionnaire

Consent form

PARTICIPANT INFORMATION SHEET

Research Project Title: Japanese encephalitis serosurvey

Investigators:

NSW Health

NSW Health Pathology

National Centre for Immunisation Research and Surveillance (NCIRS)

We invite you and your family participate in a voluntary surveillance study conducted by NSW Health and the National Centre for Immunisation Research and Surveillance to help us understand more about Japanese Encephalitis in the area. Your participation involves a once-off blood sample and completion of a short questionnaire.

What is this study about?

Japanese encephalitis virus (JEV) has recently been found in New South Wales for the first time.

As most people infected with the virus will show no or only minor symptoms, NSW Health would like to understand how many people have been exposed to the virus in this area. This is important so we can understand the spread of the virus in New South Wales, and ensure that vaccine is made available to those at most risk of exposure.

What is Japanese encephalitis?

Japanese encephalitis is a disease caused by the Japanese Encephalitis virus (JEV), which is transmitted by mosquitoes. It is a common infection in the tropical regions of Asia, and was detected for the first time in NSW in late February 2022. The virus can also infect other animals, such as birds and pigs.

We do not know how this virus became present in NSW, but it is likely the warm and wet conditions contributed to increased mosquito breeding and transmission. NSW Health is collaborating with animal and wildlife specialists to understand more about how the virus arrived in NSW.

What happens when you get JEV?

Most people with JEV have no symptoms. In 1 out of 250 people infected, it can cause a severe brain infection (encephalitis), which can lead to severe and permanent disability. In countries throughout Asia, children are reported to be particularly susceptible to this brain infection, but in places such as Australia where adults have no previous exposure to the disease, all ages are susceptible to severe infection. More information can be found at <http://www.health.nsw.gov.au/jevirus>

Why is this important?

It is important to understand JEV infection rates because it gives us a clue on how long ago the virus was introduced into New South Wales, how many people are infected and what behaviours or activities increase the risk of infection. It also helps us understand which communities and/or community groups should be prioritised for vaccination. This is important since mosquito rates are likely to increase again in the spring and summer time, and it is possible that JEV may return.

What are we asking for?

We will take a small amount of blood (approximately 1 teaspoon) from you and/or your family member and test it for antibodies in a reference laboratory in Sydney. Antibodies are developed when you have been exposed to the virus or the vaccine.

We will also ask you to complete a survey. The survey will ask questions around exposure to animals, mosquitos, vaccination and travel to countries where JEV infection is more common.

How is testing performed?

The test is performed at the NSW reference laboratory for arbovirus infections, the Institute of Clinical Pathology and Medical Research (ICPMR) at Westmead, part of Health Pathology NSW. The laboratory will screen your sample for antibodies to any arboviruses. If this is negative, you will be informed of your negative result in writing (email or SMS).

If this screening test is positive, further tests will need to be performed to identify whether the antibodies are specific to Japanese Encephalitis or another type of arbovirus infection (Barmah Forest virus and Ross River virus are common examples in NSW). A high positive to JEV antibodies indicates prior infection, and therefore immunity to infection. These tests can take some time. You will be informed of your positive result and the interpretation of the antibody test by phone.

What will happen with the results?

The results will be pooled and published together. No individual results will be published on its own. We will provide you with a summary of the published results once it's available. Your privacy and confidentiality will be protected under the the Health Records and Information Privacy Act, 2002.

What do I get out of participating?

Participants will informed of their results of the testing. This will help you identify if you have acquired immunity from exposure, or are naïve to infection. The clinic will also provide an opportunity to discuss questions about Japanese Encephalitis virus in NSW public health professionals.

By signing this form I confirm:

I have read, or had read to me in my first language, the information above and I understand its contents. I believe I understand the purpose, extent and possible risks of my involvement in this project. I voluntarily consent to take part in this research project. I have had an opportunity to ask questions and I am satisfied with the answers I have received. I understand that this project has been approved by The Sydney Children's Hospital Human Research Ethics Committee and will be carried out in line with the National Statement on Ethical Conduct in Human Research (2007).

If you have any concerns about the conduct of this serosurvey, please do not hesitate to contact the Health Protection Zoonoses team at nsw-enteric@health.nsw.gov.au.

Is the participant under 16 years.

Yes
 No

Name of parent/guardian:

Minor's Signature

Signature

Today's date

Paper Consent

Personal Details

Thank you for providing some information about yourself, your travel history, and potential exposures you may have had to Japanese Encephalitis Virus. Your participation in this questionnaire is voluntary however the information collected, together with the result from your blood test, will allow us to study and better understand the risk for Japanese Encephalitis and inform how we can best protect others in the future. We thank you for your participation.

Record ID is

JEVSS-[site_name:value]00[record_id:value].

Do you live within the township of [site_name] or within 5kms of town?

- Yes
 No

Do you work within the township or within 5kms of town?

- Yes
 No

How many days a week do you work?

- 1
 2
 3
 4
 5
 6
 7

What is your employment status?

- Retired
 Not currently studying or working
 Full time student (not working)
 Volunteer
 Employed full time
 Employed part time or casual
 Student and currently employed in workforce
 Self employed

First name

Last name

Date of birth (DD-MM-YYYY)

Age in years

Parent full name

Please add parent/guardian full name here if participant is under 16 years or if parent is completing the questionnaire.

Gender

- Male
 Female
 Other
 Prefer not to say

Do you Identify as being of Aboriginal or Torres Strait Islander origin?

- Aboriginal
 Torres Strait Islander
 Aboriginal and Torres Strait Islander
 Neither
 Prefer not to say

Residential address

Property Name: (if applicable)

House/Unit number

Street name

Street Type e.g. street, avenue

- Alley
 Arcade
 Avenue
 Boulevard
 Bypass
 Circuit
 Close
 Corner
 Court
 Crescent
 Cul-de-sac
 Drive
 Esplanade
 Green
 Grove
 Highway
 Junction
 Lane
 Link
 Mews
 Parade
 Place
 Ridge
 Road
 Right of way (ROW)
 Square
 Street
 Terrace
 Way
 Circle
 Parkway

Suburb

State

Postcode

Have you lived at this address for more than 6 months?

- Yes
 No

Previous house/unit number _____

Previous street name _____

Street Type e.g. street, avenue

- Alley
- Arcade
- Avenue
- Boulevard
- Bypass
- Circuit
- Close
- Corner
- Court
- Crescent
- Cul-de-sac
- Drive
- Esplanade
- Green
- Grove
- Highway
- Junction
- Lane
- Link
- Mews
- Parade
- Place
- Ridge
- Road
- Right of way (ROW)
- Square
- Street
- Terrace
- Way
- Circle
- Parkway

Previous suburb _____

Previous postcode _____

Contact phone number _____

(Please enter phone number without spaces including area code if landline)

Email address _____

GP contact information

Would you like a copy of your results sent to your GP? Yes
 No

GP name _____

GP Practice _____

House/Unit number _____

Street name _____

Street Type e.g. street, avenue

- Alley
- Arcade
- Avenue
- Boulevard
- Bypass
- Circuit
- Close
- Corner
- Court
- Crescent
- Cul-de-sac
- Drive
- Esplanade
- Green
- Grove
- Highway
- Junction
- Lane
- Link
- Mews
- Parade
- Place
- Ridge
- Road
- Right of way (ROW)
- Square
- Street
- Terrace
- Way
- Circle
- Parkway

Suburb _____

Postcode _____

GP phone number (if known)

 (Please enter phone number without spaces including area code if landline)

Background information

Were you born in Australia?

- Yes
- No

Country of birth

- Adelle Land (France)
- Afghanistan
- Aland Islands
- Albania
- Algeria
- Andorra
- Angola
- Anguilla
- Antigua and Barbuda
- Argentina
- Armenia
- Aruba
- Austria
- Azerbaijan
- Bahamas
- Bahrain
- Bangladesh
- Barbados
- Belarus
- Belgium
- Belize
- Benin
- Bermuda
- Bhutan
- Bolivia
- Sint Eustatius and Saba
- Bosnia and Herzegovina
- Botswana
- Brazil
- British Antarctic Territory
- Brunei Darussalam
- Bulgaria
- Burkina Faso
- Burundi
- Cabo Verde
- Cambodia
- Cameroon
- Canada
- Cayman Islands
- Central African Republic
- Chad
- Chile
- Chilean Antarctic Territory
- China (excludes SARs and Taiwan)
- Colombia
- Comoros
- Democratic Republic of
- Cook Islands
- Costa Rica
- Cote d'Ivoire
- Croatia
- Cuba
- Curacao
- Cyprus
- Czechia
- Denmark
- Djibouti
- Dominica
- Dominican Republic
- Ecuador
- Egypt
- El Salvador
- England
- Equatorial Guinea
- Entrea
- Estonia
- Eswatini
- Ethiopia
- Falkland Islands projectredcap.org

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- Faroe Islands
- Fiji
- Finland
- France
- French Guiana
- French Polynesia
- Gabon
- Gambia
- Gaza Strip and West Bank
- Georgia
- Germany
- Ghana
- Gibraltar
- Greece
- Greenland
- Grenada
- Guadeloupe
- Guam
- Guatemala
- Guernsey
- Guinea
- Guinea-Bissau
- Guyana
- Haiti
- Holy See
- Honduras
- Hong Kong
- Hungary
- Iceland
- India
- Indonesia
- Iran
- Iraq
- Ireland
- Isle of Man
- Israel
- Italy
- Jamaica
- Japan
- Jersey
- Jordan
- Kazakhstan
- Kenya
- Kiribati
- Kosovo
- Kuwait
- Kyrgyzstan
- Laos
- Latvia
- Lebanon
- Lesotho
- Liberia
- Libya
- Liechtenstein
- Lithuania
- Luxembourg
- Macau
- Madagascar
- Malawi
- Malaysia
- Maldives
- Mali
- Malta
- Marshall Islands
- Martinique
- Mauritania
- Mauritius
- Mayotte
- Mexico
- Federated States of
- Moldova

- Monaco
- Mongolia
- Montenegro
- Montserrat
- Morocco
- Mozambique
- Myanmar
- Namibia
- Nauru
- Nepal
- Netherlands
- New Caledonia
- New Zealand
- Nicaragua
- Niger
- Nigeria
- Niue
- Norfolk Island
- North Korea
- North Macedonia
- Northern Ireland
- Northern Mariana Islands
- Norway
- Oman
- Pakistan
- Palau
- Panama
- Papua New Guinea
- Paraguay
- Peru
- Philippines
- Pitcairn Islands
- Poland
- Polynesia (excludes Hawaii), nec
- Portugal
- Puerto Rico
- Qatar
- Queen Maud Land (Norway)
- Reunion
- Romania
- Ross Dependency (New Zealand)
- Russian Federation
- Rwanda
- Samoa
- American
- San Marino
- Sao Tome and Principe
- Saudi Arabia
- Scotland
- Senegal
- Serbia
- Seychelles
- Sierra Leone
- Singapore
- Sint Maarten (Dutch part)
- Slovakia
- Slovenia
- Solomon Islands
- Somalia
- South Africa
- South Korea
- South Sudan
- Spain
- Spanish North Africa
- Sri Lanka
- St Barthelemy
- St Helena
- St Kitts and Nevis
- St Lucia
- St Martin (French part)
- St Pierre and Miquelon

- St Vincent and the Grenadines
- Sudan
- Suriname
- Sweden
- Switzerland
- Syria
- Taiwan
- Tajikistan
- Tanzania
- Thailand
- Timor-Leste
- Togo
- Tokelau
- Tonga
- Trinidad and Tobago
- Tunisia
- Turkey
- Turkmenistan
- Turks and Caicos Islands
- Tuvalu
- Uganda
- Ukraine
- United Arab Emirates
- United States of America
- Uruguay
- Uzbekistan
- Vanuatu
- Venezuela
- Vietnam
- Virgin Islands, British
- Virgin Islands, United States
- Wales
- Wallis and Futuna
- Western Sahara
- Yemen
- Zambia
- Zimbabwe

Year of arrival in Australia

Have you lived or travelled, for longer than 1 month total, in any of the following countries? (Select all that apply)

- China
- Vietnam
- Philippines
- India
- Indonesia
- Nepal
- Sri Lanka
- Thailand
- Taiwan
- Papua New Guinea
- None of the above

Have you ever been vaccinated for Japanese encephalitis before? This vaccine is often offered to people who plan to travel overseas to Asia (it is not routinely given at childhood in Australia).

- Yes
- No
- Unsure

Have you ever been vaccinated for yellow fever? This vaccine is often offered to people who plan to travel to Africa, South America & Central America (it is not routinely given at childhood in Australia).

- Yes
- No
- Unsure

Have you ever been diagnosed with the following mosquito-related infections?
(Select all that apply)

- Murray Valley encephalitis virus
- Kunjin virus
- West Nile virus
- Yellow fever
- Dengue fever
- Chikungunya virus
- Zika virus
- St Louis encephalitis virus
- Barmah forest virus
- Ross River virus
- None of the above
- Unsure

Information on locations of possible exposures to mosquitoes

When responding to the exposure questions please consider the time from the 1st of December 2021 until today.

Home

Between 1 December 2021 to now have you lived or visited a farm in the [site_name] area? Yes
 No

Between 1 December 2021 to now did you keep any of the following animals on your property/backyard Pigs
 Sheep
 Horses
 Poultry
 Cattle
 None

Between 1 December 2021 to now, have you lived within 5km of a piggery? Yes
 No
 Unsure

Between 1 December 2021 to now, have you lived within 5km of any bodies of water? (Select all that apply) Dams/Lakes
 Outdoor pools (public or private)
 Irrigation systems
 Wetlands
 Rivers/Creeks
 None

Around your home is there standing water (e.g. watering cans, flowerpots, bird bath, open water container)? Never
 Some of the time
 Most of the time
 All of the time

Between 1 December 2021 to now do you recall being bitten by mosquitoes in or around your home? Yes
 No
 Unsure

Work or school

Between 1 December 2021 to now have you worked on a farm? Yes
 No

Between 1 December to now, have you worked with or in close proximity to pigs? Yes
 No

Between 1 December to now, have you worked with or spent time in close proximity to waterbirds? Yes
 No

Between 1 December to now, have you spent time in close proximity to a large amount of wildlife birds? Yes
 No

Between 1 December to now, have you worked with animals (excluding birds and pigs)? Yes
 No

Within work or school hours, how long would you spend outdoors in an average day?

< 2 hours
 Between 2-4 hours
 Between 4-8 hours
 > 8 hours

As part of work or school activities do you spend time outdoors during dawn and dusk?

Yes
 No

Between 1 December 2021 to now do you recall being bitten by mosquitoes at work or school?

Yes
 No
 Unsure

Recreation activities / other locations

Do you participate in the following activities between December and April?
(Select all that apply)

Hunting
 Fishing (fresh water)
 Camping
 Bushwalking/hiking
 Watersports
 Gardening/Horticulture
 Picnicking
 Outdoor sports & leisure e.g. football, horse-riding, golf etc.
 None of the above

How often do you participate in hunting?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in fishing (fresh water)?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in camping?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in bushwalking/hiking?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in watersports?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in gardening/horticulture?

A few times a year
 A few times a month
 A few times a week
 Daily

How often do you participate in picnicking?	<input type="radio"/> A few times a year <input type="radio"/> A few times a month <input type="radio"/> A few times a week <input type="radio"/> Daily
How often do you participate in outdoor sports?	<input type="radio"/> A few times a year <input type="radio"/> A few times a month <input type="radio"/> A few times a week <input type="radio"/> Daily
Outside of work or school activities, how long would you spend outdoors in an average day?	<input type="radio"/> < 2 hours <input type="radio"/> Between 2-4 hours <input type="radio"/> Between 4-8 hours <input type="radio"/> > 8 hours
As part of your home duties or leisure activities, do you spend time outdoors during dawn or dusk?	<input type="radio"/> Yes <input type="radio"/> No
Between 1 December 2021 to now do you recall being bitten by mosquitoes when participating in recreation activities?	<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unsure

Measures taken to protect yourself from mosquitoes

During summer, how often do you wear clothing to protect against mosquitoes when you go outside (ie. long sleeves/pants?)	<input type="radio"/> Never <input type="radio"/> Some of the time <input type="radio"/> Most of the time <input type="radio"/> All of the time
How regularly do you use insect repellent when exposed to mosquitos?	<input type="radio"/> Never <input type="radio"/> Some of the time <input type="radio"/> Most of the time <input type="radio"/> All of the time
Does your home have insect screens on all or some windows/doors?	<input type="radio"/> All <input type="radio"/> Some <input type="radio"/> None
Have you had any of the following symptoms since 1 December 2021 that remained unexplained?	<input type="checkbox"/> Fever <input type="checkbox"/> Headache <input type="checkbox"/> Rash <input type="checkbox"/> Joint pain <input type="checkbox"/> Fatigue <input type="checkbox"/> I did not have any of the above that remain unexplained
Approximately what month did your symptoms occur?	<input type="checkbox"/> December 2021 <input type="checkbox"/> January 2022 <input type="checkbox"/> February 2022 <input type="checkbox"/> March 2022 <input type="checkbox"/> April 2022 <input type="checkbox"/> May 2022 <input type="checkbox"/> June 2022

Table. Exposure risk factors and prevalence odds ratios, in five towns in New South Wales, June and July 2022

Risk Factor	JEV antibody-positive	JEV antibody negative	Prevalence odds ratio (95% CI%)
Home exposures			
Live on farm	47/80 (58.8%)	519/837 (62.0%)	0.9 (0.5-1.4)
Keep pigs	1/80 (1.3%)	17/837 (2.0%)	0.6 (0.01-4.0)
Keep sheep	12/80 (15.0%)	114/837 (13.6%)	1.1 (0.5-2.2)
Keep horses	10/80 (12.5%)	61/837 (7.3%)	1.8 (0.8-3.8)
Keep poultry	16/80 (20.0%)	125/837 (14.9%)	1.4 (0.7-2.6)
Keep cattle	5/80 (6.3%)	73/837 (8.7%)	0.8 (0.2-1.8)
No animals kept	49/80 (61.3%)	569/837 (68.0%)	0.7 (0.5-1.2)
Live nearby piggery	6/80 (7.5%)	109/837 (13.0%)	0.5 (0.2-1.3)
Live near dams/lakes	28/80 (35.0%)	344/837 (41.1%)	0.8 (0.5-1.3)
Live near outdoor pools	24/80 (30.0%)	274/837 (32.7%)	0.9 (0.5-1.5)
Live near irrigation systems	20/80 (25.0%)	253/837 (30.2%)	0.8 (0.4-1.3)
Live near wetlands	16/80 (20.0%)	137/837 (16.4%)	1.3 (0.7-2.3)
Live near rivers/creeks	36/80 (45.0%)	371/837 (44.3%)	1.0 (0.6-1.7)
No water body nearby	19/80 (23.8%)	109/837 (13.0%)	2.1 (1.1-3.7)
Standing water around the home	67/80 (83.8%)	698/837 (83.4%)	1.0 (0.5-2.1)
Mosquito bites around the home	60/80 (75.0%)	663/837 (79.2%)	0.8 (0.5-1.4)
Work or school exposures*			
Work at farm	16/55 (29.1%)	192/700 (27.4%)	1.1 (0.6-2.0)
Work at piggery	0/55 (0.0%)	44/700 (6.3%)	0.0 (0.0-1.1)
Work with/near waterbirds	9/55 (16.4%)	122/700 (17.4%)	0.9 (0.4-2.0)
Work with/near other birds	9/55 (16.4%)	123/700 (17.6%)	0.9 (0.4-1.9)
Work with animals other than pigs/birds	12/55 (21.8%)	196/700 (28.0%)	0.7 (0.3-1.4)
Work outdoors ≥ 4 hours per day	13/55 (23.6%)	197/700 (28.1%)	0.8 (0.4-1.5)
Work outside at dawn or dusk	23/55 (41.8%)	376/700 (53.7%)	0.6 (0.3-1.1)
Mosquito bites at work	29/55 (52.7%)	362/700 (51.7%)	1.0 (0.6-1.9)
Recreation exposures			
Hunting	4/80 (5.0%)	54/837 (6.5%)	0.8 (0.2-2.2)
Fishing	15/80 (18.8%)	165/837 (19.7%)	0.9 (0.5-1.7)
Camping	12/80 (15.0%)	228/837 (27.2%)	0.5 (0.2-0.9)
Bushwalking	23/80 (28.8%)	267/837 (31.9%)	0.9 (0.5-1.5)
Water sports	8/80 (10.0%)	114/837 (13.6%)	0.7 (0.3-1.5)
Gardening	51/80 (63.8%)	512/837 (61.8%)	1.1 (0.7-1.9)
Picnicking	9/80 (11.3%)	183/837 (21.9%)	0.5 (0.2-0.9)
Outdoor sports	21/80 (23.3%)	311/837 (37.2%)	0.6 (0.3-1.0)
Leisure activities outdoors ≥ 4 hours per day	22/80 (27.5%)	194/837 (23.2%)	1.3 (0.7-2.2)
Leisure activities outside at dawn or dusk	62/80 (77.5%)	661/837 (79.0%)	0.9 (0.5-1.7)
Mosquito bites during leisure activity	55/80 (68.8%)	562/837 (67.1%)	1.1 (0.6-1.8)
Any outdoor recreation activity	66/80 (82.5%)	712/837 (85.1%)	0.8 (0.4-1.7)
Protective behaviours			
Clothing	53/80 (66.3%)	633/837 (75.6%)	0.6 (0.4-1.1)
Repellent	55/80 (68.8%)	535/837 (63.9%)	1.2 (0.7-2.1)
Insect screens	15/80 (18.8%)	183/837 (21.9%)	0.8 (0.4-1.5)
No protective measures	1/80 (1.3%)	2/837 (0.2%)	5.3 (0.1-102)

CI = confidence interval; JEV = Japanese encephalitis virus.

* Participants who reported they were retired were not asked about work or school exposures.