WIV Newsletter
No.15 MAR 2017

French Prime Minister Visits Wuhan P4 Laboratory

On Feb. 23, French Prime Minister Bernard Cazeneuve visited Wuhan National Biosafety Level 4 (P4) Laboratory in Wuhan Institute of Virology (WIV), Chinese Academy of Sciences (CAS), which was his first stop in Wuhan. More than 100 people from different administrative entities including CAS, China National Accreditation Service for Conformity Assessment (CNAS), Ministry of Foreign Affairs of China and French National Institute of Health and Medical Research (INSERM) that have participated in the entire program on Sino-French Cooperation on Emerging Infectious Diseases and provided tremendous help in the past decade attended the activity.

Mr. Bernard Cazeneuve attended the ribbon-cutting ceremony and paid a visit to Wuhan P4 laboratory interior. In his speech, Mr. Cazeneuve declared France is proud of building jointly with China the first national P4 Laboratory. And he added that, as epidemic knows no borders, a united world society is a must to win over the challenge like Ebola to public health in recent years. Wuhan P4 Laboratory will be our front line of emerging infectious diseases prevention and control. France will join hands with China to firmly dedicate to operating top-notch scientific research to response to the diseases. He thanked Chinese government and all the contributors' extraordinary contributions, and wished more in-depth scientific cooperation on emerging infectious diseases around P4 laboratories will be developed by our two nations.

Mr. Zhiming Yuan introduced the laboratory to Mr. Bernard Cazeneuve.

French Prime Minister, Mr. Bernard Cazeneuve (left two), the Vice President of CAS, Mr. Yaping Zhang (left one), and the Director of Wuhan P4 Laboratory, Mr. Zhiming Yuan (left three)

Mr. Yaping Zhang, Vice President of CAS pointed out, that the laboratory will help China to strengthen the capability of preventing and controlling outbreaks of emerging infectious diseases and aid scientific research and development of

Spotlight

Mr. Cazeneuve congratulated Wuhan P4 Laborotory on being issued the certificate by CNAS.

antiviral drugs and vaccines. He hoped that the laboratory will play an active role in the Sino-French cooperation on prevention and control of emerging infectious diseases, so as to benefit not only our two peoples but also the entire world.

On behalf of CAS, Vice President Yaping Zhang made an on-site speech.

According to Mr. Zhiming Yuan, Director of Wuhan P4 Laboratory, China is positively undertaking the responsibility and obligation to ensure global public health security. He emphasized that transparency is the cornerstone of the laboratory, and an open culture is of vital importance to guarantee the security of Wuhan P4 Laboratory.

After the visit, Mr. Yves LECY, Director of INSERM, Mr. Hervé RAOUL, Director of Jean Mérieux-Inserm Biosafety Level-4 Laboratory in Lyon, Mr. Dianwen Cao, Vice President of Bureau of International Cooperation, CAS, and stakeholders from CNAS, Chinese Center for Disease Control and Prevention and WIV attended the Meeting on Sino-French Scientific Cooperation around Biosafety Level 4 Laboratories. The meeting was aimed at upgrading the bilateral strategic and cooperative partnership and further expanding the dimension and depth of cooperation between laboratories in Wuhan and Lyon.

The Deputy Director General, Ms. Yanyi Wang was granted the P4 laboratory accreditation from Mr. Jianhua Xiao, the Secretary General of CNAS.

Wuhan P4 Laboratory, as one of the mega scientific cooperation programs under the Sino-French Cooperation Framework Agreement, was designed by French and Chinese design units, and was installed and built by Chinese part. The Laboratory has been issued the certificate by CNAS in January and will be fully operational soon. As an essential platform for research and development against high contagious and infectious diseases, it will inevitably provide critical and technical supports for scientists from the world to fight against life-threatening infectious diseases.

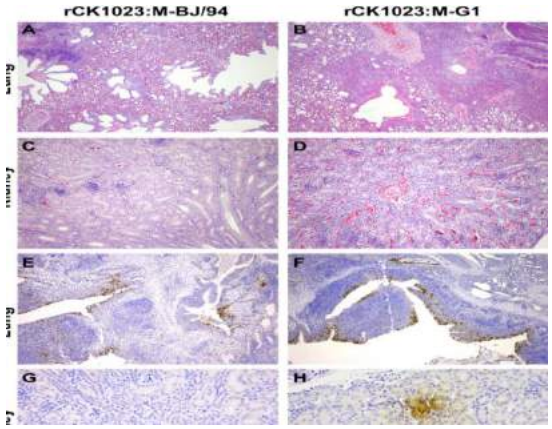
Image Source: *Changjiang Daily*

Scientists reveal that M gene reassortment in H9N2 influenza virus promotes early infection and replication

Segment reassortment and base mutagenesis of influenza A viruses are the primary routes to the rapid evolution of high fitness virus genotypes.

Scientists from China Agricultural University, University of Nottingham and Wuhan Institute of Virology recently described a predominant G57 genotype of avian H9N2 viruses that caused country-wide outbreaks in chickens in China during 2010-2013 which led to the zoonotic emergence of H7N9 viruses. One of the key features of the G57 genotype is the substitution of the earlier BJ/94-like M gene with the G1-like M gene of quail origin.

They reported on *J Virol.* the functional significance of the G1-like M gene in H9N2 viruses in conferring increased infection severity and infectivity in primary chicken embryonic fibroblasts and chickens. H9N2 virus housing the G1-like M gene, in place of BJ/94-like M gene, showed early surge in viral mRNA and vRNA transcription that were associated with enhanced viral protein production, and with early elevated release of progeny virus comprising largely spherical



rather than filamentous virions. Importantly, H9N2 virus with G1-like M gene conferred extrapulmonary virus spread in chickens. Five highly represented signature amino acid residues (37A, 95K, 224N and 242N in M1 protein, and 21G in M2 protein) encoded by the prevalent G1-like M gene were demonstrated as prime contributors to enhanced infectivity. Therefore, the genetic evolution of M gene in H9N2 virus increases reproductive virus fitness, indicating its contribution to rising virus prevalence in chickens in China.

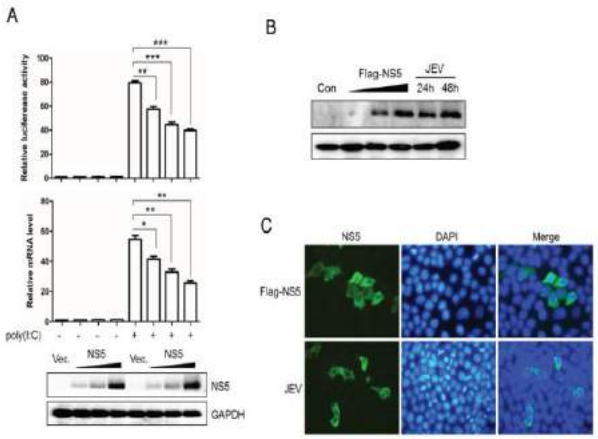
Link: *http://jvi.asm.org/content/early/2017/01/26/JVI.02055-16.long*

A novel strategy for JEV to escape the host innate immune response is revealed

JEV is the major cause of viral encephalitis in South and Southeast Asia with high mortality. However, the molecular mechanisms contributing to the severe pathogenesis are poorly understood. The ability of JEV to counteract the host innate immune response may be one of the potential mechanisms responsible for JEV virulence.

In present study, research fellows from Huazhong Agricultural University and Wuhan

Research Progress



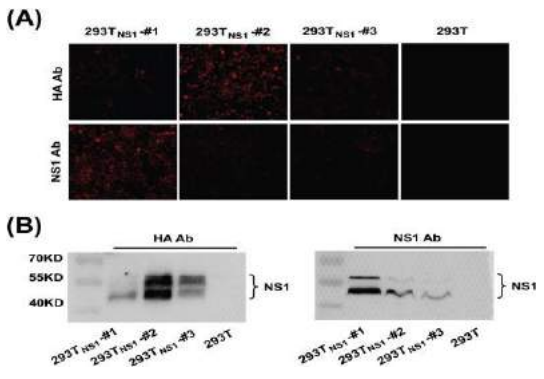
Institute of Virology found that Japanese encephalitis virus (JEV) NS5 protein could inhibit double strand RNA (dsRNA)-induced IFN-β expression in a dose-dependent manner. Their data further demonstrated that JEV NS5 suppressed the activation of IFN transcriptional factors, IRF3 and NF-κB. However, there was no defect in the phosphorylation of IRF3 and degradation of IκB, an upstream inhibitor of NF-κB, upon NS5 expression, indicating a direct inhibition of the nuclear localization of IRF3 and NF-κB by NS5. Mechanically, NS5 was shown to interact with the nuclear transport proteins, KPNA2, KPNA3 and KPNA4, which competitively blocked the interaction of KPNA3 and KPNA4 with their cargo molecules, IRF3 and p65, a subunit of NF-κB, and thus inhibited the nuclear translocation of IRF3 and NF-κB. Furthermore, overexpression of KPNA3 and KPNA4 restored the activity of IRF3 and NF-κB and increased the production of IFN-β in NS5-expressing or JEV-infected cells. Additionally, an up-regulated replication level of JEV was shown upon KPNA3 or KPNA4 overexpression. These results suggest that JEV NS5 inhibits the induction of type I IFN by targeting KPNA3 and KPNA4.

Link: *http://jvi.asm.org/content/early/2017/02/02/JVI.00039-17.long*

A stable and safe tool to handle West Nile virus outside of a BSL-3 facility

West Nile virus (WNV), a mosquito-borne flavivirus, is an important neurotropic human pathogen. As a biosafety level-3 (BSL-3) agent, WNV is strictly to BSL-3 laboratories for experimentations, thus greatly hindering the development of vaccine and antiviral drug.

Recently, the research group led by Prof. Bo Zhang in WIV, developed a novel pseudo-infectious WNV reporter virus expressing the Gaussia luciferase (Gluc). A stable 293TNS1 cell line expressing NS1 was selected for trans-supplying NS1 protein to support the replication of WNV-ΔNS1 virus and WNV-ΔNS1-Gluc reporter virus with large-fragment deletion of NS1. WNV-ΔNS1 virus and WNV-Gluc-ΔNS1 reporter virus were confined to complete their replication cycle in this 293TNS1 cell line, displaying nearly identical



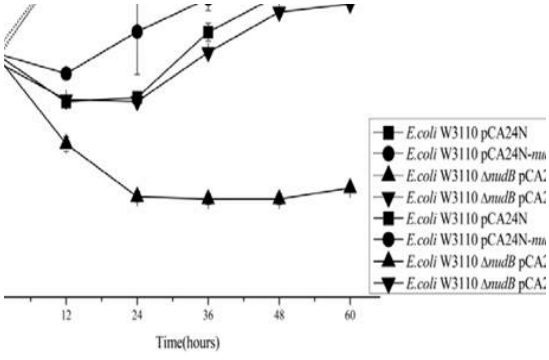
growth kinetics to WT WNV although the viral titers were lower than those of WT WNV. The reporter gene was stably maintained in virus genome at least within three rounds of passage in 293TNS1 cell line. Using a known flaviviruses inhibitor, NITD008, we demonstrated that the pseudo-infectious WNV-Gluc-ΔNS1 could be used for antiviral screening. Furthermore, a high-throughput screening (HTS) assay in a 96-well format was optimized and validated using several known WNV inhibitors, indicating that the optimized HTS assay was suitable for high-throughput screening WNV inhibitors. Our work provides a stable and safe tool to handle WNV outside of a BSL-3 facility and facilitates high throughput screening for anti-WNV drugs.

Link: *http://www.sciencedirect.com/science/article/pii/S0166354216306131*

Scientists find that deletion of nudB causes increased susceptibility to antifolates in Escherichia coli and Salmonella enterica

Co-trimoxazole, a fixed-dose combination of sulfamethoxazole (SMX) and trimethoprim (TMP), has been used for the treatment of bacterial infections since the 1960s.

Since it has long been assumed that the synergistic effects between SMX and TMP are the consequence of targeting 2 different enzymes of bacterial folate biosynthesis, 2 genes (pabB and nudB) involved in the folate biosynthesis of Escherichia coli were deleted, and their effects on the susceptibility to antifolates were tested by the reseach group led by Prof. Jiaoyu Deng in WIV, under the collaboration with Prof. Xian'en Zhang from Institute of Biophysics. the results showed that the deletion of nudB resulted in a lag of growth in minimal medium, and increased susceptibility to both SMX and TMP. Moreover, deletion of nudB also greatly enhanced the bactericidal effect of TMP. To elucidate the mechanism of how the deletion of nudB affects the bacterial growth and susceptibility to antifolates, 7, 8-dihydroneopterin and 7, 8-dihydropteroate were supplemented into the growth medium. Although those metabolites could restore bacterial growth, they had no effect on the susceptibility to the antifolates. Reverse mutants of the nudB deletion strain were isolated to further study the mechanism of how the deletion of nudB affects the susceptibility to antifolates. Targeted sequencing and subsequent genetic studies revealed that the disruption of the tetrahydromonapterin biosynthesis pathway could reverse the phenotype caused by the nudB deletion. Meanwhile, overexpression of folM could also lead to increased susceptibility to both SMX and TMP. These data suggested that the deletion of



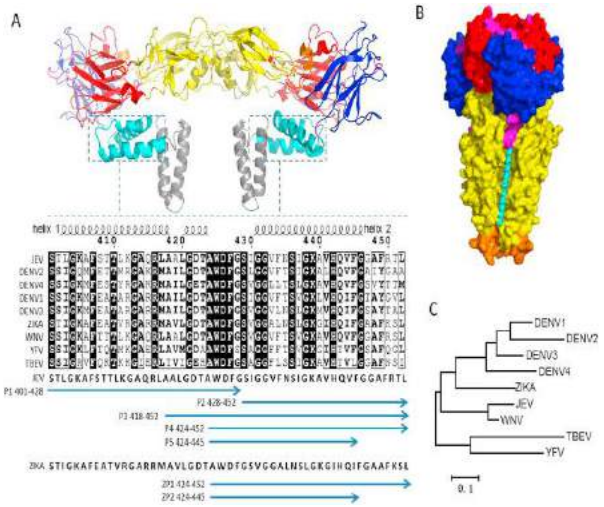
nudB resulted in the excess production of tetrahydromonapterin, which then caused the increased susceptibility to antifolates. In addition, they found that the deletion of nudB also resulted in the increased susceptibility to both SMX and TMP in Salmonella enterica Since dihydroneopterin triphosphate hydrolase is an important component of bacterial folate biosynthesis and the tetrahydromonapterin biosynthesis pathway also exists in a variety of bacteria, it will be interesting to design new compounds targeting dihydroneopterin triphosphate hydrolase, which may inhibit bacterial growth and simultaneously potentiate antimicrobial activities of antifolates targeting other components of folate biosynthesis.

Link: *http://aac.asm.org/content/early/2017/02/14/AAC.02378-16.long*

The uncoupling of catalysis and translocation in the viral RNA-dependent RNA polymerase

Japanese encephalitis virus (JEV) and Zika virus (ZIKV) are mosquito-borne viruses of the Flavivirus genus that cause viral encephalitis and congenital microcephaly, respectively, in humans, and thus present a risk to global public health. The envelope glycoprotein (E protein) of flaviviruses is a class II viral fusion protein that mediates host cell entry through a series of conformational changes, including association between the stem region and domain II leading to virion-target cell membrane fusion.

In the study led by scientists in the Research Group of HIV Viral Biochemistry in WIV, whether peptides derived from the JEV E protein stem can prevent JEV infection were investigated. Peptides from either helix 1 or 2 showed inhibitory activity against JEV in vitro; one of these—designated P5—had a 50% inhibitory concentration (IC50) of 3.9 nM in BHK-21 (baby hamster kidney) cells and protected against JEV infection in a mouse model. Consistent with the high degree of conservation among flavivirus stem sequences, they also found that P5 Inhibited ZIKV infection, with an IC50 at the micro-



molar level. Similarly, a peptide from helix 2 of the ZIKV E protein stem blocked ZIKV infection. Moreover, in a type I and II interferon (IFN) receptor-deficient mouse model, P5 was proved to reduce the histopathological damages in brain and testes resulting from ZIKV infection. These findings provide a basis for the development of peptide-based drugs to prevent JEV and ZIKV infection.

Link: *http://www.sciencedirect.com/science/article/pii/S0166354216306118*

Research Progress

The uncoupling of catalysis and translocation in the viral RNA-dependent RNA polymerase

The nucleotide addition cycle of nucleic acid polymerases includes two major events: the pre-chemistry active site closure leading to the addition of one nucleotide to the product chain; the post-chemistry translocation step moving the polymerase active site one position downstream on its template. In viral RNA-dependent RNA polymerases (RdRPs), structural and biochemical evidences suggest that these two events are not tightly coupled, unlike the situation observed in A-family polymerases such as the bacteriophage T7 RNA polymerase.

Recently, in a paper written by Prof. Peng Gong and Dr. Shu Bo in WIV and published on *RNA Biol.*, an RdRP translocation intermediate crystal structure of enterovirus 71 shed light on how translocation may be controlled by elements within RdRP catalytic motifs, and a series of poliovirus apo RdRP crystal structures explicitly suggest that a motif B loop may assist the movement of the template strand in late stages of transcription. Implications of RdRP catalysis-translocation uncoupling and the remaining challenges to further elucidate RdRP translocation mechanism are also discussed.

Link: *http://aac.asm.org/content/early/2017/02/14/AAC.02378-16.long*

Cooperation

WIV gains supports from CAS President's International Fellowship Initiative

In 2017, WIV has been approved 2 program applications from CAS President's International Fellowship Initiative (PIFI). Under these programs, two international scientists will come to WIV for carrying out collaborative researches.

As a distinguished scientist under PIFI, Prof. George R. Stark Lerner from Research Institute, Cleveland Clinic will visit the Research Group of Prion Cell Biology led by Prof. Chaoyang Li in WIV for a week in May this year. They plan to further discuss the project that involves uncovering the mechanistic underpinnings of prion regulation of interferon. Besides, Dr. Sheila Ommeh from Jomo Kenyatta University of Agriculture and Technology, will be a visiting scientist to WIV from September to December. She will work in the Emerging Viruses Group led by Zhengli Shi. They will carry out the proposed project on virus discovery of public health concern from camels, poultry and pigs in Kenya, which leads to

Cooperation

better diagnosis and effective vaccine development and will be a great milestone towards improved disease surveillance and pandemic preparedness in Kenya and the rest of Eastern Africa.

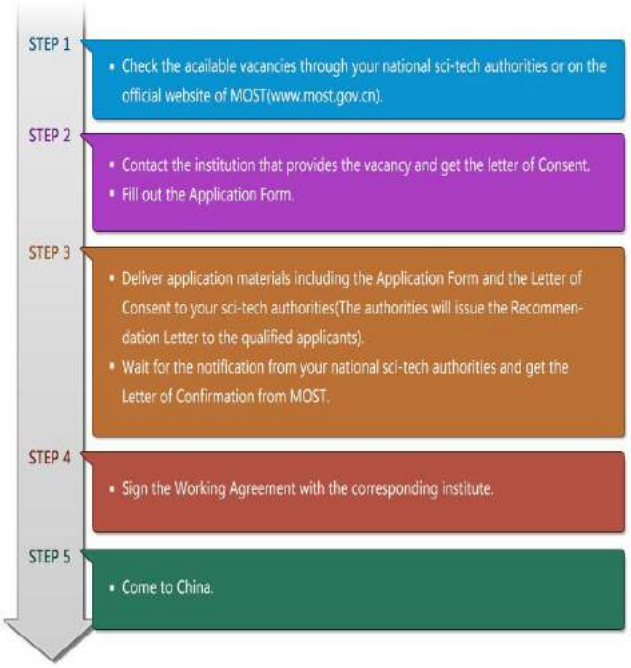
The PIFI program set by CAS aims to support highly-qualified international scientists and postgraduate students to work and study at CAS institutions and strengthen their scientific collaboration with CAS researchers. The PIFI program is available for four categories of international researchers and students: distinguished scientists, visiting scientists, postdoctoral researchers and international PhD students.

For more details: *http://english.cas.cn/cooperation/fellowships/201503/t20150313_145274.shtml*

MOST Talented Young Scientist Program

The Talented Young Scientist Program (TYSP) from Mistry of Science and Technology (MOST) supports talented young scientists, scholars and researchers from Afro-Asian countries to work in Chinese research institutes, universities or enterprises. TYSP aims to promote communication among Afro-Asian science and technology talents, nurture young science and technology leaders, and foster long-term international cooperation among research institutes, universities and enterprises in Afro-Asian countries. Ministry of Science and Technology of China (MOST) will provide each scientist with RMB ¥12500 per month for accommodation, insurance and other living expenditure during the program.

For more details: *http://www.tysp.org/English*



Science Tips

Schmallenberg Virus confirmed in Scottish sheep flocks

A virus which can cause stillbirths or birth defects in livestock has been confirmed in lambs in two Scots flocks near the border with England.

SAC Consulting veterinary services diagnosed the cases of Schmallenberg Virus (SBV) in the past two weeks. It follows the increasing numbers of affected lambs

Science Tips

Farmers with concerns over possible incidents have been advised to speak to their vet. Photo by GETTY IMAGES.

identified in England and Wakes throughout the winter. Farmers seeking advice on possible incidents of SBV in their animals have been advised to speak to their vet. SBV was first detected in the UK in southern England in January 2012. It is spread by midges and can cause brain and limb deformities in lambs and calves.

George Caldow, head of SAC Consulting veterinary services, said: "It can be difficult to predict how widespread any infection will turn out to have been or to be but there are some important points that give us an indication of the likely impact that SBV infection will have this spring on Scottish livestock.

"In winter 2016-2017 SRUC vets have not diagnosed SBV in either early lambing flocks in Scotland or in all year round calving dairy herds in Scotland. "It is therefore inferred that at the time of maximum midge activity in 2016 there was unlikely to have been SBV present in the midges in Scotland otherwise we would have seen cases in these two categories of animals." He said it could be that only a small number of ewes had been affected in the south of the country.

SAC Consulting said it expected the higher risk would be to cattle mated in the summer of 2017 and at the edge of the northward progression of infected midges. However, it said there might only be "limited spread" into Scotland as was seen in a previous epidemic.

Source: *BBC News*

Express News

Upcoming event - Second China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security

Second China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security, jointly organized by National Academy of Sciences, Chinese Academy of Sciences, Wuhan Institute of Virology and Hubei Society for Microbiology, will be held on May 17-19, 2017 in Wuhan, China.

This workshop will focus on 5 sessions:

- Gain of function research, gene editing, targeting and delivery and other novel biotechnology;
- Emerging infectious diseases and global health security;
- Public health response to outbreaks and issues;
- High-level biosafety Laboratory: construction, commissioning, and sustainment;
- Biosafety, biosecurity and bioethics.