

21 COE *Hokkaido University*

The 9th International Symposium for Zoonosis Control

Pathogenesis and Etiology of Zoonoses
Causing Encephalitis and/or Encephalopathy

| Date August 29, (Mon) 13:00-17:25

| Venue Centennial Hall at Hokkaido University

第9回人獣共通感染症制圧のための国際シンポジウム

“脳炎/脳症を起こす人獣共通感染症の原因と病因論”

日時 H17年8月29日(月) 13:00 - 17:25

場所 北海道大学 百年記念会館大会議室

主催 北海道大学21世紀COEプログラム
「人獣共通感染症制圧のための研究開発」

北大獣医学研究科COE推進室

<http://www.hokudai.ac.jp/veteri/coe>

Tel/Fax: 011-706-5294



Time Schedule

13:00-13:05 Welcoming Address
Prof. Dr. Takashi Umemura
Dean,
Graduate School of Veterinary Medicine, Hokkaido University

13:05-13:10 Opening remarks
Prof. Dr. Ikuo Takashima
COE Program leader,
Graduate School of Veterinary Medicine, Hokkaido University

13:10-14:30 Session I: Prion Diseases
Chairpersons
Dr. Mutsumi Inaba, Hokkaido University
Dr. Motohiro Horiuchi, Hokkaido University

13:10-13:50 Prevalence, Diagnosis and Eradication of Prion Infections in Ruminants 4
Dr. Martin H. Groschup,
Institute for Novel and Emerging Infectious Diseases at the
Friedrich-Loeffler-Institut, Germany

13:50-14:30 Therapeutic Trial and Diagnostic Markers of Human Prion Diseases 5
Dr. Susumu Shirabe, Nagasaki University, Japan

14:30-15:15 Coffee Break/ Poster Session

15:15-17:15	Session II: Viral Zoonoses	
	Chairpersons	
	Dr. Hirofumi Sawa, Hokkaido University	
	Dr. Ayato Takada, Hokkaido University	
15:15-15:55	West Nile Virus –Vector Interactions	6
	Dr. Stephen Higgs, University of Texas Medical Branch, USA	
15:55-16:35	Influenza-associated Encephalopathy	7
	Dr. Tsuneo Morishima, Okayama University, Japan	
16:35-17:15	Rabies in Asia: Zoonotic Threat, Problem, Control and Prevention	8
	Dr. Satoshi Inoue, National Institute of Infectious Diseases, Japan	

17:15-17:25 **Closing remarks**
 Prof. Dr. Hiroshi Kida
 Director, Research Center for Zoonosis Control,
 Professor,
 Graduate School of Veterinary Medicine, Hokkaido University

18:00- **Reception**
 Faculty House Trillium

Prevalence, Diagnosis and Eradication of Prion Infections in Ruminants

Dr. Martin H. Groschup

Institute for Novel and Emerging Infectious Diseases at the Friedrich-Loeffler-Institut, Insel Riems, Germany

Bovine spongiform encephalopathy (BSE) belongs to the group of transmissible spongiform encephalopathies (TSE), which are thought to be caused by infectious proteins, so-called prions. BSE was first time diagnosed in 1986 in the United Kingdom and has ever since led to a huge epidemic with possibly more than 4 million animals having been infected in Europe alone. While the majority of these infected animals was slaughtered in the incubation time (and went into the human feed chain in consequence) about 180,000 cattle became old enough to develop clinical signs. Until the year 2000, indigenous BSE cases were only found in few European countries, a picture which changed completely after the introduction of the obligatory BSE rapid testing in January 2001 in all EU member states. According to the EU regulations cattle older than 30 months of age and slaughtered for human consumption must be tested by using BSE rapid tests. Likewise must be fallen stock and clinically affected animals older than 24 month. This presentation gives a short overview over the incidence of BSE in Europe.

The host encoded cellular prion protein (PrP^C) plays a crucial role in the pathogenesis and in the infection cycle of TSEs. Following infection, PrP^C is converted into the pathological, putatively infectious isoform, designated (PrP^{Sc}). However, our knowledge of the route which the infectious BSE prions take from the alimentary tract of cattle to reach the CNS is still little understood. Since January 2003 we are therefore carrying out a pathogenesis study of BSE in cattle. Fifty six calves were infected orally with a macerate of BSE positive brainstem (100g per animal) and another 18 animals serve as mock controls. All animals are bled and urine is taken in two monthly intervals as well as cerebrospinal fluid every four months. In the course of serial kills, every four month 4 or 5 animals are euthanised and necropsied under TSE steril conditions to collect a large number of tissue and bodily fluid samples (i.e. more than 1.400 samples of more than 150 tissues from each euthanised animal). Selected samples are inoculated into transgenic mice, that overexpress the bovine PrP (Tgbov XV mice) and which are about 10.000 times more sensitive than wild type mice to cattle derived BSE prions. Using this novel bioassay, the spread of infectious agent from the alimentary tract to the CNS in bovines is analysed.

Moreover, following the implementation of a large scale TSE surveillance programme of small ruminants, more than 250.000 small ruminants in Germany were tested. In this national survey 186 TSE-affected sheep were found which resembled classical scrapie cases as well as a novel type, so-called atypical scrapie cases with divergent transmission and pathogenesis characteristics and with a novel biochemical phenotype of the infectious agent. According to the regulation EU 999/2001, all TSE cases in small ruminants have to be examined by strain typing methods to explore any possibility of the presence of BSE infections in the field sheep and goat population. We have therefore designed a novel biochemical typing strategy (termed FLI-test), which includes the determination of molecular masses, antibody binding affinities and glycosylation patterns of the TSE induced abnormal prion protein. By this way we were able to confirm that none of the recent German TSE outbreaks in small ruminants displays biochemical features indicative for a BSE infection.

Therapeutic Trial and Diagnostic Markers of Human Prion Diseases

Dr. Susumu Shirabe

The First Department of Internal Medicine, Graduate School of Biomedical Sciences,
Nagasaki University, Japan

1. Diagnostic markers of human prion diseases

Markers to diagnose CJD and evaluate disease progression of CJD will be discussed. 14-3-3 protein, S100 and NSE have been known as diagnostic markers. In this presentation, titers of total tau protein (t-tau) and phosphorylated tau (p-tau) levels in CSF were measured. Compare with Alzheimer patients, t-tau/p-tau ratio is diagnostic. Also, serial analysis of t-tau titer in CSFs of CJD patients showed peaks of t-tau titers in the clinical time courses. These changes of titers were compared with clinical time course and progressive MRI changes.

2. Therapeutic trial of oral pentosan polysulfate (PPS) for Prion diseases and new design of low molecular-weight PPS for an candidate as therapeutic agent for CJD.

To create effective treatment of Prion diseases, we designed clinical protocol with oral pentosan polysulfate (PPS). We treated 8 CJD patients with PPS. Five patients with probable CJD, one patient with definite (MM2 cortical), and two patients with familial CJD (V180I) were treated. One definite case showed marked improvement in walking and some higher cerebral function.

To deliver PPS into brain tissue efficiently, we planed to use of low molecular-weight PPS (LMW-PPS). Anti-prion efficiency of LMW-PPS was estimated by western blot performed by scrapie-infected neuroblastoma cells (GTFK and GT22L) to screen for inhibition of nascent PrP^{Sc} as well as the clearance of pre-existing PrP^{Sc}. We also estimated drug delivery efficiency by using in vitro blood brain barrier model (BBB kit). BBB kit was constructed with primary culture cells of astrocytes, pericytes and endothelial cells. LMW-PPS was applied on BBB kit for 6 hours, then, culture media in lower chamber were used for screening assay of anti-prion activity.

Some fractions of LMW-PPS had anti-prion effect in persistently prion-infected cell line. In certain fractions, anti-prion activity in the culture media penetrated the BBB kit, which suggested these LMW-PPS factions might be useful as a candidate of anti-prion agent.

West Nile Virus –Vector Interactions

Dr. Stephen Higgs

Department of Pathology, Center for Biodefence & Emerging Infectious Diseases, Sealy Center for Vaccine Development, & WHO Collaborating Center for Tropical Diseases, University of Texas Medical Branch, U.S.A.

Arthropod-borne viruses (arboviruses) are emerging and reemerging in many areas of the world. Arboviruses such as o'nyong-nyong have re-emerged after years of silence and are increasing in significance to human health. The reemergence and spread of diseases such as chikungunya, dengue, Japanese encephalitis, Rift valley fever, Ross river, Venezuelan encephalitis and yellow fever, may be the result of many factors. Increased travel, encroachment into new areas such as the Amazon Basin, and reduced efforts to control arthropod vectors all play a role in increasing the number of existing diseases and in the emergence of new ones. Due to the complexity and specificity of the relationships between the vector, agent and vertebrate, many questions remain unanswered despite over 100 years of research on arthropod-borne diseases.

West Nile virus (WNV) is a member of the Japanese encephalitis serological group of viruses in the genus *Flavivirus* in the family *Flaviviridae*. The virus was first described in Uganda in 1937, and it is widely distributed in the Old World, transmitted in a cycle between avian hosts and mosquitoes. Although the predominant vectors are mosquitoes in the genus *Culex*, WNV has been identified in over 50 species of arthropods, including ticks.

In 1999 WNV was identified in birds and horses that were dying in New York. That year sixty two confirmed human cases were identified with 7 deaths. The virus reappeared in 2000, probably having survived the winter in hibernating mosquitoes. Since 1999, the rapidity and extent of the spread of WNV in North America, the Caribbean, and Central America, exceeded all expectations and most predictions. WNV now occurs in all mainland U.S. States except for Washington. This spread has been driven by migrating birds. WNV is now the predominant circulating arthropod-borne virus in the US, with over 15,000 human cases and more than 600 fatalities since 1999.

The virus has now been identified in over 60 species of North American mosquitoes. Susceptible mosquitoes generally become infected when feeding on a viremic host, and following an intrinsic incubation period, salivary glands become infected. Mosquito must salivate during blood feeding because the saliva contains numerous substances that function to counter vertebrate hemostasis by preventing blood coagulation and enhancing vasodilation. The transmission of arboviruses to the vertebrate host depends upon the secretion of infectious virions with the saliva of the arthropod vector. The presentation will describe the infection process in mosquitoes, qualitative and quantitative techniques including immunohistochemistry, electron microscopy, qRT-PCR, and the use of genetically engineered infectious clones. Our recent demonstration of non-viremic transmission of WNV between co-feeding mosquitoes and influences of salivary factors on WN disease will also be discussed.

Influenza-associated Encephalopathy

Dr. Tsuneo Morishima, and The Collaborative Study Group of Influenza-associated Encephalopathy in Japan

Department of Pediatrics, Okayama University Graduate School of Medicine and Dentistry, Japan

Recently, the number of reports of encephalitis/encephalopathy associated with influenza has increased in Japan. The abrupt onset of seizure and coma within a few days after development of high-grade fever is a prominent indicator of CNS involvement during influenza infection. In order to investigate the various parameters of the disease outbreak, we have studied on influenza-associated encephalopathy during 1999 and 2004. Case definition of the disease was based on altered or loss of consciousness associated with influenza. In total, 879 cases have been reported and approximately 80% of them were virologically confirmed by virus isolation, antigen detection and/or significant rise of HI titer. 78% of them are younger than 6 yr old, and the mortality was 30 % in 1999- 2000 and 15 % in 2001-04. The incidence of disease in H3N2 outbreak was significantly higher than those in H1N1 or B outbreak. Elevation of pro-inflammatory cytokines (TNF- α and IL-6) and the damage of vascular endothelial cells were demonstrated. Recent study of our group showed that apoptosis rapidly occurred in nerve and in liver tissues. Astroglia cells were activated probably due to locally produced cytokines. Influenza virus was undetectable in the brain by careful examinations including immuno-staining and PCR. Virus DNA and/or antigens of HSV, HHV-6, HHV-7, CMV and EBV could not be detected by PCR in CSF and brain tissues. In 2003/2004 flu season, more than 150 children died in USA due to influenza, and some of them showed neurological signs and symptoms. We now continue the genome project using DNA tips and SNPs to find genetic variations in children with influenza-associated encephalopathy.

Rabies in Asia: Zoonotic Threat, Problem, Control and Prevention

Dr. Satoshi Inoue

Department of Veterinary Science, National Institute of Infectious Diseases, Japan

Rabies is observed not only in dogs but also in all mammals as well as in humans (zoonosis). The mortality in both humans and animals contracting this ailment is 100%. Over 50,000 people and over 100,000 animals reportedly die of rabies every year. It is widely recognized that the number of deaths officially reported in most developing countries greatly underestimates the true incidence of disease, with several factors contributing to widespread underreporting. In turn, underreporting leads to lack of attention by national authorities in much of Africa and Asia, and by the international organizations concerned. According to WHO, over 90% of humans dying of rabies has been found in Asia. The animals causing rabies outbreaks are mainly dogs (especially stray dogs) in Asian countries, although dogs, jackals and mongooses in Africa; dogs and bats (mainly vampire bats) in Latin America; raccoons, skunks, coyotes and bats in North America; mainly foxes in Europe. In general, in case of rabies, the virus excreted in the saliva penetrates the nerve tissues from the bite wound and the surface of the mucous membrane through the bite and it causes infection. Besides the incubation period is long and lasts 1 to 2 months on average or even sometimes up to 7 years, and it is impossible to detect the virus before the onset of the disease. Therefore wild animals from regions where rabies is endemic should not be imported, bred or moved to other destinations, even though they may be healthy. Nonetheless it is possible to prevent human rabies onset through post-exposure vaccination of subjects suspected of having been infected by this disease, or to eliminate occasions of infection in humans by vaccinating animals having a high risk of transmission (dogs). Every year over 10 million people are immunized after exposure, and the majority is in Asia. It is important to proactively take measures concerning the animals transmitting the infection to humans combined with action concerning people. In the WHO Expert Consultation on Rabies met in Geneva from 5 to 8 October 2004, it was pointed out that the disease has not been brought under control throughout most of the affected countries. A major factor in the low level of political commitment to rabies control is a lack of accurate data on the true public health impact of the disease. Disparities in the affordability and accessibility of post-exposure prophylaxis, levels of rabies awareness and risks of exposure to rabid dogs result in a skewed distribution of the disease burden across society, with the major impact falling on members, particularly children, of poor rural communities.

