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# The detection of Japanese encephalitis virus (JEV) in the Murray region, New South Wales: a public health investigation

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# The detection of Japanese encephalitis virus (JEV) in the Murray region, New South Wales: a public health investigation

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# Abstract

The detection of Japanese encephalitis virus (JEV) in pigs, at four piggeries in the Murray region in February 2022, prompted a public health investigation (PHI) by the New South Wales Department of Health (NSW Health) to identify people at greatest risk of infection. The PHI included three components: a vaccination clinic and accompanying clinic questionnaire; a serological investigation; and a cross-sectional study for consenting Australian-born participants who completed an extended questionnaire after receiving their serological results. The goals were to vaccinate a presumably naïve population to reduce associated risk and to understand the seroprevalence among Australian-born piggery workers.

A total of 322 farm workers and/or residents attended clinics organised by NSW Health; 311 received a JEV vaccine (96.6%); and 302 (94%) completed a clinic questionnaire. Of 178 people from whom serology was collected (55.3%), a total of 165 returned Defined Epitope Blocking enzyme-linked immunosorbent assay (DEB ELISA) results; 153/165 of those returning DEB ELISA results were Australian born. The study's cross-sectional component involved 129 participants, ten of whom were seropositive.

The overall seropositivity for 153 Australian-born participants across the identified piggeries was 6.5% (95% confidence interval [95% CI]: 3.4–12.0%), suggesting that JEV was circulating in piggeries, and plausibly more broadly, within the Murray region prior to serology collection. Male sex and working on, or visiting, a farm other than their regular workplace were both associated with JEV seropositivity (odds ratio [OR]: 5.4; 95% CI: 0.94–137.1 and OR: 37; 95% CI: 0.92–22.08 respectively).

JEV vaccination uptake was high among piggery workers in the Murray region. Further studies are needed to determine if piggery workers have an increased risk of developing JEV compared to people who do not work or live on JEV-affected piggeries. The reasons for the emergence of JEV in pigs in the Murray region remain unclear.

Keywords: emerging diseases; infectious diseases; Japanese encephalitis; flaviviruses; mosquito; cross-sectional study; vaccines; sero-survey; rapid investigation

# Introduction

Japanese Encephalitis Virus (JEV) belongs to the *Orthoflavivirus* genus<sup>1</sup> within the *Flaviviridae* family,<sup>2,3</sup> and is most closely related to other flaviviruses such as Murray Valley encephalitis virus (MVEV), Alfuy virus (ALFV), St Louis encephalitis virus (SLEV) and West Nile virus (WNV), including Kunjin virus (KUNV).<sup>2</sup>

Many infections are asymptomatic or mild, with only one in 100 cases thought to develop encephalitic neurological illness, and this can lead to the underreporting of JEV cases globally, as most cases are reported with severe clinical symptoms of JEV.<sup>4-6</sup> Severe symptoms include neck stiffness, paralysis, mental status changes such as disorientation, movement disorders and light sensitivity.<sup>7,8</sup> Treatment is through supportive therapies only.

JEV is maintained through an enzootic cycle between mosquito vectors and reservoir hosts.<sup>2,9</sup> The major Australian JEV vector is *Culex annulirostris*, but other species are also thought to be capable vectors.<sup>10,11</sup> The virus is transmitted through the bite of an infected mosquito.9 Humans and horses are 'incidental deadend' hosts: mosquitoes cannot be infected by biting an infected human or horse, due to their low-level and brief viraemia.<sup>10,12</sup> Therefore, human-to-human transmission is extremely unlikely. The role of wading birds in transmitting JEV in Australia is considered important, but difficult to quantify due to challenges in testing wild birds. Amplifying hosts, such as pigs, play an important role in viral transmission in many countries, although in Australia, their role in transmission is not yet well understood.<sup>12</sup>

JEV causes endemic disease and is the leading cause of encephalitis in countries including China, Philippines, and other parts of Asia.<sup>13</sup> In endemic countries, JEV is mostly detected in children in proximity to rice and/or pig farming.<sup>2,12,14</sup> Most adults in these areas have acquired immunity either through vaccination or natural infection.<sup>15,16</sup> In other countries, cases are sporadic and often imported by travellers.

The World Health Organization (WHO) has recommended JEV vaccination programs in countries where JEV poses a risk to public health. In endemic countries, vaccination programs primarily target children.<sup>14</sup> Fifteen countries in Asia have national or subnational JEV public vaccination programs, including the outer Torres Strait Islands of Australia.<sup>17</sup> Four JEV vaccine classes are currently available worldwide. A live vaccine (Imojev) and an inactivated vaccine (JEspect)<sup>10,18</sup> are approved for use in Australia. Both have high immunogenicity and a low incidence of adverse events.<sup>10,18</sup> Prior to recognition of locally acquired cases in Australia, JEV vaccination was recommended for Australians travelling to known endemic countries for a month or longer; for laboratory workers with potential occupational exposure to JEV; and for people living or working on the outer islands of the Torres Strait.<sup>18</sup>

Prior to 2021, JEV had never been detected in southern mainland Australia. Two previous outbreaks had been detected in the relatively sparsely populated very northern tip of Australia in 1995 and 1998. The 1995 outbreak resulted in three confirmed human cases of JEV; the 1998 outbreak resulted in five cases with two deaths, and was attributed to cyclonic-wind-transported mosquitoes from Papua New Guinea into the Torres Strait.<sup>10,19</sup> Subsequent sentinel pig surveillance and mosquito trapping since 1998 indicated that JEV was now endemic in the Torres Strait.<sup>20</sup>

JEV became nationally notifiable in Australia in 2001.<sup>21</sup> Fifteen cases were reported in Australia from 2012 to early 2022, of whom 14 were returned overseas travellers.<sup>21,22</sup> One case was locally acquired in the Tiwi Islands of the Northern Territory. No locally-acquired human cases had previously been detected in the south-eastern areas of Australia, including in New South Wales, prior to 2022.<sup>23</sup>

In February 2022, JEV was detected in pigs at six piggeries in New South Wales,<sup>24</sup> including at four piggeries in the Murray region. Investigations into the increased numbers of pig abortions and deaths in many areas<sup>25</sup> resulted in 70 affected farms being identified across the Australian states of Victoria, South Australia, Queensland, and New South Wales by the end of 2022.<sup>26</sup>

Investigations into humans with encephalitis and other clinically consistent illnesses since January 2021 resulted in the detection of 45 human infections in Australia across five jurisdictions (as of June 2023).<sup>27</sup> Eight confirmed JEV cases were residents of, and exposed within, the Murray region of southern New South Wales, with onsets from December 2021 to February 2022.<sup>28</sup> JEV was declared a Communicable Disease of National Significance on 4 March 2022.<sup>29</sup> On 24 March 2022, the Australian Government Department of Health and Aged Care recommended and funded JEV vaccine for people with direct exposure or proximity to pigs and mosquitoes, and/or high-level occupational exposures, in addition to the existing eligible groups.<sup>29</sup>

#### **Aims and objectives**

The primary goal of this public health investigation (PHI) was to urgently vaccinate workers and residents onsite at Murray region piggeries where JEV had been detected. Through additional opportunistic study components, we were also able to explore the prevalence of JEV among Australian-born piggery workers and/or residents and the characteristics of sero-positive cases.

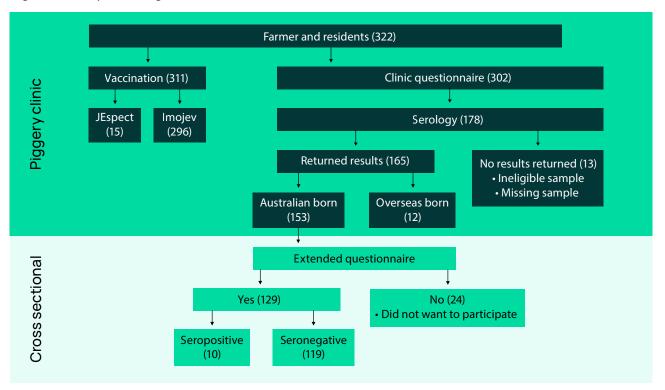
# Methods

This PHI was conducted in March 2022 after notification from the New South Wales Department of Primary Industries (DPI) to the New South Wales Department of Health (NSW Health) on the detection of JEV in pigs and sows of local piggeries. The investigation had three components: vaccination against JEV, serological investigation collection, and a cross-sectional study (Figure 1). This PHI was conducted as part of the public health response under the *Public Health Act, 2010* (NSW) to control the risk to public health by the new local detection of a notifiable condition.<sup>25</sup> The Greater Western Human Research Ethics Committee reviewed the study protocol and was satisfied with the Chief Health Officer (CHO) authorisation provided by the investigation order.<sup>30</sup>

#### **Study population (enrolment)**

Workers and contractors at identified local piggeries in the Murray region were eligible to participate because of the risk of occupational exposure to JEV. Staff had varied roles, including working directly with pigs, administrative, and office-based roles. Non-worker residents at these farms were also eligible to participate. The study population was the piggery workers and/or residents who attended NSW Health-organised vaccination clinics. The clinic questionnaire participation rate was 94%. Previous JEV vaccination was not an exclusion criterion for vaccination at the clinic. Convenience sampling from those attending the vaccination clinics provided participants for the JEV serology and crosssectional components.

#### Figure 1: Study flow diagram, March 2022



#### **JEV vaccination**

Written consent for vaccination was obtained for all participants (via a guardian for children aged less than 18 years). Consenting participants completed a self-administered clinic questionnaire. The clinic questionnaire collected information on each participant's demography, travel history, symptoms, pregnancy status and workplace history.

Two clinics offering vaccination and serology collection were held by NSW Health for the JEV infected properties. The first clinic was held at a local hospital on 8 March 2022, and the second clinic was held onsite at a piggery on 10 March 2022. JEV vaccination was administered by a medical doctor or by a nurse under the order of a medical officer. Attending medical practitioners screened consenting participants for contraindications and documented vaccination administration on signed participant consent forms. Vaccines were administered following the Australian Immunisation Handbook Guidelines; participants were monitored for 15 minutes for adverse events following vaccine administration.<sup>18</sup>

#### **JEV** serology

The serological component of the study was open to any who attended the clinics. In addition to the introduction letter, for convenience sampling, those who consented for serology also completed a clinic questionnaire (via a guardian in children aged less than 18 years).

Participants aged over 18 years had 8 mL whole blood collected prior to vaccine administration. Samples were refrigerated before transportation to the New South Wales arboviral reference laboratory, the Arbovirus Emerging Diseases Unit at the Institute of Clinical Pathology and Medical Research (ICPMR). At ICPMR, participant samples were screened using an in-house Defined Epitope Blocking (DEB) enzyme-linked immunosorbent assay (ELISA) for JEV and other flaviviruses. Positive samples were then screened for MVEV and KUNV also using DEB ELISA techniques. Positive samples also underwent retesting in titration series using the DEB ELISAs and had immunoglobulin M (IgM) analysis performed using immunofluorescence assay (IFA) techniques. Participants returning positive results by DEB ELISA, but negative IgM, were reported as having evidence of past infection or vaccination. Those positive by DEB ELISA and positive IgM were reported as having recent infection or vaccination.

Participants were notified of their serological results by a medical practitioner or a public health officer via telephone in April 2022. Uncontactable participants were re-contacted over a period of two weeks and provided with their results.

#### **Cross-sectional study**

The cross-sectional study comprised consenting Australian-born unvaccinated workers and/or residents who had serology collected. After receiving their serological results, participants were invited to complete an extended questionnaire to collect further risk exposures (including occupation, leisure activities and mosquito protection) retrospectively for December 2021 to March 2022.

#### **Data analysis**

Univariate statistics were computed to describe the distribution of the clinic attendees by selected sociodemographic characteristics, JEV vaccination status (history and administration) and risk exposures; and to determine the seroprevalence of JEV among the Australian-born clinic attendees. The statistics included means and proportions with associated 95 percent confidence intervals (95% CI). Prevalence calculations excluded anyone who was born in, or who reported ever travelling to a JEV endemic country, and/or anyone who reported previous yellow fever or JEV vaccination. Selected socio-demographic and risk exposures were analysed among clinic attendees with or without JEV sero-positive test results using bivariate analysis; the differences were assessed by Fisher's Exact test with a statistical significance threshold of 0.05. The un-adjusted prevalence odds ratios (POR) were calculated to estimate association between JEV seropositivity and the risk exposures. Bivariate analyses comprised those participants who were Australian born, provided samples for JEV serology and completed the extended questionnaire. Data analyses were performed in R-Studio,<sup>i</sup> version 4.02, with the package epitools.<sup>ii</sup>

i https://posit.co/products/open-source/rstudio/.

ii https://epitools.ausvet.com.au/.

# Results

Three hundred and twenty-two piggery workers and/ or residents presented to NSW Health-organised clinics from the affected piggeries for vaccination and/or serological investigation.

## Vaccination

Three hundred and eleven participants (96.6%) across all four farms received a JEV vaccine. Fifteen (4.8%) received JEspect and 296 (95.2%) had Imojev (Figure 1). Four respondents (1.3%) reported previous JEV vaccination.

## **Clinic questionnaire**

Clinic questionnaires were completed by 302/322 workers and residents (93.8%; Table 1). Among clinic questionnaire respondents, 270 were Australian born (89.4%); of these, four identified as being of Aboriginal and/or Torres Strait Islander origin. Most respondents (208/302; 69.7%) resided in the state of New South Wales. The Philippines was the most reported country of birth (n = 13) for the 32 overseas born participants. A majority of respondents (195/302; 64.6%) were in the 25–54 years age group; the median age was 45 years (range 3–75 years). Males predominated (204/302; 67.5%).

# Serological

Within the study population, 178/322 participants (55.3%) had sera collected; however, there were only 165 returned serological results among all participants (Figure 1). Thirteen samples did not return results, as they were either ineligible or were not received by the laboratory. Of those 165 workers and/ or residents, 153 were Australian born (Figure 1). The JEV sero-prevalence among Australian-born participants was 6.5% (95% CI: 3.4–12.0%).

## **Risk factor analysis**

Of the 153 Australian-born participants who had serological results, 129 verbally consented to complete the extended questionnaire. The average age of extended questionnaire respondents was 43.5 years (95% CI: 41.1–46.0 years). Among the respondents, 87/129 (67.4%) lived in NSW (95% CI: 60.4–76.6%) and 80/129 (62.0%) were male (95% CI: 53.6–70.4%).

Ten of the extended questionnaire respondents (7.8%) had JEV-positive serology (Table 2; Appendix A, Table A.1). No risk exposures had PORs reaching statistical significance, although elevated PORs were found for those who worked on or visited a farm other than the workplace piggery and for those who were male (Table 2).

	Numberv	vaccinated		from who as collected		ompeting stionnaire	Number attending
Farm	n	% <sup>a</sup>	n	% <sup>a</sup>	n	% <sup>a</sup>	vaccination clinic
Farm A	8	100.0	1	12.5	1	12.5	8
Farm B	23	92.0	18	72.0	23	92.0	25
Farm C	275	96.8	155	54.6	274	96.5	284
Farm D	5	100.0	4	80.0	4	80.0	5
Total	311	96.6	178	55.3	302	93.8	322

 Table 1: Total vaccines administered, serology collection, clinic questionnaires completed for attendees at Murrumbidgee Local Health District JEV clinics, 2022

a Denominator for percentages is the number attending the vaccination clinic from the indicated farm(s).

Table 2: Summary of selected risk factors of in any setting, at work and recreationally/residentially in Australian-born participants with prevalence odds ratios, and *p*-values, March 2022

		Seropositive (N =10)	sitive 10)	Seronegative (N = 119)	gative 119)			
Setting type		c	%	c	%	POR <sup>a</sup>	95% CI <sup>b</sup>	<i>p</i> value
	Exposure to bodies of water (e.g. rivers, lakes, creeks)	7	70.0	85	71.4	0.91	0.23-4.67	1
	Age > 54 years	9	60.0	87	73.1	1.88	0.44–7.25	0.50
In any setting	Worked on or visited a farm other than a workplace piggery $^{\epsilon}$	7	70.0	46	38.7	3.7	0.92-22.08	0.09
(work and/or recreational/	Directly worked – pigs (residential and recreational)	7	70.0	78	65.5	1.19	0.30-6.10	1
residential)	Male	6	90.0	71	59.7	5.4	0.94–137.1	0.09
	Worked outside at either dawn or dusk	7	70.0	67	56.3	1.81	0.45–7.35	0.51
	Worked outside for more than 8 hours a week	7	70.0	76	63.9	1.28	0.33-6.56	1
	Exposure to stagnant water (e.g., standing water, irrigation channels etc)	Q	60.0	76	63.9	0.84	0.22–3.59	-
At work only	Exposure to bodies of water (e.g., rivers, lakes, creeks)	2	20.0	34	28.6	0.66	0.09–2.88	0.72
	Work directly with animals – at work only	7	70.0	70	58.8	1.58	0.41-8.08	0.74
	Work provided with insect repellent – protective behaviours	6	90.0	108	90.7	0.83	0.13-22.01	1
	Lives on a farm	2	20.0	21	17.6	1.22	0.16-5.48	1
	Lives in New South Wales	9	60.0	71	59.7	0.71	0.19–3.07	0.73
Recreational/ residential only	Direct contact with animals outside of work (pigs, horses, cattle, water birds)	7	20.0	24	20.2	1.04	0.14-4.62	-
	Gardening at home	8	80.0	94	79.0	1.01	0.22-7.73	1
	Camping	-	10.0	40	33.6	0.24	0.01–1.42	0.17
<ul><li>a POR: prevalence odds ratio.</li><li>b 95% CI: 95% confidence interval.</li><li>c Excludes people who live on a farm.</li></ul>	dds ratio. Jence interval. 10 live on a farm.							

# Discussion

Although JEV transmission is well-established throughout South-East Asia, widespread transmission of JEV is not known to have occurred in mainland Australia before 2021/2022.<sup>31</sup> The detection of JEV at commercial pig farms in the Murray region of New South Wales in February 2022 was of public health concern, given that the presumably naïve population was likely highly susceptible to infection and to associated morbidity and mortality. NSW Health's rapid PHI among piggery workers and residents in the Murray region achieved a 96.6% JEV vaccination rate among farm staff and/or residents who attended.

The seropositivity rate was 6.5% among Australianborn workers and/or residents at the piggeries with JEV detections, in a sub-population with no previous known JEV or yellow fever vaccination and no overseas travel, suggesting circulation of JEV within the Murray region. While there is no evidence that participants had previous exposure to JEV, this study was unable to confirm the naïvety of participants prior to the detection of JEV in pigs in February 2022, nor was the timing of seroconversion able to be ascertained. It cannot be confirmed that JEV infection was acquired in the Murray region after February 2022, though it is a plausible explanation for the results.

After the initial JEV detections, other humans, mosquitoes and sentinel chickens<sup>31</sup> within the Murrumbidgee Local Health District (MLHD) were found to be infected. By 5 May 2022, pigs in 70 piggeries across four states had JEV detections.<sup>32</sup> A study by Baruah et al (2018)<sup>33</sup> demonstrated the seasonal correlation between mosquito abundance, JEV pig seroconversion and JEV human outbreaks. This study suggests that monitoring of pig seroconversion, along with monitoring of the abundance of JEV carrying mosquitoes, can assist in the prediction of human infections.<sup>33</sup>

While it is plausible that human JEV infection was acquired on piggeries with JEV infected pigs, it is possible that workers were exposed to JEV in their non-work, local environment, as it is likely that infected mosquitoes were in non-occupational locations. A subsequent serosurvey across New South Wales showed a crude seropositivity of 8.7% among 917 sampled volunteers in towns where human JEV cases had not been detected in the 2021–2022 mosquito season,<sup>32</sup> suggesting circulation of JEV in non-occupational settings. This compares with the overall observed 10.3% seropositivity in this investigation

among all participants (regardless of country of birth or vaccination history).

Cases were unable to be randomly selected for the serological or cross-sectional components, and participants voluntarily gave blood for serological testing, which may have created a bias towards people, for example, more compliant with health advice and initiatives, including occupational mosquito prevention measures. The convenience sample is an underrepresentation of the broad population of piggery workers, as we only looked at those who attended the NSW-Health-organised clinics. The representativeness of this participating cohort with the entire piggery population is unknown. As this study involved workers, there is an increased risk of volunteer bias and the healthy worker effect,<sup>34</sup> which may have influenced the type of people who participated, which may not be a true representation of the general Murray population.

Risk exposures associated with JEV seropositivity, for previously unvaccinated Australian-born piggery workers without travel to JEV-endemic countries, were explored through a cross-sectional study. While no risk exposures were statistically associated with JEV seropositivity, the highest prevalence odds ratios for seropositivity were for being male (POR = 5.4) and for working on or visiting a farm other than a workplace piggery between December 2021 and March 2022 (POR = 3.7). This is a small observational study with only one seropositive female participant so caution should be exercised when drawing conclusions about the differences between sexes. Results may have been affected by recall bias and potential misclassification of exposure status, as questionnaires were administered up to three months after the exposure period. There was a lack of specificity around exposure risk factors due to the potentially ubiquitous exposure to mosquitoes and the long period of interest.

The cross-reactivity of human flaviviruses can lead to inconclusive results.<sup>36</sup> The effect of this cross-reactivity on a study can be dependent on the biological assays utilised for diagnosis and on the level of exposure, within a population, to other co-circulating flaviviruses.<sup>37</sup> Knowledge of study participants' prior flavivirus exposure and vaccination history, and knowledge of local disease epidemiology, can thus both assist in further understanding the possible effects of flavivirus cross-reactivity on a study.<sup>38</sup>

Such considerations are particularly important in locations where multiple antigenic flaviviruses can co-circulate: the more flavivirus infections a person has been exposed to, the lower the specificity of the results.<sup>39</sup> There are limitations in the serological assays currently available for use: there has been no development of individualised tests for each flavivirus. However, the use of in-house ELISAs can confirm unvalidated serological data or when no licensed kits for specific flaviviruses are yet available, and the neutralisation IFA can allow for distinction between flaviviruses.<sup>35</sup> A 2019 study by Maeki et al.<sup>36</sup> demonstrated the cross-reactivity of JEV sera with other flaviviruses; neutralisation testing among flaviviruses was shown to be more specific in diagnosing JEV than was the use of ELISA.<sup>36</sup>

Assessing 95% confidence intervals indicated that the cross-sectional study participants were representative of those who completed a clinic questionnaire. As stated above, no risk exposures met the study's criterion for statistical significance at  $p \le 0.05$ . As a study with small numbers, it is reasonable to expect wider confidence intervals and weaker significance in associations due to the cross-sectional study's limited statistical power. However, despite these limitations, the study holds value as an initial investigation into an emerging disease in a presumably naïve population. The findings and conclusions drawn in the paper are reasonable, given the operational nature of the PHI. The cross-sectional design of this study provides weak evidence between the piggery farm exposures and the outcome of JEV; we cannot be certain whether exposure at a piggery farm preceded the detection of JEV.

The relationship between occupation and exposure is unclear and warrants further exploration. As outlined by Yakob,<sup>40</sup> reported Australian human cases to date do not appear to be associated with an occupational hazard, suggesting that it is exposure to mosquitoes, rather than to infected pigs, that dictates the current risk. Although suggestive, our study was unable to determine if JEV exposure was more likely to occur at work or at other nonwork activities in the community or at farm sites (including residences). Workers on larger commercial farms with robust biosecurity may have less exposure to JEV-infected mosquitoes compared to workers on smaller farms with little or no mosquito control; farm workers may also engage in protective behaviours (such as wearing of protective clothing and use of mosquito repellent) to a greater or lesser extent than other members of the community. Further investigation into the behaviours and environments associated with JEV seropositivity should be conducted using larger populations and adequately powered investigations to better understand JEV exposure in the Australian context.

In this context, a study by Ren et al. (2017)<sup>41</sup> suggests that commercial piggeries in China, which are equipped with rigorous security measures and equipment, have a reduced risk of JEV infection among the workers. Ren et al.41 recommended that pigs should be in pig farms far away from dwellings, and farmers should cease working with pigs or pig farming in places of residence, to reduce the risk of JEV infection. A 2010 study by Liu et al.<sup>42</sup> looked at the risk factors associated with JEV in Bali Indonesia, which found the proximity to rice fields, and pig ownership (either by family or next door), to be independently associated with JEV. Although the risk is increased, the direct connection between pigs as the sole cause of infection remains unknown. Pigs are known as amplifying hosts for JEV; however, there is limited evidence to suggest that the infection of JEV within the Murray is directly linked with commercial piggeries.

As recently outlined by Howard-Jones et al.,<sup>31</sup> understanding of JEV seroprevalence and the incidence of mild or non-encephalitic disease in Australia is lacking, and ongoing iterative serosurveys in humans are essential to further characterise the prevalence and distribution of JEV infection in Australia in the coming years. Ideally, these should encompass targeted testing of 'high-risk' regions and populations, such as repeating this survey among the broader piggery worker population in the Murray region as well as in the Australian population at large, to gain an understanding of JEV incidence. This is particularly important as, given the geographical extent of virus activity and evidence the virus was present prior to the widespread 2021-2022 outbreak, it is unlikely that elimination of JEV from mainland Australia is possible.32

A variety of factors may have contributed to the recent expansion of JEV in Australia. The presence of multiple consecutive years of La Nina weather events, resulting in very high rainfall during the Australian summer, created new temporary wetlands; this may have impacted the movement and distribution of JEV-infected wading birds dispersing from the north of the country, as well as creating optimal habitats for the proliferation of *Culex annulirostris* mosquitoes.<sup>43</sup> The spread may have been aided by intensive pig farming and a large feral pig population.<sup>40</sup> In light of the new establishment of disease and a naïve population, serosurveys will be critical to identify risk factors for infection and to guide implementation of management strategies such as vaccination, changes in animal husbandry, and mosquito control,<sup>32</sup> particularly as a warming climate and future extreme flood events may intensify transmission and may increase the frequency and severity of outbreaks.

This serological investigation demonstrated that a presumably naïve population had a JEV antibody prevalence of 6.5%, indicating likely transmission of JEV in the summer of 2021-2022 in the Murray region. It shows, at a minimum, that those who work in the initially identified piggeries in parts of the Murray region were at risk of JEV. Although specific risks associated with seropositivity were not identified, prevention strategies against mosquito bites, including JEV vaccination, should be developed and communicated widely across at-risk occupations and geographical areas. The prevalence of JEV among Australian-born participants and characteristics were also explored through additional opportunistic study components. Vaccination is known as the most effective way to prevent and reduce the burden of JEV for humans and has been known to be a great public health success, particularly in endemic countries.18 This study aimed to urgently vaccinate the workers and residents of the affected piggeries, achieving 96.6% vaccine coverage among those who attended the NSW-Health-organised clinics.

Ongoing surveillance of potential vectors and hosts such as pigs and birds, including also humans, is required to determine whether affected populations change in coming seasons and whether JEV will become endemic in southern mainland Australia.

Overall, this exploratory study serves as an important stepping-stone for future research and metaanalyses to further understand the particular risk factors associated with JEV among individuals working on or residing on or near piggeries.

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# Appendix A

Table A.1: Exposures among Australian-born serological participants reporting no previous exposure or vaccination, March 2022

		Serop (N	Seropositive (N =10)	Seron (N =	Seronegative (N = 119)			
Setting type	Risk variable	۶	%	۶	%	POR <sup>a</sup>	95% Cl <sup>b</sup> p value	<i>p</i> value
	Visited or worked at agricultural show	0	0.0%	0	0.0%	Ι	Ι	I
Recreational/ residential only	Walks along river	m	30.0%	50	42.0%	0.59	0.15-2.40	0.52
	Use of insect repellent	8	80.0%	101	84.9%	0.71	0.14–3.63	-

POR: prevalence odds ratio.

b 95% CI: 95% confidence interval.