JEV Ecology

Diyar Mailepessov (NEA/EHI)

Japanese Encephalitis Virus: Emerging Global Threat to Humans & Livestock

October 17-19, 2022

Biosurveillance: Risk assessment & forward defense for emerging and re-emerging vector-borne zoonotic diseases in Singapore

Identification of emerging & re-emerging threats

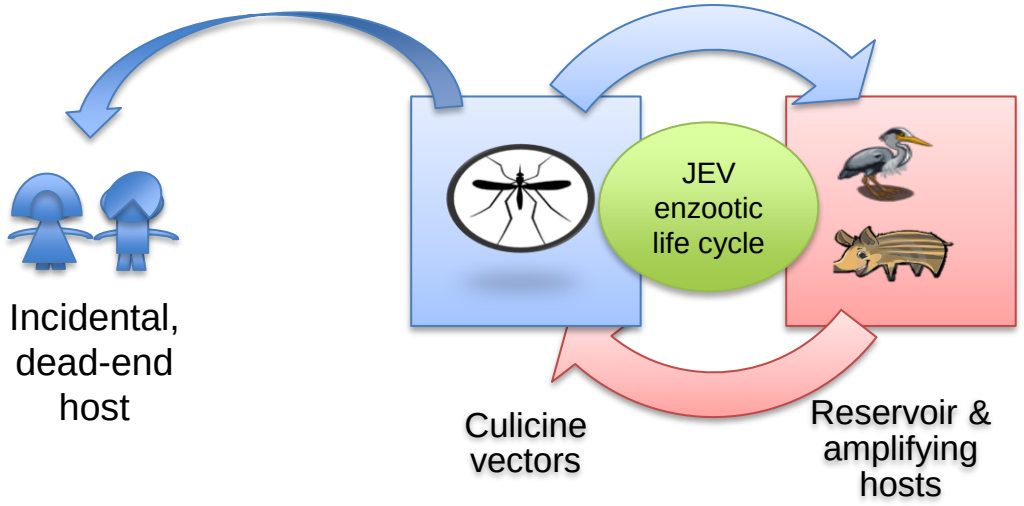
Hosts and modality of transmission

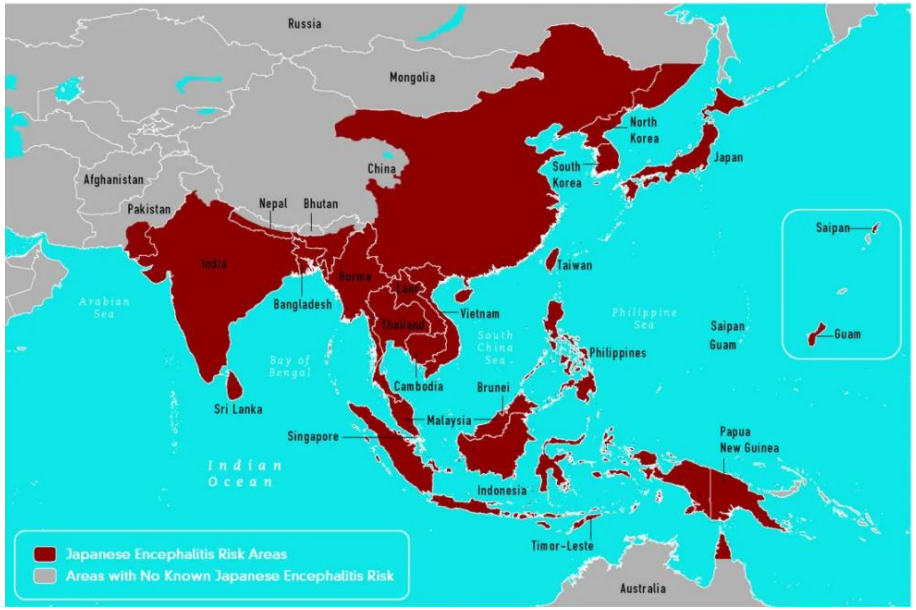
Key determinants of transmission

Mitigation plans & strategies

Understanding the risk of emerging vector-borne diseases

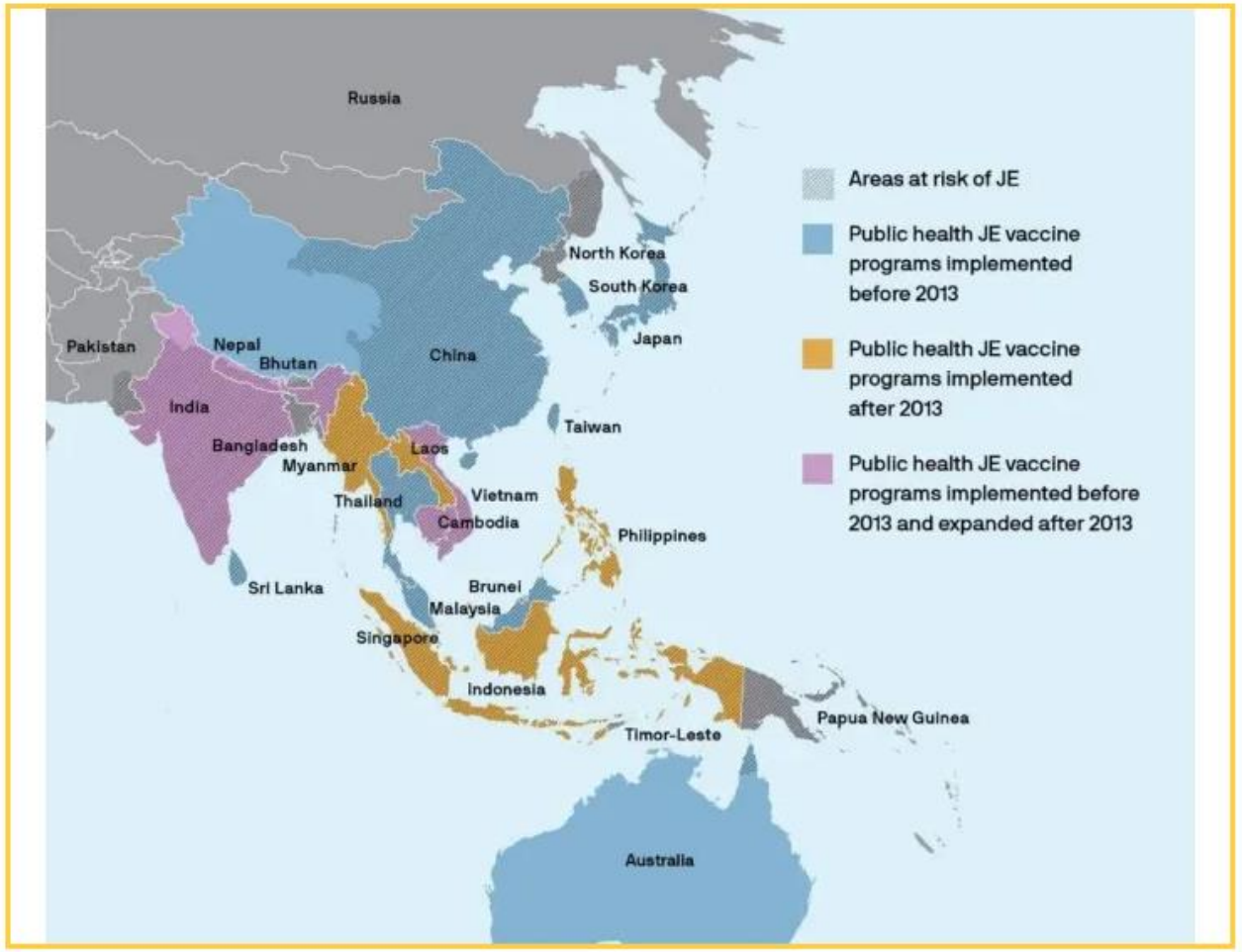
Transmission of Japanese Encephalitis in Singapore





- Human case of Japanese encephalitis was last reported in 2005
- Singapore is located within JEV endemic region
- Both reservoir and amplifying hosts and mosquito vectors of JEV are present on the island
- Located along EAAF

Could JEV be a public health issue in the future?



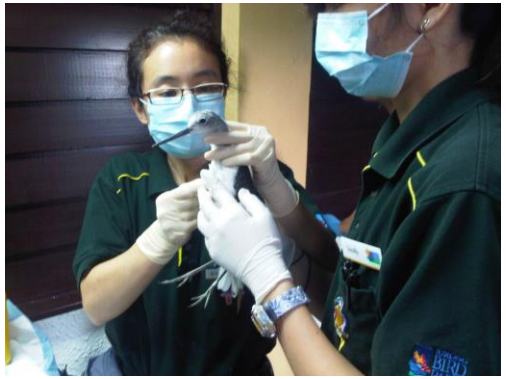
- Poor JE vaccination coverage
- Lack of systematic studies evaluating cost effectiveness
- Poor surveillance and vaccination data
- Vaccination efficacy/boosters
- Emerging genotypes

Vannice KS, Hills SL, Schwartz LM, Barrett AD, Heffelfinger J, Hombach J, Letson GW, Solomon T, Marfin AA. The future of Japanese encephalitis vaccination: expert recommendations for achieving and maintaining optimal JE control.

Presence of JEV specific antibodies suggested transmission in the wildlife

Animals (Surveillance period)	No. of samples tested	No. of positive samples (Year collected)	Seropositivity rate (%)	Neutralization Titer (Range)
Resident birds (2010-2017)	700	12 (2010-2013)	1.7	Not determined
Migratory birds (2010-2018)	819	24 (2010-2013)	2.9	Not determined
Wild boars (2013-2016)	154	10 (2013-2015)	6.49	10-1000
Sentinel chickens (2013-2018)	180	6 (2013-2014)	3.3	10-100

- Resident and migratory birds were positive for JEV antibodies
- Wild boars were positive for JEV neutralizing antibodies, often with high titers
- Naive sentinel chickens seroconverted (acquired JEV specific antibodies) after being placed at northwestern Nature Reserve



Yap G, Lim XF, Chan S, How CB, Humaidi M, Yeo G, Mailepessov D, Kong M, Lai YL, Okumura C, Ng LC. Serological evidence of continued Japanese encephalitis virus transmission in Singapore nearly three decades after end of pig farming. Parasites & vectors. 2019 Dec;12(1):1-7.

Both local and migratory birds were positive for JEV antibodies

Resident birds positive for JEV neutralizing antibodies highlight the risk of JEV transmission in areas where they nest

Sample ID	Bird Species	Location found	Date
DB2	Crested goshawk (Accipiter trivirgatus)	Central-western residental area adjoining CCNR	March 2011
DB3	White-bellied sea eagle (Haliaeetus leucogaster)	International Airport	March 2011
DB4	Changeable hawk-eagle (Nisaetus cirrhatus)	Western residential area 1	April 2011
DB9	Crested serpent eagle (Spilornis cheela)	Not available	May 2011
DB13	Oriental Honey-buzzard (Pernis ptilorhyncus)	Western Urban Park	May 2011
DB14	Black-crowned Night Heron (Nycticorax nycticorax)	Easten Urban Park	May 2011
DB20	White-bellied sea eagle (Haliaeetus leucogaster)	Northern transit hub adjoining Nature Reserve	Not available
DB31	White-bellied sea eagle (Haliaeetus leucogaster)	Central Catchment Nature Reserve (CCNR)	April 2011
DB38	White-bellied sea eagle (Haliaeetus leucogaster)	Western industrial area	March 2012
DB41	Cattle Egret (Bubulcus ibis)	Western residential area 2	July 2012
DB59	Little Egret (Egretta garzetta)	Western Urban Park	October 2012
DB71	Heron (not identified to species)	Avian Sanctuary	Not available

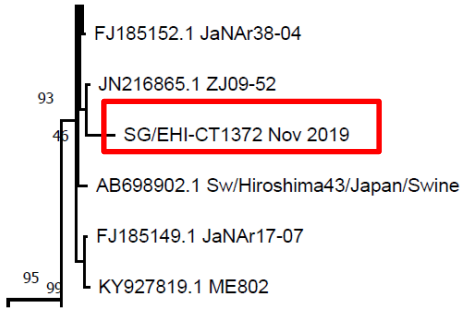
Migratory birds positive for JEV neutralizing antibodies highlight the risk of introduction of new JEV strains and other viruses

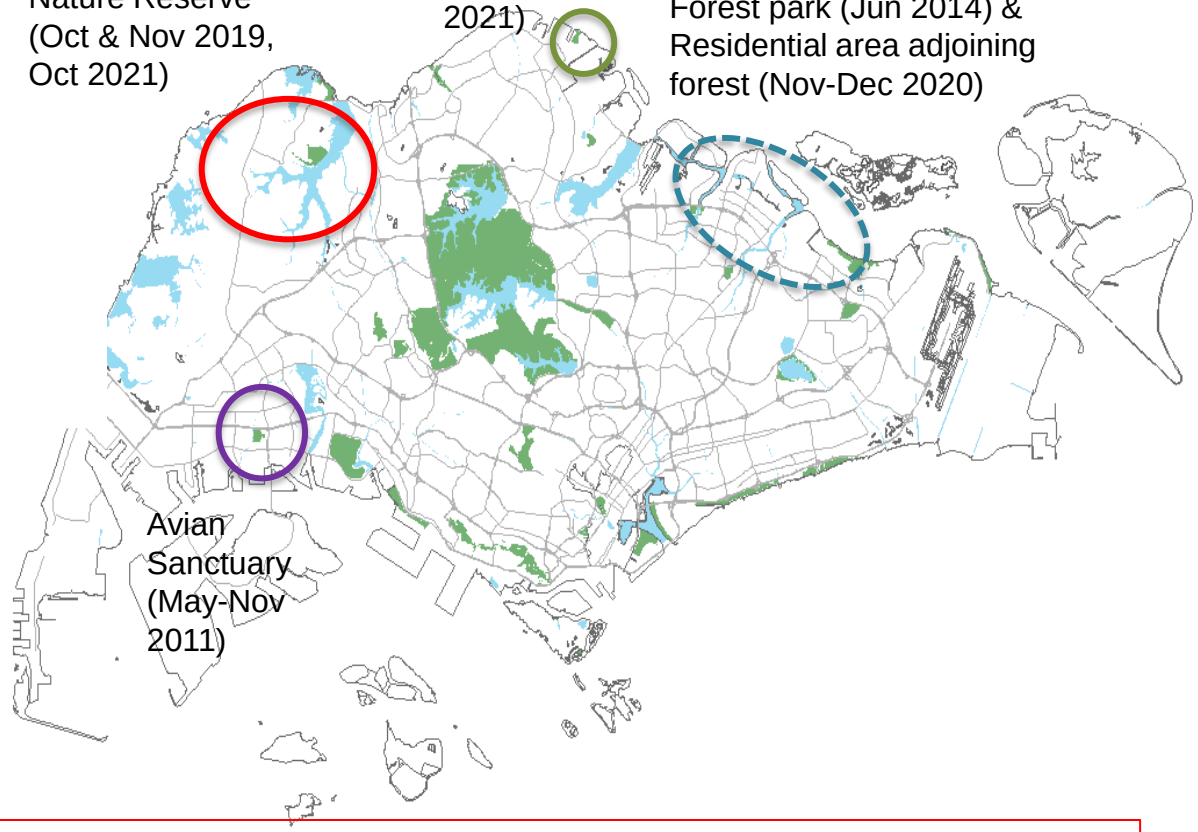
	Sero-positivity rate % (seropositive sera/total sera tested)				
Bird Species	2010-2011	2011-2012	2012-2013	2013-2014	2014-2015
Plovers	11.1 (1/9)	7.7 (3/39)	18.2 (2/11)	0 (0/2)	0 (0/4)
Common Redshank	29.4 (5/17)	52.9 (9/17)	26.6 (4/15)	0 (0/12)	0 (0/77)

Several JEV strains have been detected over the years

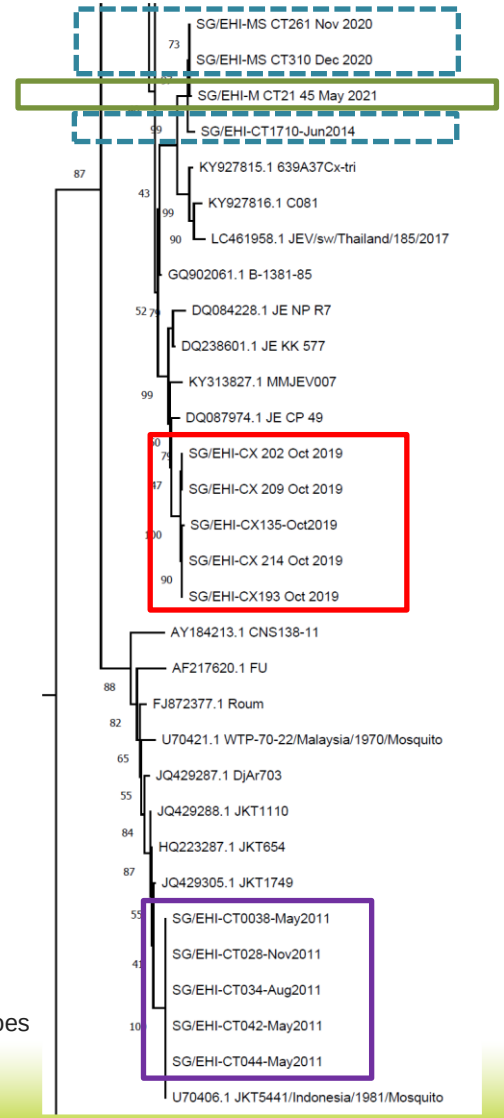
- **Phylogenetic analysis suggests continuous transmission of the same strains within the same area and spread to other areas in Singapore**

E-gene sequence that clusters with East Asian sequences





E-gene sequences that cluster with South-east Asian sequences



All strains were detected in *Culex tritaeniorhynchus* pools

Yap G, Mailepessov D, Lim XF, Chan S, How CB, Humaidi M, Yeo G, Chong CS, Lam-Phua SG, Lee R, Okumura C. Detection of Japanese encephalitis virus in culex mosquitoes in Singapore. The American journal of tropical medicine and hygiene. 2020 Sep;103(3):1234.

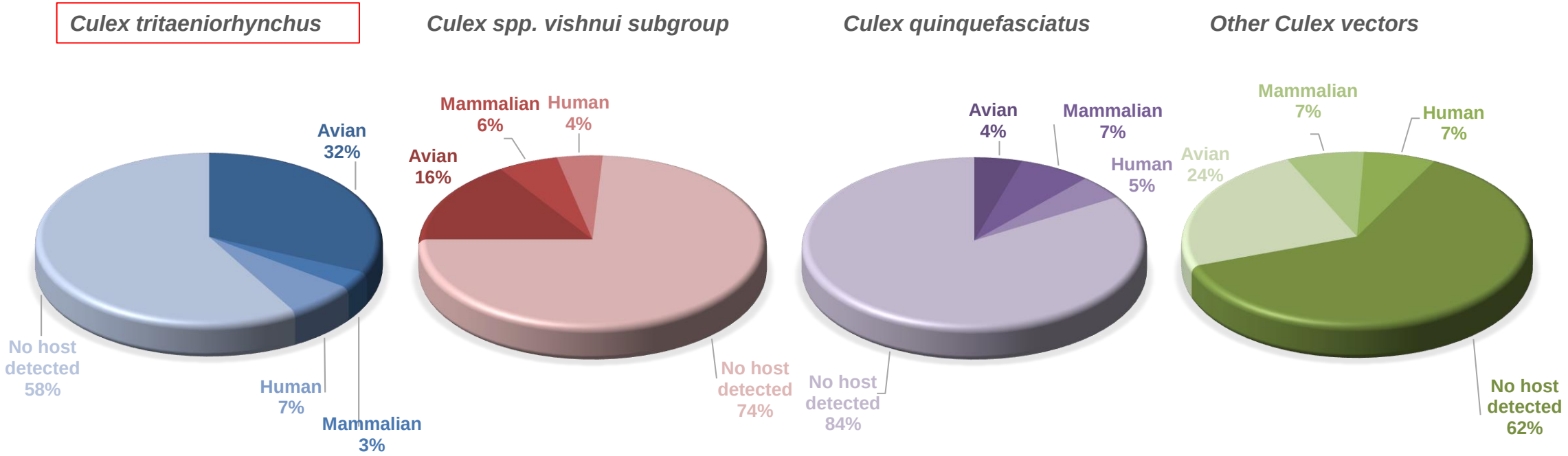
Lim MJ, Loh ZY, Yeo HL, Yenamandra SP, Kong M, Pang HY, Lee MH, Humaidi M, Chua C, Griffiths J, Ng LC, Hapuarachchi HC, Mailepessov D. Isolation and genetic characterization of Japanese encephalitis virus two decades after its elimination in Singapore, submitted for publication

Several JEV strains have been detected over the years

- **While low, the detection rate of JEV positive pools has increased over the years**

Study	Number of mosquitoes screened	Number of positive pools	Percent detection	Species virus detected in	Remarks
2010-2013 (Nature Reserve, Avian Sanctuary, Offshore Island, Farmway)	31,557	4	0.010%	*Culex tritae*	*Culex tritae* represented ~3% of all mosquitoes caught
2013-2016 Snapshot and Forest Park	85,692	1	0.001%	*Culex tritae*	*Culex tritae* represented ~91% of all mosquitoes caught
2019-2021 (Nature Reserves, Urban parks)	27,479	6	0.022%	*Culex tritae*	*Culex tritae* represented ~50% of all *Culex* mosquitoes caught
2020-2021 MSU mosquitoes (throughout the island)	4,137	3	0.072%	*Culex tritae*	*Culex tritae* represented ~61% of all *Culex* mosquitoes caught

Molecular analysis of bloodmeals reveals stronger preference of Cx. tritae for avian hosts, compared to other *Culex* vectors



- Study conducted from January 2011 to December 2012 at Nature Reserve, Farmway, Urban Park, and Offshore Island (generally in northern regions)
- 21,287 mosquitoes pooled 1- 50 per pool
- 29% of all mosquito pools were positive for host DNA
- Avian 57% of positive pools
- Mammalian 40% of positive pools

Yeo G, Chan S, How CB, Humaidi M, Lim XF, Mailepessov D, Chong CS, Phua-Lam SG, Lee R, Hapuarachchi HC, Ng LC. Molecular analysis of the bloodmeals of culex spp. mosquitoes at natural habitats in Singapore to investigate the potential risk of Japanese encephalitis virus and West Nile Virus transmission. Vector-Borne and Zoonotic Diseases. 2020 Sep 1;20(9):703-14.

Summary and risk assessment

- Detection of JEV/JEV antibodies in field mosquitoes and wildlife suggests that JEV is enzootic in Singapore

- Phylogenetic analysis suggests continuous circulation of certain JEV strains, with spread to other areas in Singapore

- Detection only in *Cx tritaeniorhynchus* to date suggests that this species should be prioritized for further study/control
  - Strong preference for avian bloodmeals may contribute to its role as a vector

- Risk of transmission to humans is considered low
  - Detection rates in *Cx* mosquitoes are very low (<0.1%)
  - *Cx tritaeniorhynchus* are nocturnal biters, and virus and the vectors are present in rural areas, often inaccessible to public at night

- However, continued surveillance/research is warranted
  - Increasing rate of JEV detection in mosquitoes suggests higher transmission rate of JEV compared to the past years
  - Climate change may impact vector life cycles and wildlife migration patterns
  - Would increasing push for a “City in Nature” concept create habitats for both hosts (birds) and mosquito vectors closer to/within urban settlements?

Future studies

- Understanding JEV mosquito vectors spread, seasonality and habitat
- JEV vector competence studies to rule out JEV spread by non-traditional species
- Continuous surveillance for JEV and other emerging viruses (Flaviviruses, Alphaviruses)
- Understanding the impact of climate change on mosquito/animal habitats in Singapore
- Understanding the impact of closer contact with JEV hosts and vectors on JEV transmission

Acknowledgement

EHI:
Ng Lee Ching
Sim Shuzhen
Wilson Tan
Joel Aik
Chee Seng Chong
Chanditha Hapuarachchi
Surya Pavan Yenamandra
Setoh Yin Xiang
Jerald Yam
Jeslyn Wong
Majhalia Torno

EHI Field Ecology Branch:
Low Zhi Yang
Lim Ming Jie
Yeo Hui Ling
Pang Hao Yang
Lee Meng Han
Sivaraman Sivanantham

Past members:
Grace Yap
Lim Xiao Fang
Mahathir Humaidi
Cliff Chua
Marcella Kong
Gladys Yeo

Malaria Surveillance Unit:
Rama Chandramogan
Ahmad Khair ABDUL AZIZ

Sharon Chan
Choon Beng How

Discussion