

Domestic Pigs and Japanese Encephalitis Virus Infection, Australia

Andrew F. van den Hurk, Scott A. Ritchie,
Cheryl A. Johansen, John S. Mackenzie,
and Greg A. Smith

To determine whether relocating domestic pigs, the amplifying host of Japanese encephalitis virus (JEV), decreased the risk for JEV transmission to humans in northern Australia, we collected mosquitoes for virus detection. Detection of JEV in mosquitoes after pig relocation indicates that pig relocation did not eliminate JEV risk.

Japanese encephalitis virus (JEV) is a major cause of viral encephalitis in Southeast Asia; >50,000 cases are reported annually (1). Ardeid wading birds are the primary maintenance hosts, pigs are the main amplifying hosts, and *Culex* mosquitoes are the primary mosquito vectors. Suppression of JEV disease in humans is generally considered to be best achieved through vaccination of humans or swine, mosquito control, or a combination of these strategies (2). An alternative approach of moving domestic pigs away from human habitation has been suggested as a potential method of reducing JEV transmission to humans (1,3). Although this strategy could be considered a logical way to limit human exposure to infected vectors, the actual effect that removing domestic pigs would have on mosquito infection rates has not been established.

Since the emergence of JEV in northern Australia in 1995, we (the authors) have investigated the ecology of JEV on Badu Island in the Torres Strait, where most human, pig, and animal infections have occurred. Intense transmission on this island has been linked to domestic pigs, which until late 1998 were housed in small backyard pens (Figure 1, panel A), and high populations of *Culex sitiens* subgroup mosquitoes (4). In Australia, members of the *Cx. sitiens*

Author affiliations: Queensland Health, Coopers Plains, Queensland, Australia (A.F. van den Hurk, G.A. Smith); The University of Queensland, St. Lucia, Queensland, Australia (A.F. van den Hurk); Queensland Health, Cairns, Queensland, Australia (S.A. Ritchie); James Cook University, Cairns (S.A. Ritchie); The University of Western Australia, Nedlands, Western Australia, Australia (C.A. Johansen); and Australian Biosecurity Cooperative Research Centre for Emerging infectious Diseases, Perth, Western Australia, Australia (J.S. Mackenzie)

DOI: 10.3201/eid1411.071368

subgroup, from which *Cx. annulirostris* is considered to be the most important species, are the primary Australian JEV vectors (5). During an outbreak in 1998, the virus was shown to be widespread on Badu Island; isolates were obtained from mosquitoes collected throughout the community (6). A vaccination program initiated on the outer Torres Strait islands in 1995, including Badu Island, appears to have limited the number of human clinical cases (5).

To further reduce JEV risk for Badu Island residents, after the 1998 outbreak domestic pigs were removed from the Badu Island community to a piggery located ≈2.5 km away (Figure 1, panel B). This relocation led to a significant reduction in the proportion of *Cx. annulirostris* feeding on pigs, and speculation was that this might reduce the number of JEV-infected mosquitoes (7). We report on the effects of pig relocation away from human habitation on virus infection rate in *Cx. sitiens* subgroup mosquitoes.

The Study

In response to JEV activity, as evidenced by human clinical cases or the seroconversion of sentinel pigs, adult mosquitoes were collected on Badu Island during 1995, 1998, and 2003 (6,8, and this article, respectively). Badu Island is located at 10°07'S and 142°09'E in the central western region of the Torres Strait and is a granite island of ≈101 km²; its ecology has been described (6–8).

The mosquitoes were collected with Centers for Disease Control (CDC) miniature light traps (Model 512; John

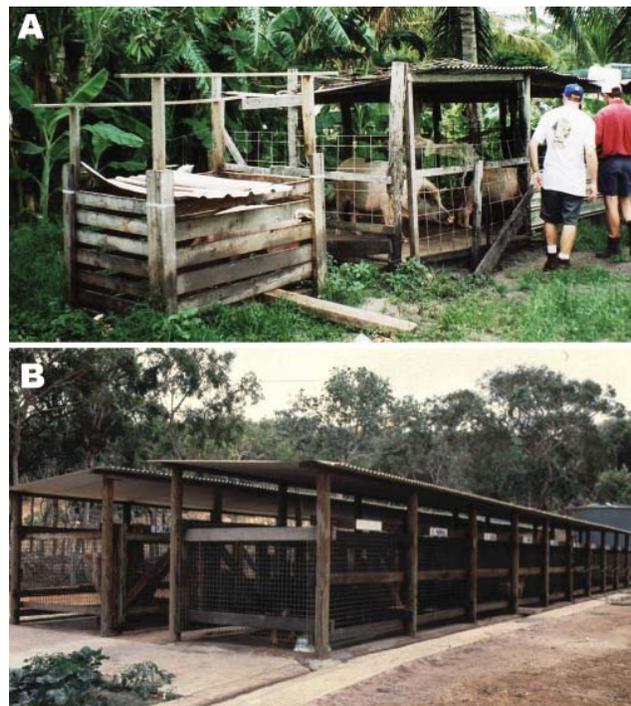


Figure 1. Pig housing in Badu Island. A) Typical backyard pig pen in community before removal in 1998 and B) Badu Island piggery, where pigs have been housed since late 1998.

W. Hock Co., Gainesville, FL, USA) baited with either CO₂ alone or in combination with 1-octen-3-ol. Mosquitoes were killed on dry ice and placed in liquid nitrogen dry shippers or on dry ice in insulated containers for transport to Cairns for storage at -70°C. Mosquitoes were placed on a refrigerated table for species or taxonomic group identification before being placed in pools of ≤200 mosquitoes and sent to Queensland Health Forensic and Scientific Services or the University of Queensland, Brisbane, for JEV detection.

The virus isolation protocols used in 1995 and 1998 have been described (6,8). In 2003, virus RNA was detected by using a real-time TaqMan reverse transcription-PCR (9).

To facilitate the comparison of virus distribution on Badu Island, trap locations were grouped into 3 general areas: within 1.2 km of the piggery, within the area of human habitation (the community), and at a rubbish dump located ≈1.5 km from the community and 4.0 km from the piggery (Figure 2). Dumps are a potential focus of JEV activity because mosquitoes, feral pigs, and wading birds congregate at them (10). Maximum-likelihood estimation of mosquito infection rates with 95% confidence intervals were calculated for each of these general areas by using the PooledInRate statistical software package (11).

Because of the difficulty in morphologically separating the members of the *Cx. sitiens* subgroup, data for the 3 members of the group found in Australia—*Cx. annulirostris*, *Cx. palpalis*, and *Cx. sitiens*—were pooled for analysis. However, PCR restriction fragment length polymorphism analysis of a subsample of 135 *Cx. sitiens* subgroup mosquitoes collected in 2003 showed that *Cx. annulirostris* was the dominant member of this group on Badu Island and comprised 94.1% of polymorphic specimens processed; the other 4.4% and 1.5% were identified as *Cx. palpalis* and *Cx. sitiens*, respectively.

A total of 44,328 *Cx. sitiens* subgroup mosquitoes were processed for detection of JEV; 2,871, 24,592, and 16,865 were processed from 1995, 1998, and 2003, respectively (Table). JEV was detected in 66 pools of *Cx. sitiens* subgroup mosquitoes; the highest maximum-likelihood estimation of mosquito infection rate was obtained from mosquitoes collected at the dump in 1998. Despite removal of the domestic pigs, JEV was still detected in 5 pools of *Cx. sitiens* subgroup mosquitoes collected within the community in 2003. However, the point estimates of infection rates were lower than those obtained in 1995 and 1998, when domestic pigs were present within the community, although the slight overlap in 95% confidence intervals indicates that this difference in infection rate was not significant.

Conclusions

We demonstrated that although removing domestic pigs from areas of human habitation may reduce contact between amplifying hosts and vectors (7), it does not elimi-

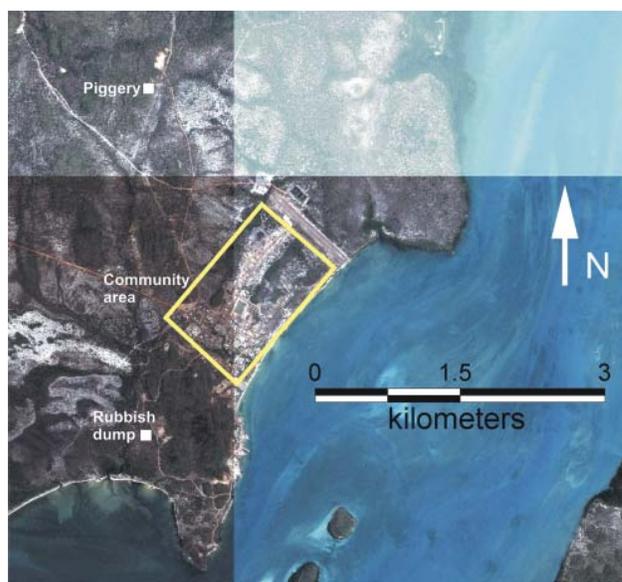


Figure 2. Aerial photograph of Badu Island showing the location of the community, piggery, and rubbish dump.

nate the presence of JEV-infected mosquitoes. Thus, pig removal does not negate JEV risk for humans. Indeed, evidence for low-level virus transmission to humans is provided by Hanna et al., who found a low level (32%) of natural boosting immunity in Badu residents who had received an inactivated mouse brain-derived JEV vaccine 3 years earlier (12).

As has been observed elsewhere in the absence of pigs (13), mosquitoes may have become infected by feeding on viremic herons and egrets, populations of which are found on Badu Island (14). Analysis of host feeding patterns demonstrated that birds accounted for 23% of blood meals of *Cx. annulirostris* identified from the dump in 2003 (S. Hall-Mendelin and A.F. van den Hurk, unpub. data). Alternately, feral pigs in the community and at the dump are a potential source of virus for mosquitoes, although <1% of mosquito blood meals were from swine at these locations.

Mosquitoes could become infected by feeding on viremic pigs at the piggery and then disperse to other areas on the island. The mean flight distance of *Cx. annulirostris* is 4.4 km; some females traverse up to 12 km (15), which is considerably farther than the 2.5 km between the piggery and the community on Badu Island. Solomon recommends that domestic pigs be moved >5 km from human habitation to limit JEV transmission to humans (3). Indeed, if infected mosquitoes were flying from the piggery to the community, then the data from our study support this recommendation. However, we suggest that domestic pigs be removed far enough away from human habitation to encompass the flight range of the local *Culex* vectors.

Table. Mosquito infection rates during 3 recognized incursions of Japanese encephalitis virus, Badu Island, northern Australia*

Collection location	Pigs located within community						Pigs relocated outside community		
	1995			1998			2003		
No.†	No. detected‡	Infection rate (95% CI)	No.†	No. detected‡	Infection rate (95% CI)	No.†	No. detected‡	Infection rate (95% CI)	
Community	2,871	8	3.02 (1.43–5.74)	23,467	38	1.69 (1.21–2.29)	7,019	5	0.75 (0.28–1.66)
Piggery	NS	NS	NS	NS	NS	NS	3,316	5	1.61 (0.61–3.56)
Dump	NS	NS	NS	1,125	4	3.68 (1.20–8.85)	6,530	6	0.99 (0.41–2.07)

*Mosquito infection rates determined by maximum-likelihood estimation; 1995, Apr 8–9 and 20–21, 30 trap nights; 1998, Mar 5–6, 25 trap nights; 2003, Mar 13–19, 92 trap nights; CI, confidence interval; NS, mosquitoes not sampled from this location during the year of collection.

†Total no. mosquitoes processed.

‡No. Japanese encephalitis virus–positive pools detected by virus isolation or TaqMan reverse transcription–PCR.

Acknowledgments

We thank the Badu Island council, particularly Sean Lawlor in 2003, for supporting our investigations. We also thank Brian Montgomery for assistance with mosquito collections, Paul Zborowski for mosquito sorting, and Debra Nisbet for assistance with processing of samples. Finally, we thank Jeffrey Hanna, Craig Williams, and Jay Nicholson for suggestions for the manuscript.

Aspects of this study were funded by the Australian Health Minister's Advisory Council Priority Driven Research Program and Queensland Health.

Dr van den Hurk is a research entomologist in the Virology Section of Forensic and Scientific Services, Queensland Health, Coopers Plains, Australia. His primary research interest is the study of mosquito-borne pathogens, with a focus on the entomologic aspects of arbovirus transmission cycles.

References

- Mackenzie JS, Williams DT, Smith DW. Japanese encephalitis virus: the geographic distribution, incidence, and spread of a virus with a propensity to emerge in new areas. In: Tabor E, editor. Emerging viruses in human populations. Amsterdam: Elsevier; 2007. p. 201–68.
- Igarashi A. Control of Japanese encephalitis in Japan: immunization of humans and animals, and vector control. *Curr Top Microbiol Immunol.* 2002;267:139–52.
- Solomon T. Control of Japanese encephalitis—within our grasp? *N Engl J Med.* 2006;355:869–71. DOI: 10.1056/NEJMp058263
- Hanna JN, Ritchie SA, Phillips DA, Shield J, Bailey MC, Mackenzie JS, et al. An outbreak of Japanese encephalitis in the Torres Strait, Australia, 1995. *Med J Aust.* 1996;165:256–60.
- Mackenzie JS, Johansen CA, Ritchie SA, van den Hurk AF, Hall RA. Japanese encephalitis as an emerging virus: the emergence and spread of Japanese encephalitis virus in Australasia. *Curr Top Microbiol Immunol.* 2002;267:49–73.
- Johansen CA, van den Hurk AF, Pyke AT, Zborowski P, Phillips DA, Mackenzie JS, et al. Entomological investigations of an outbreak of Japanese encephalitis virus in the Torres Strait, Australia, in 1998. *J Med Entomol.* 2001;38:581–8.
- van den Hurk AF, Nisbet DJ, Johansen CA, Foley PN, Ritchie SA, Mackenzie JS. Japanese encephalitis on Badu Island, Australia: the first isolation of Japanese encephalitis virus from *Culex gelidus* in the Australasian region and the role of mosquito host-feeding patterns in virus transmission cycles. *Trans R Soc Trop Med Hyg.* 2001;95:595–600. DOI: 10.1016/S0035-9203(01)90090-2
- Ritchie SA, Phillips D, Broom A, Mackenzie J, Poidinger M, van den Hurk A. Isolation of Japanese encephalitis virus from *Culex annulirostris* in Australia. *Am J Trop Med Hyg.* 1997;56:80–4.
- Pyke AT, Smith IL, van den Hurk AF, Northill JA, Chuan TF, Westcott AJ, et al. Detection of Australasian flavivirus encephalitic viruses using rapid fluorogenic TaqMan RT-PCR assays. *J Virol Methods.* 2004;117:161–7. DOI: 10.1016/j.jviromet.2004.01.007
- van den Hurk AF, Montgomery BL, Northill JA, Smith IL, Zborowski P, Ritchie SA, et al. The first isolation of Japanese encephalitis virus from mosquitoes collected from mainland Australia. *Am J Trop Med Hyg.* 2006;75:21–5.
- Biggerstaff BJ. PooledInfRate: a Microsoft Excel add-in to compute prevalence estimates from pooled samples. Fort Collins (CO): Centers for Disease Control and Prevention; 2003.
- Hanna JN, Smith GA, McCulloch BG, Taylor CT, Pyke AT, Brookes DL. An assessment of the interval between booster doses of Japanese encephalitis vaccine in the Torres Strait. *Aust N Z J Public Health.* 2005;29:44–7. DOI: 10.1111/j.1467-842X.2005.tb00747.x
- Soman RS, Rodrigues FM, Guttikar SN, Guru PY. Experimental viraemia and transmission of Japanese encephalitis virus by mosquitoes in ardeid birds. *Indian J Med Res.* 1977;66:709–18.
- Draffan RDW, Garnett ST, Malone GJ. Birds of the Torres Strait: an annotated list and biogeographical analysis. *Emu.* 1983;83:207–34.
- Bryan JH, O'Donnell MS, Berry G, Carvan T. Dispersal of adult female *Culex annulirostris* in Griffith, New South Wales, Australia: a further study. *J Am Mosq Control Assoc.* 1992;8:398–403.

Address for correspondence: Andrew F. van den Hurk, Virology, Forensic and Scientific Services, Queensland Health, PO Box 594, Archerfield, Queensland, 4108, Australia; email: andrew_hurk@health.qld.gov.au

Search past issues of EID at www.cdc.gov/eid