APNES Webinar

Date: 27th May (Thu), 2021

Time: 10:00-12:00AM (Taipei Time)(GMT +8)

Venue: Conference Rm 1, National Health Research Institutes, Miaoli, Taiwan

Attendees

Dr. Veasna Duong (Institut Pasteur du Cambodge, Cambodia), Prof. Dr. David Perera (Universiti Malaysia Sarawak), Dr. Nhan Le Nguyen Thanh (Children's Hospital No.1, Vietnam), Dr. Yoke-Fun Chan (Faculty of Medicine, University of Malaya), Dr. Min-Shi Lee (NHRI, Taiwan), Dr. Yeh-Sheng Chien (NHRI, Taiwan), Dr. Han-Jie Wu (NHRI, Taiwan), Dr. Steven Su (Unimed, Taiwan), Dr. Wen-Chiung Chang (NHRI, Taiwan) and Yao Chang (NHRI, Taiwan).

Chairperson: Dr. Min-Shi Lee

Enterovirus surveillance updates

Taiwan (Presented by Dr. Min-Shi Lee)

Taiwan had a large outbreak of EV-A71 in 1998. Since then, several enterovirus surveillance systems were set up as a counter measure. This includes EV infections with severe complications (report within 7 days), contract labs for EV-like illness (HFMD & Herpangina), real-time outbreak and disease surveillance system (RODS), and national health insurance outpatient claim data. In 2020, the Taiwan Centre for Disease Control has started using CODEHOP for pilot studies, expanding the current techniques in addition to virus isolation. However, the number of enterovirus isolates in 2020 were low. For cases with severe complications, only 6 cases were detected (5 EV-A71, 1 CV-A6) in 2020. Based on surveillance of mild enterovirus infections, several EV-A71 outbreaks occurred in 2012, 2016, and 2019. Taiwan has around 1000-2000 isolations every year, except 2020 which had around 190 isolations. Overall, the number of EV-A71 isolation decreased gradually and other enterovirus serotypes (CV-A6, CV-A10, CV-A16) become prevalent. In 2021, about 65 enterovirus infections were detected and most of them were related to coxsackievirus type A. In week 20, 4 EV-A71 cases were detected.

Vietnam:

(1) Hospital-based enterovirus surveillance in HCMC, Vietnam (Presented by Dr. Wen-Chiung Chang)

NHRI has collaborated with Children Hospital 1 (CH1) in Ho Chi Min city (HCMC) to conduct hospital-based enterovirus surveillance system since 2011. CH1 collected throat swab and serum specimens of about 30 HFMD cases per month. Virus culture was conducted in CH1 to determine EV and EV-A71 positivity. In addition, the specimens of throat swab and serum would be shipped to NHRI for conducting CODEHOP, a molecular test, and measuring neutralizing antibody against EV-A71. The average (range) of EV positive rate detected by CH1 was 26.2% (9.8-42.0%) and the average (range) positive rate of EV-A71 was 7.4% (1.6-29.6%). It is well observed that the positive rate by using CODEHOP was much higher, it was 54.7% on average, and 13.7% was EV-A71. Overall, the top 5 serotypes detected by using CODEHOP were CV-A2, CV-A6, CV-A10, CV-A16, and EV-A71. In addition, a seroprevalence study was conducted in 2012-2013 to collect serum samples of non-EV related inpatients for measuring the neutralizing antibody against EV-A71. About 23.5% of 1-year-old children in Southern Vietnam has been infected by EV-A71, and the median age of infection was estimated to be 3 years. No significant antigenic variation could be detected among the three EV-A71 genogroups (PLOS Neglected Tropical Diseases, Volume 14, Issue 3, 2020).

(2) HFMD in Children's Hospital 1(CH1-VN) (2019-2021) (Presented by Dr. Nhan Le Nguyen Thanh)

Children's Hospital 1 is a tertiary care, teaching, and research center for pediatrics in southern Vietnam, and is currently under expansion. In 2019 there were about 2,737 hand-foot-mouth disease (HFMD) admission cases in CH1-VN followed by 1,613 and 985 cases in 2020 and 2021, respectively. In Feb-June 2020 the number of HFMD inpatients decreased significantly, which may be related to prevention measures for Covid-19. However, the HFMD inpatients increased significantly after August 2020. The number of severe HFMD inpatients increased from 49 in 2019, to 51 in 2020 and 167 in 2021 (by May). The median age for severe HFMD in 2019-2021 was 18.3 months with interquartile range of 12.7 to 25.8 months. CH1 uses PCR and sequencing for identifying serotype and pathogens of HFMD inpatients. The top three pathogens identified in

June-Dec 2020 were untypable enteroviruses (40.6%), CV-A6 (22.9%) and EV-A71 (15%). In Apr-May 2021, majority of the HFMD inpatients were caused by EV-A71 (64.6%).

Cambodia (Presented by Dr. Veasna Duong)

EV-A71 continues to circulate predominantly among HFMD patients in Cambodia. The genotypes of circulating EV-A71 were mainly C4a with a few B5, starting with several possible introductions from China, Vietnam, and Thailand. Then it has become endemic in the country since 2017 and caused outbreak again in 2019. Epidemic cycle of HFMD is around every 2-3 years. EV-D68, a species of interest, since 2009 has circulated in Cambodia and their genotypes belong to Clade A2 which is phylogenetically related to the Philippines, and Clade B1 and B3 phylogenetically related to China. Current ongoing work also focuses on sequencing other non EV-A71 isolated in 2014-2016 and recent EV-A71 strains in the 2019 outbreak. On the other hand, surveillance effort ended after 2019 due to the lack of funding.

Malaysia

(1) Enterovirus Surveillance in East Malaysia (Presented by Prof. David Perera)

No single case of HFMD was reported in East Malaysia 2020. Since March 2020, Malaysia was placed under the Movement Control Order with the closing of schools and kindergartens. The main paediatrics ward that supplied samples to Prof. David Perera's lab only had up to 10% occupancy rate for infectious diseases. In 1997, Sarawak had an enterovirus outbreak and in 1998, Malaysia established surveillance programs in major hospitals in south central Sarawak and northern Sarawak. However, this was then discontinued in 2010 due to funding issues. As for now, the main methods being used for identifying of pathogens by the 2 main hospitals in Kuching and Sibu, we currently collaborate with is RT-PCR and sequencing. The state of Sarawak has recently announced the setting up of an infectious disease related institute (e.g., national emerging infectious disease institute), under the state of government, in which Prof. David Perera and Dr. Mong How Ooi would be involved. The institute is expected to begin operation by 2023/24.

(2) Enterovirus Surveillance in West Malaysia (Presented by Dr. Yoke-Fun Chan)

Similar to East Malaysia, there are currently no funding available for HFMD surveillance. Samples have not been received for the past 2 years, we have not isolated any enteroviruses. One of the

ongoing work is to investigate enterovirus in adults. Though viral pathogens (rhinovirus/enterovirus) were detected in severe acute respiratory infection (SARI) patients, further investigations are still needed to confirm the causation. It is known that enterovirus causes infection in adults and many were found with HRV-A, HRV-B, HRV-C, EV-D68, CV-B3 and a newer one EV-C104. In general, we use multiplex PCR and NGS for identification of enteroviruses in adult.

Thailand (Slide provided by Dr. Jiratchaya Puenpa)

Epidemics of enterovirus in Thailand follow a 2-3 years of cycle. The first large-scale HFMD outbreak was in 2012, which affected primarily infants and children. Another nationwide HFMD outbreak was in 2017 though not as severe as the early one. In 2019, the majority of EV cases are related to CV-A6 (29 cases) and CV-A16 (15 cases), followed by EV-A71 (17 cases). Genotypes of circulating EV-A71 in 2019 includes B5 (9), C1 (7) and C4 (1). In 2020, a total of 123 suspected cases were tested, enterovirus results were positive for 34 (27.6%) HFMD patients, including CV-A6 (7), CV-A5 (4) and EV-A71 (4). Genotypes of circulating EV-A71 in 2020 includes C4 (2), C1 (1), B5 (1). In 2021 a total of 7 suspected cases were tested and 5 of them were CV-A16.

Updates on Oxford Nanopore Platform for Enterovirus Surveillance by NHRI, Taiwan (Presented by Dr. Yeh-Sheng Chien)

The Oxford Nanopore sequencing platform was used to establish the enterovirus molecular surveillance system. This can be applied to:

1) Genome sequencing of enterovirus isolates.

Human enterovirus is composed of 4 species, including more than 100 serotypes. Due to frequent recombination, it is important to conduct genome sequencing for monitoring enterovirus evolution. The current technology using NGS for genome sequencing is expensive and labor-intensive. We established Nanopore platform (MinION) for genome sequencing of enterovirus. In this study, we tested 72 samples which includes 34 historical samples (sequenced by Illumina NGS before) and 38 new samples. Among the 34 historical samples, 100% of them could be sequenced successfully

using the Nanopore platform, with identity of more than 99.9% between the 2 sequencing platforms. Among the 38 new samples, 35 samples (92%) could be successfully sequenced using the Nanopore platform. Overall, the successful rate is 95.8% (69/72). Among the 3 samples which cannot be sequenced using the Nanopore platform, the cDNA quantity is very low (<0.2 ng/uL). Hence, we have established an economic (1/3 of Illumina NGS cost) and high-throughput platform for enterovirus genome sequencing, which is beneficial to the development of enterovirus research.

2) Establishment of rapid enterovirus serotyping platform using clinical samples

Traditional methods for detection and serotyping of enterovirus infections are virus isolation and immunofluorescence assay (VI-IFA), which are labor-intensive and time-consuming. Recently, *VP1* gene has been targeted to develop a CODEHOP-based RT-PCR (VP1-CODEHOP) for the same purpose. Previously, we conducted a 5-year enterovirus surveillance comparing the VI-IFA and VP1-CODEHOP tests and found that the VP1-CODEHOP is more reliable for detection of human enteroviruses than the VI-IFA. However, the VP1-CODEHOP relies on Sanger sequencing which requires expensive equipment and is time consuming. Therefore, we established Nanopore platform (Flongle) for sequencing of enterovirus partial *VP1* gene. In this study, we tested 93 positive (11 serotypes) and 3 negative samples based on traditional Sanger sequencing. The agreement between the two sequencing platforms is 88.5% (85/96). Interestingly, the Nanopore platform Flongle sequencing could only detect 56% (5/9) of the CV-A2 samples. It would be important to identify the reasons of low detection rate for CV-A2 virus. In conclusion, the Nanopore platform could determine serotypes of enterovirus infection within 24 hours using clinical samples and does not need expensive equipment. This Nanopore platform could be useful for enterovirus surveillance, especially in remote areas.

Recorded by: Yao Chang (NHRI, Taiwan)