

Spatial Analysis of Japanese Encephalitis Virus Transmission Risk Factors in Singapore

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Abstract

Background Singapore was previously endemic for JEV but the threat of JE remains. There is continued JEV transmission in animal hosts despite the banning of pig farming in 1992. Surveillance of mosquitoes, ardeid birds and swine gives public health officials useful warning signs for the monitoring of JEV. In this study, we aim to develop a JE Risk Map based on the data gathered by spatial and quantitative survey of the JE-associated vectors and animal hosts.

Methods For over a year across the island, the distribution and population of herons were visually surveyed and the distribution of wild boars was captured with wildlife camera traps. Mosquito population data was collected from 16 sites with ecological factors suitable for JE transmission. The animal and vector data were then spatially analysed using open-sourced GIS software.

Results Four JE high risk clusters were interpolated from the combined risk factors of data points. A JEV positive *Culex tritaeniorhynchus* pool was subsequently detected in the largest of these JE high risk clusters.

Introduction

Japanese encephalitis virus (JEV) is the most important member of the Japanese encephalitis (JE) serogroup of the genus flavivirus, family Flaviviridae with up to 68,000 cases and 20,400 deaths annually. It is prevalent throughout Eastern and Southern Asia and the Pacific Rim. [1–3]. Genetic studies of JEV show that the Indonesia-Malaysia region has all the genotypes of JEV circulating. This suggests that JEV originated from its ancestral virus in the Malay Archipelago and evolved into the 5 different genotypes which spread across Asia [2].

In temperate areas, JEV transmission peaks in summers and falls, while in the subtropics and tropics, JEV transmission occurs all year-round [4]. Proximity to rice fields has been shown to be an important environmental risk factor for JEV transmission, with JEV-infected encephalitis patients 2.93 times more likely to be living near rice fields than non-infected patients [5]. Out of the 1.9 billion people living in rural JE-prone areas, 220 million people live nearby rice irrigation schemes. Richards et al. have shown positive links between the proportion of rice fields and mosquito abundance [5].

JEV can be isolated from over 30 species of mosquitoes but the paddy breeding mosquitoes of the *Culex vishnui* subgroup, specifically *Cx. tritaeniorhynchus*, are the major vectors of the virus. Other species such as *Cx. gelidus*, *Cx. fuscocephala* and *Cx. annulirostris*, have also been implicated as important secondary or regional vectors. Other *Culex* species that are competent vectors in the laboratory include *Cx. quinquefasciatus*, *Cx. pipiens pallens*, *Cx. pseudovishnui*, *Cx. gelidus*, *Cx. fuscocephala*, *Cx. annulirostris* and *Cx. sitiens* [6]. There has also been reports of JEV isolates from midges, *Forcipomyia* (Lasiohelea) *taiwana* and *Culicoides* spp. but it is not clear what role these arthropods play in the transmission of JEV [7, 8].

Ardeid wading birds are the primary enzootic hosts of JEV. Extensive field and immunologic studies by Buescher et al. have established the role of the black-crowned night heron (*Nycticorax nycticorax*), plumed egret (*Egretta intermedia*), little egret (*Egretta garzetta*) and the Asian cattle egret (*Bubulcus ibis coromandus*) in the ecology of JEV [2, 9–11]. Domestic and wild pigs and boars have been shown to be amplifying hosts of JEV due the following reasons ; i) JE vectors are highly attracted to pigs, ii) Infection rates of up to 98–100% have been observed in pigs, iii) Pigs can produce high levels of viremia 24 h post infection, which can last up to five days. In addition, all domestic pigs are able to infect mosquitoes, iv) The turnover of pigs is rapid and the older and presumably JE immune population is replaced with younger pigs [12–15].

Air transport of infected vectors had been implicated in the introduction of JEV to the Pacific islands of Guam and Saipan [16, 17]. With the advent of cheap air travel, we may expect increased risk of JEV introduction into new areas. Migratory birds may also play an expansionary role in JEV spread, as shown in the existence of JEV in India, Pakistan and Nepal where there is little pig farming [18, 19]. Mani et al. found that JEV seroconversion rates of children in rice eco-agro systems with presence of herons were 50–56% while systems without herons in close proximity were 0–5% [20]. The East Asian-Australasian (EAA) flyway is one of the great flyways that migratory birds take during their seasonal migrations. Migratory herons transit through this flyway which passes through JE endemic areas [21].

Climatic condition is a major factor in the geographic and temporal distribution of vectors, their life cycle characteristics and the resultant dispersal patterns of JEV, and its transmission efficiency from vector to host. Effects of climate change such as global warming may increase vector population and increase transmission efficiency leading to rapid disease emergence in new areas [22, 23].

Surveillance of mosquitoes, migratory birds, swine, and sentinel chickens gives public health officials useful warning signs for the monitoring of JEV [24]. Singapore was previously endemic for JEV before the complete phasing out of pig farming in 1992. Although only six cases of JE infection in humans were reported from 1991 to 2005, JEV antibody prevalence in working dogs and chicken were reported in 2004. Further evidence of JEV seropositivity in ardeid birds, raptors, wild boars and sentinel chickens from 2010 till 2017 demonstrated that JEV transmission in animal hosts has continued despite the abolishment of pig farms[25–27].

In this study, we aim to develop a JE Risk Map based on the data gathered by the spatial and quantitative survey of JE-associated vectors and animal hosts. Field data collected from cross-sectional studies at 16 sites that have ecological factors suitable for JE transmission were analysed using GIS. The ecological drivers that we identified at these sites include the presence of i) heron nesting, roosting and feeding, ii) wild boar, iii) *Culex* mosquitoes and iv) the primary JEV vector, *Culex tritaeniorhynchus* species.

Materials And Methods

Distribution and population count of herons.

Preliminary survey of all possible *Ardeidae* roosting, nesting and feeding areas in Singapore was conducted from 5 September 2013 to 21 January 2014. Choice of locations and their priorities were derived from the island wide Heron Watch survey conducted on 31 August 2013, with the addition of potential estuaries, ponds, golf courses and reservoirs not included in the survey. Census walks were conducted in the mornings (7 am – 11 am) and evenings (4 pm – 6 pm). Birds were located through direct sightings and movements. When a heron, egret or bittern was sighted, surveyors stopped and noted down: 1) species and count; 2) location plotted on a GPS device; 3) if flying, the direction in which the bird was flying to and from; and 4) whether it was a (potential) roosting and/or nesting site. A roosting site was suspected when many birds were found resting on tree(s) at the same spot and confirmed when the birds were found returning to the roosting spot just before sunset (6.30 pm – 7 pm). A nesting site was suspected when juveniles were spotted and confirmed only when nests were observed. A suspected roosting or nesting site was revisited just before sunrise (6.30 am – 7.30 am) and/or just before sunset (6 pm – 7 pm) for verification. When it was not possible to visit at dawn or dusk (e.g. in the case of golf courses which entrance was allowed only at 8am), survey was done at the site at the earliest time possible. As black-crowned night herons are nocturnal, the roosting and nesting sites were verified during the day (7.30 am – 6 pm).

Population size and range determination

For a more accurate count and range determination, concurrent surveys were conducted at every roosting, nesting and feeding location in areas with more than 5 herons. Due to manpower limitations, the island was divided into four sectors (North-East, North-West, South-East and South-West) and concurrent surveys were done sector by sector. For the purpose of data comparison, all identified locations were surveyed once in the peak of the migratory (December to January) and non-migratory (June to July) season respectively. Surveys were conducted in the following periods: 9 December 2013–21 January 2014; 30 June 2014–7 July 2014; and 6 January 2015–13 January 2015. Two surveyors were assigned to each location and counts were made concurrently at sunrise. After which, surveyors observed the 1) species and count; and 2) flight direction of birds at their respective locations until 10 am. Surveys are conducted continually over the course of the year to search for potential roosting and nesting sites and coordinated surveys conducted every half a year to monitor the population and ranging behaviour at the roosting and nesting sites.

Distribution of wild boars

From 2013 to 2015, surveys to assess the presence of wild boar at various sites across mainland Singapore were selected based on previous reliable reports of wild boar, and the presence of large tracts of potential habitat (e.g. unmanaged forest patches or secondary scrub), with a focus on sites located close to urban areas. These sites were selected for camera trapping, where 5-6 Reconyx PC900 camera traps were deployed in selected locations during each session. Camera locations were selected based on areas with evidence of recent wild boar activity, as well as the presence of trees of suitable size for the cameras to be attached. Each camera was secured to the trunk of a tree with a Masterlock Python

Adjustable Locking Cable 8413DPF, 40 to 50 centimetres above the ground. Upon activation, camera traps were then left in the field for 14 days to record images of wild boar.

Distribution and abundance of Culex mosquitoes

Sixteen mosquito trapping sites (figure 3) were selected based on the presence of animals hosts as identified by our Nparks collaborators.

The sites selected are : Bishan Park (BP), Cattle Farm (CF), Coney Island (CI), Jurong Lake (JL), Laguna Country Club (LG), Lower Peirce Reservoir (LPR), Mandai Mudflat (MM), Mandai Road (MR), Pasir Ris Park (PR), Punggol 17 (P17), Sengkang Riverside (SR), Sengkang West (SW), Singapore Turf Club (STC), Springleaf (SL), Sungei Pandan (SP), Yishun Pond (YP).

Mosquitoes were trapped using the Centre for Disease Control (CDC) light traps baited with dry ice at ten selected locations in each trapping site. Every trapping session comprised of two consecutive nights and each night lasted for 16 hours which includes two hours of dusk and two hours of dawn.

Mosquitoes were collected after each 16-hour trap night and transported back to the laboratory on ice. These mosquitoes were sorted and identified to species when possible and to species group for morphologically similar species. The entire process was performed on ice. Samples of the same species were pooled in groups of 3 and kept at -80°C until further experiments were performed.

Spatial analysis of JE risk factors

QGIS (Open source software) was used to render the spatial and quantitative field data into a JE Risk Map. Data layers included the following : 1) Singapore map boundaries, 2) Culex mosquitoes catch data, 3) Heron population and distribution data, and 4) Wild boar distribution data. The quantitative data from the vectors and animal hosts was tabulated into a JE risk matrix (Table 2) which assigns JE risk score for every spatial data point. An interpolation method called Inverse Distance Weighting (IDW) was used to generate a continuous JE risk map that shows colour-coded risk levels of the different locations.

Table 2
JE Risk matrix

Risk Factor (Population)	Risk Score
Culex	
i) Presence	1
ii) 95 percentile	1
Culex tritaeniorynchus	
i) Presence	1
ii) 95 percentile	1
Hérons	
i) Presence	1
ii) 50 percentile	1
Wild Boar	
i) Presence	2

Results

Distribution and population count of herons

A total of 572 herons were sighted throughout the survey. Of them, 372 were feeding, 145 were roosting and 55 nesting. Fourteen different species of herons sighted were a) Black bittern b) Black crowned night heron c) Cinnamon bittern d) Chinese egret e) Chinese pond heron f) Great billed heron g) Grey Heron h) Intermediate egret i) Javan pond heron j) Little egret k) Purple heron l) Pacific reef egret m) Striated heron n) Yellow bittern. In Fig. 1, we have shown the distribution of the herons sighted in 2014. We will subsequently use this data to determine the risk factor as contributed by the presence of herons at a point on the map.

Distribution of wild boars

A wild boar distribution map in Singapore was built based on the wild boar survey (Figure 2) The map showed similar wild boars distribution as the one constructed by Yong et al. in 2010, however, with a few newly identified areas in the eastern part of the island such as Sengkang area [28].

Distribution and abundance of *Culex* mosquitoes

7840 mosquitoes were trapped and identified from the 16 selected sites (Figure 3). 5380 were of the genus *Culex* and 2119 were the primary JEV vector, *Culex tritaeniorynchus*. *Culex* mosquitoes were present at all trapping sites while *Culex tritaeniorynchus* were trapped in all but Lower Peirce Reservoir (LPR) and Mandai Road (MR) (Table 1.1 and Table 1.2).

Table 1.1 *Culex* abundance

Location	Count
Bishan Park	28
Cattle Farm	681
Coney Island	2898
Jurong lake	242
Laguna Golf Course	95
Lower Peirce Reservoir	255
Mandai Road	56
Mandai Mudflat	254
Pandan Reservoir	110
Pasir Ris Park	393
Punggol 17	1396
Sengkang Riverside Park	595
Sengkang West/Thanggam	548
Springleaf	174
STC	85
Yishun Pond	30
Total	7840

Table 1.2 *Culex tritaeniorhynchus* abundance

Location	Count
Bishan Park	1
Cattle Farm	234
Coney Island	1199
Jurong lake	25
Laguna Golf Course	12
Mandai Mudflat	1
Pandan Reservoir	12
Pasir Ris Park	4
Punggol 17	438
Sengkang Riverside Park	141
Sengkang West/Thanggam	32
Springleaf	1
STC	16
Yishun Pond	3
Total	2119

Detection of JEV-positive mosquito in Coney Island

A pool of *Cx. tritaeniorhynchus* screened was JEV positive. This sample was collected from Coney Island (CI) and was subjected to E-gene sequencing before comparing the sequences with JEV genotypes I to V sequences retrieved from GenBank. From the phylogenetic analysis, the positive pool of JEV is genotype I virus and is closely related to those circulating in Japan in 2004 and 2007 [Manuscript submitted]. These genotype I viruses were shown to be introduced to Japan from Southeast Asia and continental East-Asia [29].

Spatial analysis of JE Risk factors

There are four clusters of Very High JE Risk in the JE Risk map in the north western and south western parts of Singapore. These are areas where the Sungei Buloh Wetland Reserve (SBWR) and the Avian Sanctuary (AS) are located respectively. The high abundance of herons in these areas contribute

significantly to the JE Risk. We have also previously detected JEV in *Culex tritaeniorynchus* sampled from AS [Manuscript submitted]. The hotspot near Mandai Road (MR) site can be attributed to the presence of herons in the vicinity of the zoo and the presence of mosquito vector and wild boar at MR site itself. The JE Risk map indicates to us that the north eastern part represents a large cluster of Very High JE Risk. The JEV detected in our mosquito sample originates from this area of Singapore. Three of the JE Risk factors are represented in this cluster. The abundance of *Culex tritaeniorynchus* is the highest from among the 16 mosquito trapping sites in addition to the presence of herons and wild boars. Within this cluster, Sungei Tampines has a large population of roosting and nesting herons. The north eastern cluster extends into an offshore island, Pulau Ubin and the risk factor there is mainly contributed by the presence of herons on the island. At the eastern region, the cluster near Laguna Golf Course (LG) has an interface of mosquito vectors and herons (Figure 4).

Discussion

The risk map can also be further developed to include spatial and temporal indicators of infection risks. Spatial indicators of infection risks are associated with ecotones which are highly connected locations at the interface of circulating vectors, hosts or pathogens. Temporal indicators deal with biological mutation of pathogens, invasion of vectors, animal host population change, immigration of naïve human hosts, sudden land use and changes, political and economic changes and rapid climatic changes [30]. Detailed and accurate land cover mapping is required to include the role of landscape features in JE risk modelling. With the absence of rice fields as the primary habitat of *Culex tritaeniorynchus*, we need to locate areas in Singapore where there are similar environmental or landscape features. Waterlogged areas with abundant grass cover can be a potential environmental feature. However more comprehensive ecological surveys are needed to accurately characterise the local habitat of wild *Culex tritaeniorynchus*. Remote sensing technology will be essential in the search for such ecologically analogous areas in urban Singapore. Using ecological niche modelling, we can move on to estimate the abundance of *Cx. tritaeniorynchus* throughout the whole island of Singapore and develop a more accurate JE risk prediction models [31].

Before the last pig farm was shut down in 1992, the main bulk of pig farming activity was located in the north eastern part of Singapore, namely Punggol. The abundance of amplifying hosts and presence of primary vectors would most likely influence JEV to be previously endemic in this area. More than twenty years later, we are still able to detect active JEV transmission of the Genotype I variant in Coney Island which is located within the vicinity of the historical pig farms [25]. We are unable to ascertain as to whether the detected JEV is related to the strain that was previously endemic in Punggol. However, seroprevalence of local wild pigs in Singapore remains relatively high at 15.2% for a country without recent clinical cases. From our study, it is shown that local birds have JEV seropositivity rate of 3.2% and the JEV antibody is detected mainly in herons and raptors [27]. This indicates possible sylvatic transmission between the vector and both the amplifying and reservoir hosts.

The limited scope of this snapshot study does not account for the seasonality of mosquito abundance. The distribution and abundance profiles can vary temporally, which can be characterised in the scope of a future study. Singapore is a land-scarce country and development often encroach into the natural habitats of the host animals. The animal host distribution and abundance data have to be actively monitored at sites where there is future development. Setting up these active surveillance systems will require substantial resources, as the animals need to be tagged with geolocation devices and mosquitoes are regularly sampled.

It is difficult to assess and predict JE emergence as there are various biotic and abiotic environmental factors that interact synergistically to influence JEV transmission dynamics. The abolition of pig farming has greatly reduced the impact of the amplifying hosts as a risk factor. However, there are wildlife such as herons and wild boars which continue to be risk factors in JEV transmission. Other factors such as climate change might accelerate JEV transmission [32, 33]. The implementation of active JEV surveillance and the modelling of JE risk maps will enable us to predict and mitigate the re-emergence of JE in Singapore.

Conclusion

The spatial distribution of JEV-vectors and animals hosts, and the quantitative data obtained from our cross-sectional studies were integrated and a JE risk map was generated. In the areas where Very High JE Risk clusters were indicated, we were able to locate previous detection of JEV transmission at AS, and a pool of JEV positive mosquitoes was later discovered in Coney Island, a location quite a distance away from AS. We have thus demonstrated the utility of the JE risk map in identifying clusters of JE transmission risk in Singapore.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

1. Mahathir wrote the manuscript, analyzed the data and generated the risk map. He was also involved in mosquito sample collection and identification.
2. Ivan planned and conducted Wild boar survey and wrote of methods section.
3. Xinli planned and conducted the heron survey and wrote of methods section.
4. GY planned project and reviewed the manuscript.
5. XF was involved in mosquito sample collection and identification.
6. JE was involved in mosquito sample collection and identification.
7. GLY was involved in mosquito sample collection and identification.
8. CCS provided mosquito taxonomy expertise.
9. HCB supervised the animal surveys.
10. DY supervised and oversaw the manuscript submission. He was also involved in mosquito sample collection and identification.

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References

1. Solomon, T., *Flavivirus encephalitis*. N Engl J Med, 2004. **351**(4): p. 370-8.
2. Solomon, T., et al., *Origin and evolution of Japanese encephalitis virus in southeast Asia*. J Virol, 2003. **77**(5): p. 3091-8.
3. Campbell, G.L., et al., *Estimated global incidence of Japanese encephalitis: a systematic review*. Bull World Health Organ, 2011. **89**(10): p. 766-74, 774A-774E.
4. Fischer, M., et al., *Japanese encephalitis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)*. MMWR Recomm Rep, 2010. **59**(RR-1): p. 1-27.
5. Liu, W., et al., *Risk factors for Japanese encephalitis: a case-control study*. Epidemiol Infect, 2010. **138**(9): p. 1292-7.
6. van den Hurk, A.F., S.A. Ritchie, and J.S. Mackenzie, *Ecology and geographical expansion of Japanese encephalitis virus*. Annu Rev Entomol, 2009. **54**: p. 17-35.

7. Pan, X.L., et al., *Emergence of genotype I of Japanese encephalitis virus as the dominant genotype in Asia*. J Virol, 2011. **85**(19): p. 9847-53.
8. Wang, H.Y., et al., *Molecular epidemiological analysis of Japanese encephalitis virus in China*. J Gen Virol, 2007. **88**(Pt 3): p. 885-94.
9. Scherer, W.F., E.L. Buescher, and C.H. Mc, *Ecologic studies of Japanese encephalitis virus in Japan. V. Avian factors*. Am J Trop Med Hyg, 1959. **8**: p. 689-97.
10. Buescher, E.L., et al., *Immunologic studies of Japanese encephalitis virus in Japan. III. Infection and antibody responses of birds*. J Immunol, 1959. **83**: p. 605-13.
11. Buescher, E.L., et al., *Ecologic studies of Japanese encephalitis virus in Japan. IV. Avian infection*. Am J Trop Med Hyg, 1959. **8**: p. 678-88.
12. Gresser, I., J.L. Hardy, and W.F. Scherer, *The growth curve of Japanese encephalitis virus in the vector mosquito of Japan, Culex tritaeniorhynchus*. Jpn J Exp Med, 1958. **28**(4): p. 243-8.
13. Scherer, W.F., J.T. Moyer, and T. Izumi, *Immunologic studies of Japanese encephalitis virus in Japan. V. Maternal antibodies, antibody responses and viremia following infection of swine*. J Immunol, 1959. **83**: p. 620-6.
14. Scherer, W.F., et al., *Ecologic studies of Japanese encephalitis virus in Japan. VI. Swine infection*. Am J Trop Med Hyg, 1959. **8**: p. 698-706.
15. Williams, D.T., et al., *Experimental infections of pigs with Japanese encephalitis virus and closely related Australian flaviviruses*. Am J Trop Med Hyg, 2001. **65**(4): p. 379-87.
16. Hammon, W.M., et al., *Epidemiologic studies of concurrent virgin epidemics of Japanese B encephalitis and of mumps on Guam, 1947-1948, with subsequent observations including dengue, through 1957*. Am J Trop Med Hyg, 1958. **7**(4): p. 441-67.
17. Mitchell, C.J., et al., *Japanese encephalitis on Saipan: a survey of suspected mosquito vectors*. Am J Trop Med Hyg, 1993. **48**(4): p. 585-90.
18. Weaver, S.C. and A.D. Barrett, *Transmission cycles, host range, evolution and emergence of arboviral disease*. Nat Rev Microbiol, 2004. **2**(10): p. 789-801.
19. Soman, R.S., et al., *Experimental viraemia and transmission of Japanese encephalitis virus by mosquitoes in ardeid birds*. Indian J Med Res, 1977. **66**(5): p. 709-18.
20. Mani, T.R., et al., *Surveillance for Japanese encephalitis in villages near Madurai, Tamil Nadu, India*. Trans R Soc Trop Med Hyg, 1991. **85**(2): p. 287-91.
21. <http://www.eaaflyway.net/about/the-flyway/>, *East Asian Australasian Flyway - About the Flyway*. 2015.
22. Reiter, P., *Climate change and mosquito-borne disease*. Environ Health Perspect, 2001. **109** Suppl 1: p. 141-61.
23. Dash, A.P., et al., *Emerging and re-emerging arboviral diseases in Southeast Asia*. J Vector Borne Dis, 2013. **50**(2): p. 77-84.

24. Solomon, T. and M.J. Cardoso, *Emerging arboviral encephalitis. Newsworthy in the West but much more common in the East*. BMJ, 2000. **321**(7275): p. 1484-5.
25. Koh, Y.L., et al., *Japanese encephalitis, Singapore*. Emerg Infect Dis, 2006. **12**(3): p. 525-6.
26. Ting, S.H., et al., *Seroepidemiology of neutralizing antibodies to Japanese encephalitis virus in Singapore: continued transmission despite abolishment of pig farming?* Acta Trop, 2004. **92**(3): p. 187-91.
27. Yap, G., et al., *Serological evidence of continued Japanese encephalitis virus transmission in Singapore nearly three decades after end of pig farming*. Parasites Vectors, 2019. **12**(244): p. 7.
28. D. L. Yong BPY-HL, A.A., and K. H. Tan, *The status on Singapore Island of the eurasian wild pig *Sus scrofa**. Nature in Singapore, 2010. **3**: p. 227-337.
29. Nabeshima, T., et al., *Evidence of frequent introductions of Japanese encephalitis virus from south-east Asia and continental east Asia to Japan*. J Gen Virol, 2009. **90**(Pt 4): p. 827-32.
30. Lambin, E.F., et al., *Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts*. Int J Health Geogr. **9**: p. 54.
31. Miller, R.H., et al., *Ecological niche modeling to estimate the distribution of Japanese encephalitis virus in Asia*. PLoS Negl Trop Dis. **6**(6): p. e1678.
32. Yen, A. and T.H.H. Chen, *Impact of climate on Japanese Encephalitis*. Epidemiol Infect, 2008. **136**(7): p. 980-987.
33. Florence, F. and J.C. Reeder, *Impact of past and on-going changes on climate and weather on vector-borne diseases transmission: a look at the evidence*. Infect Dis Poverty, 2019. **8**(51): p. 9.

Figures

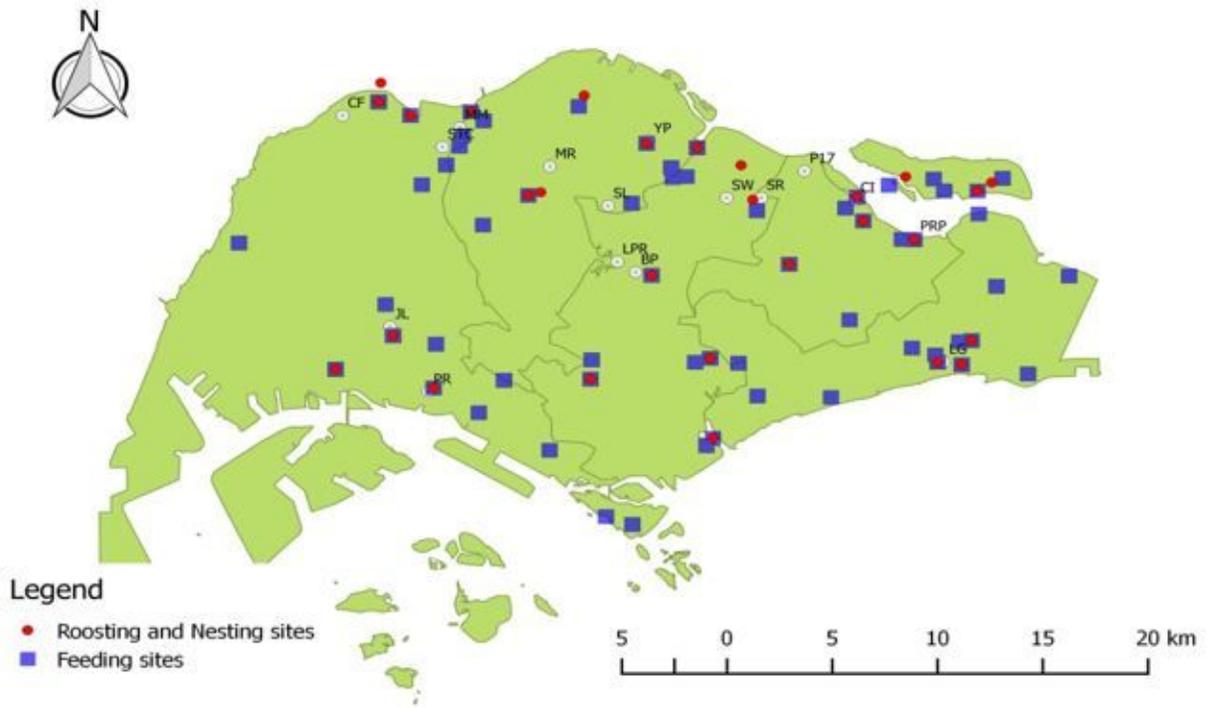


Figure 1

Distribution of heron sightings. 372 feeding, 145 roosting and 55 nesting sites were sighted. A total of 14 different species of herons were identified.

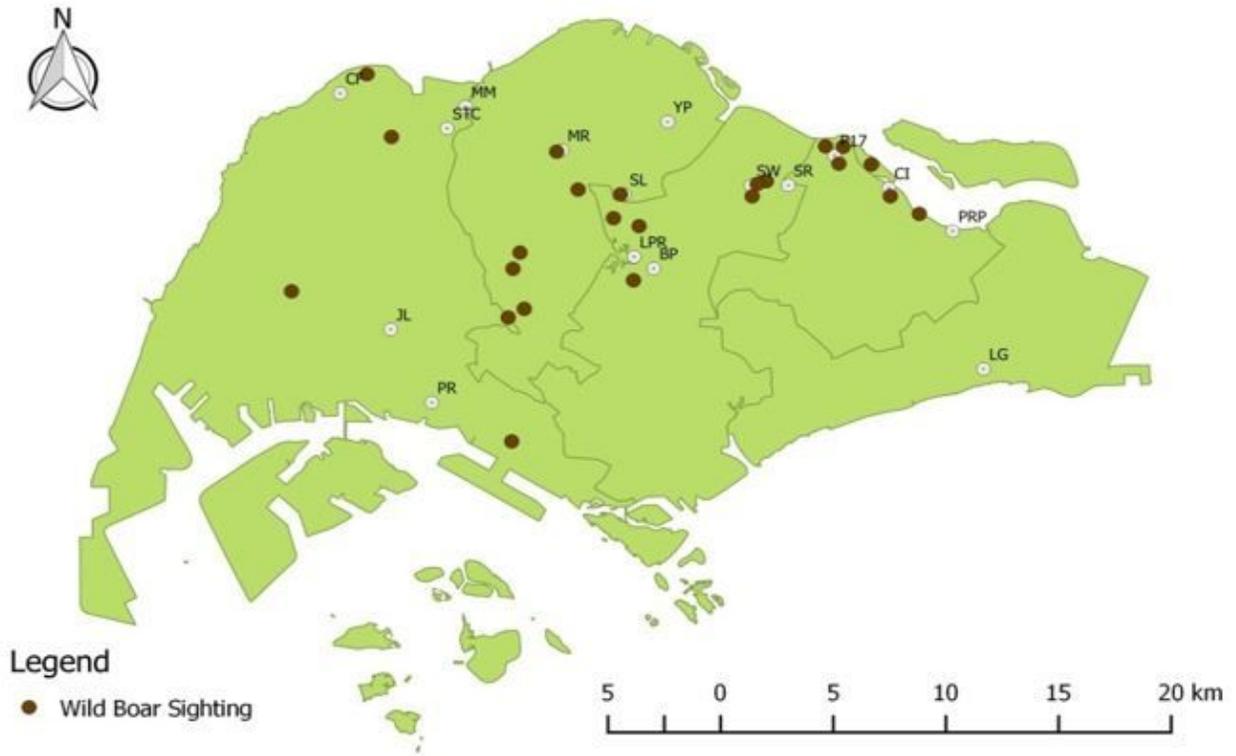


Figure 2

Location of wild boars detected by RECONYX PC900 camera traps. All locations reflected on the map present suitable habitat for wild boars.

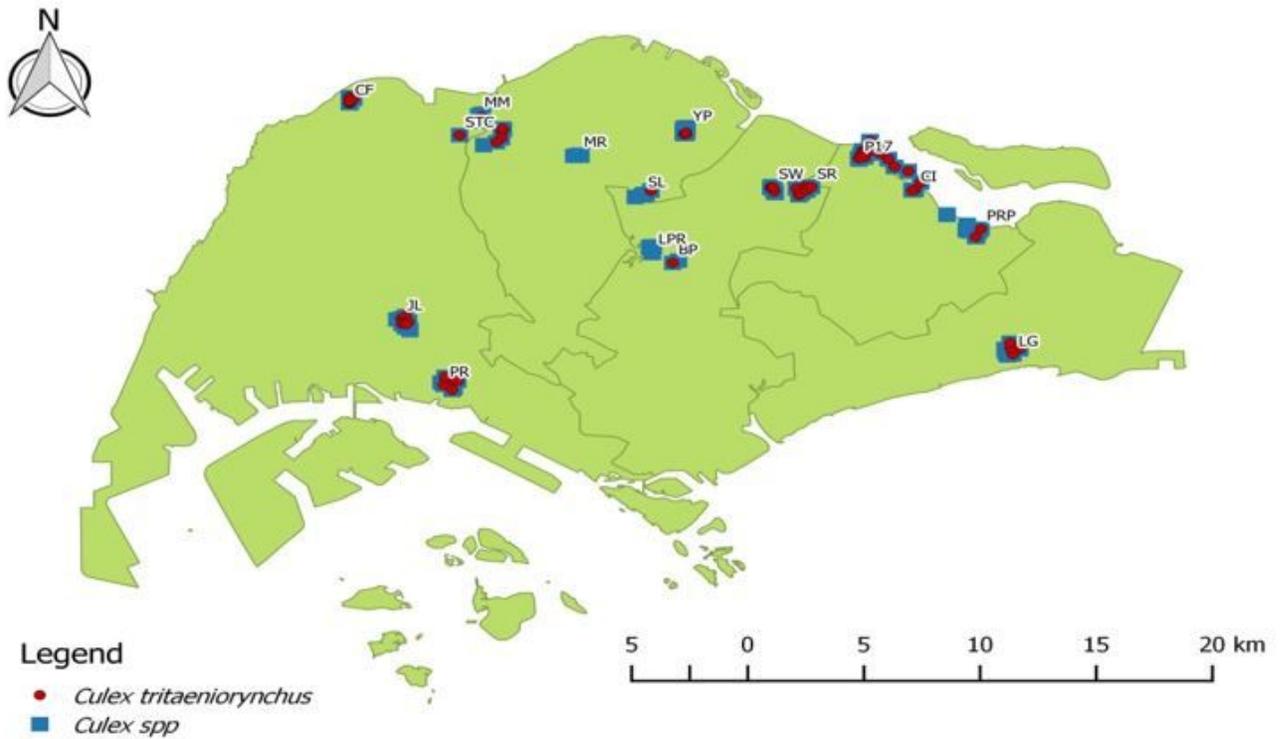


Figure 3

Culex and *Culex tritaeniorynchos* distribution in the 16 trapping sites. *Culex* vectors are present at all sites and the highest abundance of these vectors are mainly in the north-eastern part of Singapore

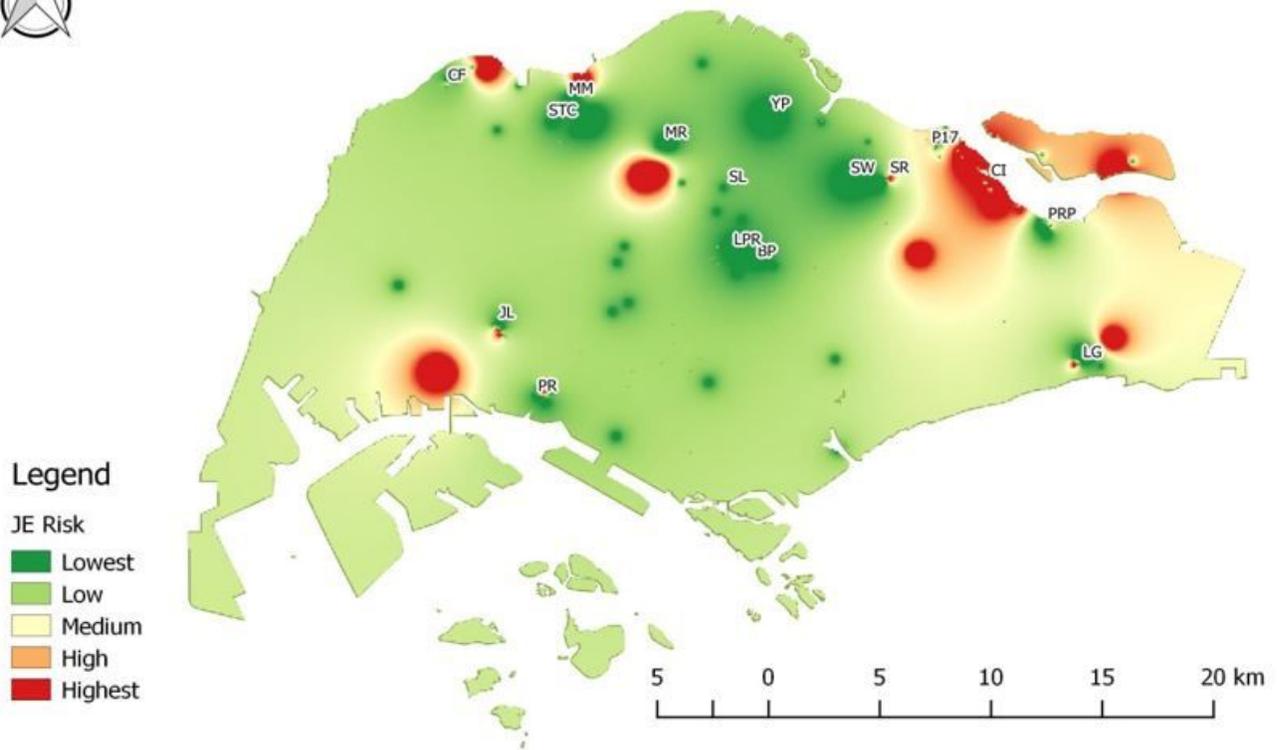


Figure 4

Spatial analysis of JE Risk Factors. The north-eastern part of Singapore represents the largest cluster of highest JE Risk. This is contributed by the abundance of all three Risk factors over a large area. The western part of Singapore carries the highest JE Risk near a wetland reserve in the north and an Avian Sanctuary in the south. The JE hotspot near MR is in the vicinity of the zoo there is a population of free-roaming herons.