Name：Jen-Ren Wang

Lab place：5786 room 4F, Department of Medical Laboratory Science and Biotechnology

E-mail：jrwang@mail.ncku.edu.tw

Research Interest：

My research focuses on viral surveillance, Genomic data establishment, virulence gene and pathogenesis analysis of influenza virus, enterovirus A71 and dengue virus. Surveillance of genetic evolution and identification of the virulence determinants may contribute to reduce the threat of virus infections.

Influenza viruses：

Influenza viruses can cause mild or severe clinical symptoms and even death as circulating in human population. Antigenic drift which accumulated mutations of viral RNAs may result in the antigenic change and escape from immune system. We continuously monitor viral activities and analyze gene evolution of influenza in Taiwan for years. We found that clinical isolates in southern Taiwan usually appear two or three seasons ahead of influenza vaccine recommendation by World Health Organization (WHO). We also analyze the antigenicity of circulating isolates to investigate whether there is antigenic change by using hemagglutination inhibition assay (HI). We found that sometimes the vaccine strains were mismatched with local strains and resulted in reduced antibody titer to circulating viruses after influenza vaccination.

We are also interested in the effect of viral evolution and genetic variations on viral properties and pathogenesis. For example, the multiple functions of nucleoprotein (NP) are to form homo-oligomers, encapsidate the virus genomes to maintain the structure and play roles in viral replication and transcription. To understand how NP contributes to the evolution of influenza virus, in our recent study, we analyzed NP gene of influenza A H3N2 viruses isolated from 1999 to 2017 and several evolutional substitutions were identified. The mini-genome assay was used to examine the effects of genetic variations of NP on polymerase activity. Among substitutions of the strain which displayed the highest polymerase activity, we found that the substitutions on body and head domain of NP gene have great impact on viral properties and may play the roles in epistasis in the viral evolution.

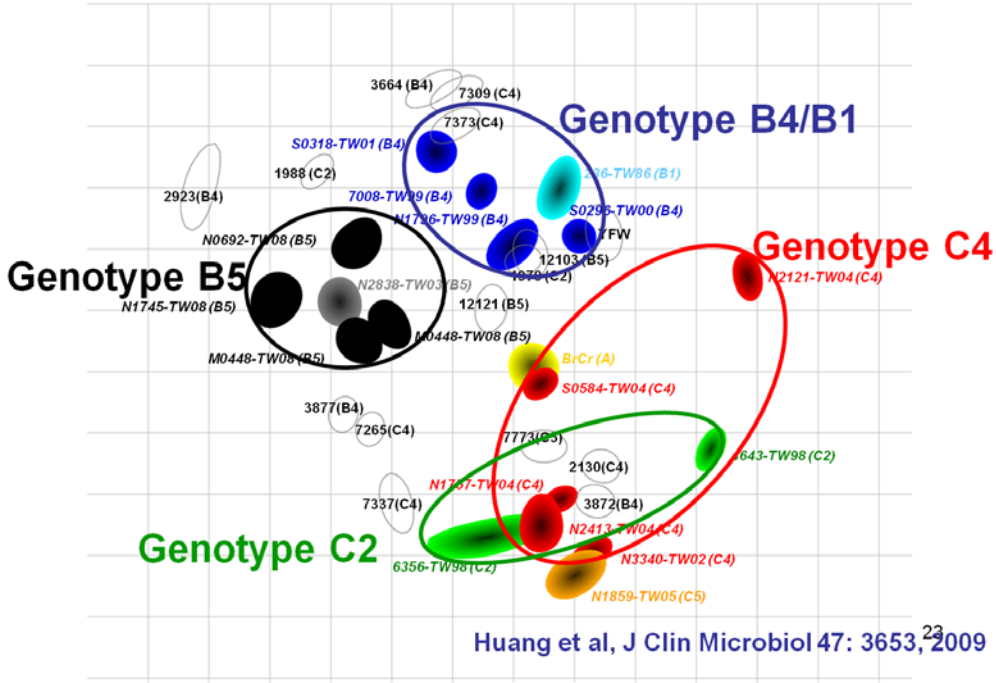
Enterovirus A71：

1. Evolutions and genetic variations of EV-A71

EV-A71-associated outbreaks have been reported worldwide and cause of numerous outbreaks of hand-foot-and-mouth disease. Phylogenetic studies indicated that the dominant EV-A71 strains circulating in the Asia-Pacific region varied genetically, suggesting that the virus was evolving. We performed full-length genomic sequences of EV-A71 circulating in Taiwan and found the occurrence of intra- and interserotypic recombination occurred frequently in the region encoding the nonstructural proteins and could potentially influence the replication, tissue tropism, and virulence of EV-A71.

2. Antigenic map

Dominant genotype of EV-A71 changes from B to C or C to B occurred at least three times between 1986 and 2008. Furthermore, antigenic cartography of EV71 by using neutralization tests revealed that the reemerging EV71 genotype B5 strains formed a separate cluster which was antigenically distinct from the B4 and C genotypes. Therefore, continuous surveillance of EV71 including the monitoring of genetic evolution and antigenic changes is recommended and may contribute to the development of a suitable vaccine for EV71.



3. Antigenic determinants

Phylogenetic studies have classified EV-A71 into genotypes A, B, and C, which can be further subdivided into subgentotypes B1 to B5 and C1 to C5. Neutralizing activities of human antisera against the various subgenotypes of EV-A71 indicate the antigenic changes of EV-A71. The structural protein VP1 contains key binding residues to host cell receptors

and antigenic sites for antibody recognition. We identified substitutions at residues 98, 145, and 164 in the VP1 capsid protein as antigenic determinants. This study provides an approach to map antigenic determinants of EV-A71 as well as to predict potential low antibody reactive mutations that may arise and cause a public health concern upon rolling out of EV-A71 vaccination programs.

4. Virulence determinants

a. 5’UTR-U158C

By comparison of two EV-A71 Isolates 237 and 4643 were isolated in Taiwan as of 1986 and 1998, we found that clinical isolate 4643 caused severe disease in humans; clinical isolate 237 caused only mild human infection. Accumulated reports have stressed contributions of 5’-UTR in viral replication and virulence of enteroviruses whereas evidence on the importance of 5’-UTR to EV-A71 virulence is limited. In our study, C158 identified in 5'-untranslated region plays a pivotal role of virulence determinant on virus translation *in vitro* and EV-A71 virulence in mouse.

b. 3D-T251I

The most severe outbreak occurred in 1998 resulting in 78 deaths in Taiwan but prior to the outbreak in 1998, sporadic cases of EV-A71 infection were reported in 1980 and 1986, which were not associated with death. Previous studies have shown the importance of the 3D region, which encodes the RNA dependent RNA polymerase, in virus replication. In this study, we identified that 3D-I251T mutation resulted in a strong temperature-sensitive phenotype. This phenomenon suggested the presence of the I251T mutation within the 3D region might contribute to attenuation in virulence of clinical virus isolates. EV-A71 in 1998 outbreak acquires the 3D-251I mutation which increases viral fitness and virulence.

c. VP1- Q145E and VP2-K149M

Mouse-adapted EV-A71 strain MP4 exhibited lower lethal dose than clinical isolate 4643 for intraperitoneal infection model in neonatal mice. We established a mouse-adapted EV-A71 infectious clone system to study mouse adaptation of EV-A71 and plotted correlation between infectivity and cytotoxicity *in vitro* and mouse lethality *in vivo*. The results showed that mutant virus with lysine to methionine substitution at VP2-149 (VP2-L149M) or glutamine to glutamic acid substitution at VP1-145 (VP1-Q145E) showed greater viral titers and apoptosis. Synergistic effect of VP2-149M and VP1-145E double mutations enhanced viral binding and RNA accumulation in infected Neuro-2a cells. The dual substitution mutants markedly reduced value of 50% lethal dose in neonatal mice infection, indicating they raised mouse lethality *in vivo*.

d. VP1-D31G

Due to low fidelity of RNA-dependent RNA polymerase, EV-A71 contains diverse quasispecies which may arise viral virulence depending on high genetic flexibility to adapt the selection pressures. As dissecting viral haplotypes from various tissues of fatal and

severe infected cases, we identified a novel haplotype containing a non-synonymous substitution at the VP1-31 residue in central nervous system. *In vitro* viral growth and fitness analyses indicated that VP1-31G conferred growth and a fitness advantage in human neuronal cells, whereas VP1-31D conferred enhanced replication in human colorectal cells. A higher proportion of VP1-31G was also found among fatal cases, suggesting that it may facilitate central nervous system infection in humans. Our data provide the first glimpse of EV-A71 quasispecies from oral tissues to the central nervous system within humans, showing broad implications for the surveillance and pathogenesis of this reemerging viral pathogen.

Dengue Virus：

1. Dengue Virus Clinical Test Comparison and Analysis (Tsai HP, et al., PLoS Negl Trop Dis. 2016. 12;10(10):e0005036.)

Every year, dengue virus has caused epidemics in Taiwan in varying degrees. In 2015, a large outbreak of dengue virus was reported in Tainan, with 112 deaths (0.5% mortality) among 22,563 infected patients. Dengue virus infection can cause diseases ranging from asymptomatic to severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), and even death. Therefore, correct, rapid and early detection of dengue virus infection is very much needed for clinical care. We analyzed 8989, 8954, and 1581 samples during a pandemic in 2015 and performed detection of NS1 antigen, IgM/IgG antibodies, and qRT-PCR virus levels, respectively. A total of 1581 samples were simultaneously tested for NS1 antigen, IgM/IgG antibodies, and qRT-PCR virus levels, among which 41.8%, 11.2%, 6.9%, and 40.2% were NS1, IgM, IgG, and qRT-PCR positive for virus, respectively. Comparing the NS1 antigen detection with the qRT-PCR viral load (LightMix assay), a positive correlation of 88.9% (1405/1581) was found. The sensitivity and specificity of the two methods were 89.4% and 100%, and 84.7% and 100% respectively. In this study, 79.5% of patients with severe illness were found to be ≥71 years old, 82.3% (14/17) had NS1/IgM/IgG (+/-/-) test results, and the virus titre was 10^6–10^9 copies/mL which is significantly higher than those of other age groups. Taken together, the above results show that the LightMix assay dengue viral load is conducive to the diagnosis of dengue virus infection and elderly patients, the high viral load of dengue virus can be used as a positive indicator of disease severity and mortality.

2. Dengue Virus Genetic Evolution, Virus Quasispecies and Disease Severity

Many studies point to the correlation between the severity of the disease and the dengue virus gene sequence variations. Due to the nature of RNA viruses, their high mutation rates produce a population of closely related but genetically diverse viruses, termed quasispecies. The role of quasispecies in DENV disease severity is yet to be determined. It is helpful to understand the relationship between gene evolution and changes in the characteristics of

dengue virus, and to understand the pathogenesis of the virus. In 2015, a large DENV outbreak in southern Taiwan resulted in over 43,000 dengue cases, where over 22,000 cases with 112 deaths were reported in Tainan City alone. From the 2015 outbreak, 22 DENV (10 mild, 12 fatal) were isolated, amplified with RT-PCR, and sequenced with Next Generation Sequencing (NGS: Illumina Miseq). Using QuasiRecomb and LoFreq programs, the sequenced data were analyzed. A total of 250, 51, and 4 positions with p-values <0.05, <0.01, and <0.001 respectively, were filtered out with QuasiRecomb. On the other hand, analysis by LoFreq showed 6 positions with $p<0.05$. Taking $p<0.001$ and $p<0.05$ from QuasiRecomb and Lofreq respectively, a total of 10 positions were selected, of which 1 and 9 positions were located in the structural and non-structural (NS) region, respectively. The NS region of DENV, like many other Flaviviruses, is responsible for the replication of new viruses. Therefore, with respect to the analyzed data, the 10 positions may provide information on the difference in disease severity during the DENV epidemic.