SPECIAL FEATURE: RESEARCH ON ZIKA VIRUS (ZIKV)

The outbreak of Zika virus (ZIKV) had taken centre-stage in healthcare news all over the world earlier this year. It represents such a serious emerging threat to public health that the World Health Organisation (WHO) declared it a global emergency in February 2016. Singapore had also experienced a major ZIKV outbreak in 2016, which was of great concern to the local residents and international travellers.

With the pressing need to understand the virus for development of disease control and preventive measures against this fearsome threat, research on the virus went into full throttle.

ZIKV and the Eye

A mosquito-bourne arvovirus of the Flavivirus genus was first isolated from a rhesus monkey during a study of wild yellow fever in Uganda in 1947 and first reported in humans in 1952 (Uganda). **Due to its self-limiting nature and similar clinical presentation as Dengue and Chikungunya viruses, the detection of disease is usually underwhelming.** With the spread of ZIKV globally, it calls for higher surveillance to prevent potential outbreaks.

ZIKV is transmitted mainly by Aedes mosquitoes in both urban and wild areas. Aedes aegypti (typically found in the tropics and subtropics) and Aedes albopictus (native to Europe, particularly Mediterranean countries) are the most common vectors. Aside from humans, mice and monkeys have been proven to be non-human hosts of ZIKV. ZIKV shares similar traits and life cycle as Dengue Virus (DENV) in urban environments, utilising mosquitoes as vectors and humans as hosts for viral propagation. **With ZIKV proliferation, it is possible that other known mosquito-bourne diseases like yellow fever, chikungunya, dengue, epidemic polyarthritis and new viruses might rise in numbers.**

Non-vector dependent modes of transmission include sexual transmission, perinatal trans-placental transmission and animal bites. Other routes of transmission yet to be explored are blood transfusion, lactation and bodily-fluid contact. **Blood transfusion is a potential route of transmission as 2.8% of blood donors had asymptomatic acute ZIKV infection.** Although viral RNA is detected in breast milk, no infected cases have been reported yet. ZIKV can stay in seminal fluid up to 2 months, indicating the importance of protected sex during and post-illness.

What is worth-noting is the latest discovery that in patients with conjunctivitis, uveitis or neuroretinitis, ZIKV can be shed from lacrimal glands or cornea, evidenced by presence of ZIKV RNA in mice tears, making this another potential route of transmission. Experimental infection of Zika virus in mice causes panuveitis and the presence of ZIKV RNA was detected in the tears and it could be also possible in humans with Zika virus infection and the infected tears may play a role in spreading the disease further. But the presence of ZIKV RNA in human tears in patients with Zika virus infection was not reported so far.

ZIKV can spread across blood-brain and ocular barriers to cause ocular symptoms.

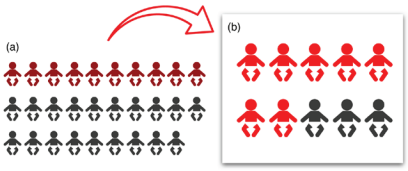
Ocular manifestations in adults

ZIKV can cause nonpurulent conjunctivitis in infected patients. Moreover, it may be linked to uveitis, unilateral acute idiopathic maculopathy and hypertensive iridocyclitis. In a patient with ZIKV RNA positive on reverse transcriptase - polymerase chain reaction (RT-PCR), bilateral nongranulomatous keratic precipitates and the cells in anterior chamber are seen. A patient with strongly-positive value on a serum plaque reduction neutralization technique (PRNT) experienced macular retinal pigment epithelium (RPE) changes with a grey annulus around the fovea on posterior segment examination and disruption of outer retinal and RPE integrity in the central macula evidenced on optical coherence tomography (OCT). Another case report discussed about a patient having bilateral hypertensive iridocyclitis after suspected ZIKV infection.

Ocular manifestations in newborns

Ocular manifestations of ZIKV are described in infants with mothers who experienced ZIKV symptoms during pregnancy. A case report from Brazil described 3 infants with presumable intrauterine ZIKV infection (evidenced by microcephaly and intracerebral calcifications) presenting with changes in the macula. All infants had gross macular pigment mottling and loss of foveal reflex, while one had a well-defined macular neuroretinal atrophy. No ocular presentations were noted in their mothers. Both mothers and children were only diagnosed clinically and no laboratory tests were performed to confirm ZIKV infection.

Another Brazilian study conducted in infants with microcephaly associated with suspected intrauterine ZIKV infection also showed similar results. Ocular findings were observed in 10 out of 29 infants (34.5%) and 7 in 10 had bilateral lesions. The abnormalities elaborated were posterior pole focal pigment mottling of the retina and chorioretinal atrophy in the macular area, optic nerve abnormalities such as optic nerve hypoplasia and severe optic disc cupping. One infant had iris coloboma and lens subluxation, though it is not clear if ZIKV was the cause. Out of 29 mothers, 23 (79.3%) had clinical signs and symptoms of ZIKV infection. The remaining 7 mothers might have asymptomatic ZIKV infection or have some other possible pathogenic agents for causing microcephaly and ocular lesions in infants. While reviewing potential congenital ZIKV cases, clinicians must bear in mind to rule out other differentials of chorioretinal lesions such as toxoplasmosis, cytomegalovirus, syphilis, rubella and herpes simplex virus.



Ocular findings were observed in 10 out of 29 infants (ref. a) (34.5%) and 7 in 10 had bilateral lesions (ref. b).

Ocular involvement is observed more in babies with mothers reporting infective symptoms in the first trimester and smaller cephalic diameter at birth. Latest interim guidance for evaluation and management of congenital ZIKV recommends performing an ophthalmology examination on infants with suspected congenital ZIKV infection within the first month of life. If the results are normal, a follow-up examination (with retinal assessment) should be done at 3 months of age. Any abnormalities will warrant an urgent referral to an ophthalmologist. Subsequent visits to the paediatrician should be accompanied by a visual screening.

It is imperative that in-utero ZIKV infection should be paid close attention to, as macular and chorioretinal disease in infants can have dire visual consequences, including blindness from chorioretinal scarring. As ZIKV is a relatively new disease trend, not many studies have been established. More clinical research studies should be carried out including proper serologic confirmation for ZIKV. It is likely that with more research, there will be more variations in ocular presentations. Ophthalmic screening of newborns in epidemic areas may be considered when more findings strengthen the correlation between congenital ZIKV infection and ophthalmic abnormalities.

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Understanding ZIKV - A Promising Step to Therapeutics

With the growing threat to public health, both protective vaccines and antiviral drugs are therefore urgently needed in order to control the spread of the ZIKV. **In collaboration with Experimental Therapeutic Centre, A*STAR, Asst Prof Luo Dahai (Nanyang Assistant Professor and Principal Investigator, Molecular Mechanisms of Viral Infection & Host Defense Laboratory, LKCMedicine) and his team have studied the Zika protein and found that the viral NS2B-NS3 protease that processes viral polyprotein within the host cell constitutes prime drug target.** Precise 3D information of the protease in presence of inhibitors will not only provide better understanding of the virus pathogenesis but also aid structure based drug discovery. It is found that the molecular structure of the NS2B-NS3 protease greatly advance the current understanding of the ZIKV protease dynamics and should accelerate structure-based antiviral drug discovery against ZIKV. The viral enzyme was captured along with a peptide inhibitor which provides an attractive starting point for further development of antivirals, against ZIKV and related flaviviruses like dengue virus. **Asst Prof Luo believes that the next step is to identify a suitable drug candidate to prevent the virus from replicating.**

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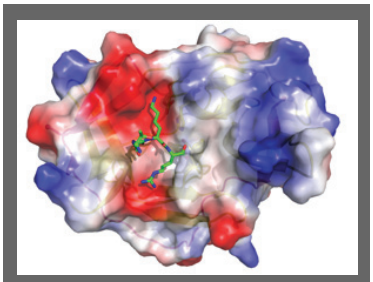
Contributed By **Nanyang Assistant Professor Luo Dahai,** LKCMedicine



Molecular structure of Zika virus NS2B-NS3 protease in complex with a peptide inhibitor (green sticks).

Outbreak Research Capabilities and Studies for ZIKV at the Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital

The Communicable Disease Centre (CDC) is an integrated part of the Institute of Infectious Diseases and Epidemiology (IIDE) and the research unit is one of its key departments. The unit has made a significant impact in conducting research during outbreaks and epidemics of emerging infectious diseases, such as the one caused by ZIKV in August to September 2016. The research unit is funded by a mix of NMRC Centre Grant and other grant funding, and comprises of the Infectious Diseases Research Office (IDRO), Infectious Diseases Research Clinic (IDRC) and Infectious Diseases Research Laboratory (IDRL), with data management and biostatistics support from staff at the Department of Clinical Epidemiology (DCE) and laboratory support from Tan Tock Seng Hospital's Department of Laboratory Medicine. The IIDE Research Unit is currently led by Asst Prof Mark Chen and supported by Dr Pang Junxiong Vincent, with guidance from Prof Leo Yee Sin, who is also the Principal Investigator of the CDC Centre Grant.

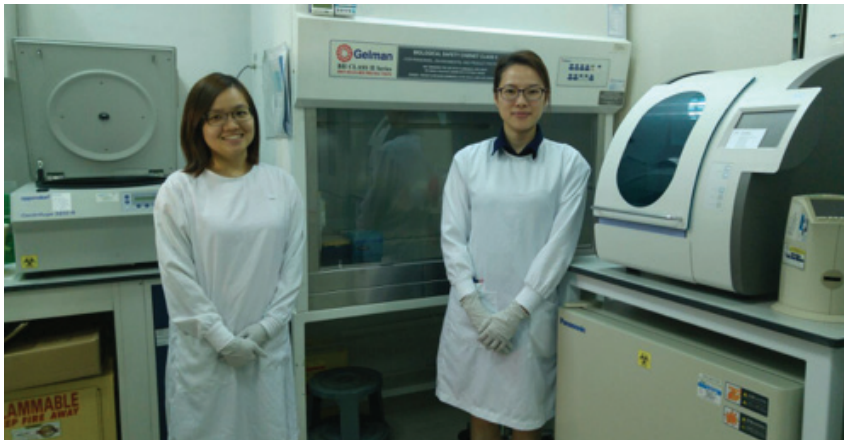
IIDE Research Unit (which houses IDRO and IDRL) at Block 812, Communicable Disease Centre 1.

Research Infrastructure

The Infectious Diseases Research Clinic was established in 2009 in response to a need to recruit patients from the Communicable Disease Centre for non-respiratory pathogen research studies such as dengue research. This research clinic has four well-furnished consultation rooms that provide full privacy for principal investigators to concurrently recruit and perform physical examination on eligible patients. It is also equipped with one phlebotomist room for blood draws as well as a cosy lounge for subjects who consented to research studies as they await research procedures during their follow-up visits.

The facilities in IDRC

The Infectious Diseases Research Laboratory (IDRL) @ IIDE is well-equipped with freezers, laboratory equipment and a biosafety cabinet for basic biological sample processing and storage in a safe and controlled environment. These samples are either transferred directly to collaborators for downstream scientific applications or stored at -80 degrees Celsius for future collaborations. The IDRL at IIDE is currently led by Dr Shawn Vasoo, and supported by Dr Pang Junxiong, Vincent, with guidance from Asst Prof Ng Oon Tek.



Staff at IDRL (From left): Miss Loh Xinyi and Ms Wong Lai Har

The facilities at IDRL

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Research Manpower & Development

Outbreak research is part of the portfolio of the Emerging and Viral Infectious Diseases cluster at IIDE. This is currently led by Asst Prof Yeo Tsin Wen, and supported by project manager Ms Linda Lee, with guidance from Prof Leo Yee Sin. **A mechanism has been put in place where the partnership between clinical and research staff enhances research recruitment, which is vital during outbreaks where we need to maximise recruitment of earlier subjects to facilitate early characterisation for a given infectious agent.** Research personnel in this research cluster are highly experienced and can respond within 24 hours for outbreak research (including after-office hours and weekends). They are also equipped to engage patients and explain the need for research in outbreak research studies. Most of them had a rich clinical or nursing background prior to joining IIDE, and that has helped them build a strong rapport with both the patients and clinicians in the Communicable Disease Centre to perform outbreak research.

All the research staff are also trained and updated in Good Clinical Practice, ethics as well as our internal outbreak research protocol: "A Multi-centered Prospective Study to Detect Novel Pathogens and Characterize Emerging Infections" ("PROTECT"). The aim of this protocol is to facilitate research of novel, previously undescribed pathogens and characterize associated clinical features. The protocol also enables us to prospectively characterize the transmission risk, clinical features, host and pathogen interactions and natural and treated history of emerging infectious disease pathogens, and allows for outbreak associated patients where a diagnosis has not yet been established, as may be the case in emerging infectious disease outbreaks. **The first ZIKV patient was recruited under this protocol before the diagnosis of Zika virus was confirmed after admission to TTSH.**

Research Staff involved in Zika Studies (From left): Dr Vincent Pang Junxiong, Mr Htet Lin Htun Danny, Miss Hsu Jung Pu, Ms Nadiah Bte A. Karim, Ms Linda K Lee and Miss Ling Wei Ping. Not in photo: Ms Tan Bee Har, Diana

The future of IIDE Research Unit

Moving forward to 2018, the IIDE Research Unit will also progressively transit into the new building at the National Centre for Infectious Diseases (NCID). NCID will be well-furnished with a Research Office with capacity for 60 staff with a full-fledged and integrated research clinic with five consultation rooms plus an in-house sample processing laboratory. In addition, there will be a multi-disciplinary research laboratory of net floor area 862m^2 co-sited at NCID with the Biosafety Level 3 (BSL-3) National Public Health Laboratory on the same floor, which will allow for better integration of laboratory-based research into emerging infectious disease pathogens. Lastly, there will be an inpatient Research Facility Phase 1 trial ward layout of about 20 beds. With these facilities, NCID aims to provide the necessary platforms to generate new knowledge and understanding of novel pathogens during outbreaks so as to guide decisions for prompt outbreak response and control.

Research Collaborations & Studies

By tapping onto the well-established network of collaborators we had previously built up through IIDE's Dengue Research Programme, we were able to rapidly initiate multiple collaborations with the aim to investigate the epidemiological, clinical, molecular and immunological characteristics of ZIKV infection.

Current collaborations with Prof Leo Yee Sin as site-PI include:

- The ZIKV patient cohort study with collaborators from KK Women's and Children's Hospital (A/Prof Chong Chia Yin), Singapore Immunology Network (SIgN) at A*STAR (A/Prof Lisa Ng) and TTSH Eye Department (Asst Prof Rupesh Agrawal);
- Collaboration with Health Science Authority to validate nucleic acid amplification techniques for blood bank testing;
- Collaboration with the Environmental Health Institute (A/Prof Ng Lee Ching) to validate commercial serology test kits;
- Collaboration with the Institute of Bioengineering and Technology at A*STAR (Dr William Sun and Prof Jackie Ying) to validate a Zika point-of-care test kit; and
- Study of risk factors and serological attack rate in the community with collaborators from Saw Swee Hock School of Public Health, NUS (Asst Prof Mark Chen)

Contributed By:
Dr Pang Junxiong Vincent, Ms Linda Lee, Asst Prof Mark Chen, Prof Leo Yee Sin, Institute of Infectious Diseases and Epidemiology, Tan Tock Seng Hospital

Congratulations on clinching the National Medical Research Council (NMRC) Research Training Fellowship (RTF) Award

Dr Barnaby Young
Consultant
Department of Infectious Diseases
Institute of Infectious Diseases and Epidemiology
Tan Tock Seng Hospital

Dr Young will be pursuing his PhD at the Lee Kong Chian School of Medicine, Nanyang Technologicial University, Singapore

Mr Li Ruijie
Senior Research Analyst
Health Services & Outcomes Research
National Healthcare Group

Mr Li will be pursuing his PhD at King's College, London

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