GCOE ZOONOSIS CONTROL Hokkaido University

Global COE (Centers of Excellence) Program
Establishment of International Collaboration Centers
for Zoonosis Control

Information

on the Advanced Training Course for Zoonosis Control 2010

Organizer: Global COE Program
Graduate School of Veterinary Medicine
Hokkaido University
Sapporo, 060-0818 Japan

Preface

During the 20th century, tremendous progress was made in the prevention and control of infectious diseases.   Improvements in sanitation, living conditions, vaccines and antibiotics, as well as the accumulation of information on infectious agents and infectious diseases, have contributed to a reduction in the risk of contracting such conditions.   Despite this success, however, emerging and reemerging infectious diseases such as influenza, Ebola and other viral hemorrhagic fevers, West Nile fever, antibiotic-resistant bacterial infections, prion diseases and others appear frequently, and remain a serious threat to public health.   Changes in human behavior, economic development, increased travel and commerce, and changes in the global environment all contribute to disease emergence and reemergence.   Importantly, most of the emerging and reemerging infectious diseases are zoonoses, which are transmissible from animals to humans.   In light of the present situation regarding zoonosis outbreaks worldwide and the advance of borderless globalization, international collaboration for the control of zoonoses needs to be emphasized.

Recognizing the intense interest in the importance of zoonoses in terms of science and public health, the 21st Century COE (Centers of Excellence) *Program of Excellence for Zoonosis Control* (executed from 2003 to 2007) extensively investigated the mechanism by which zoonotic agents are perpetuated in nature, and made efforts to develop novel methods for the diagnosis, prevention and treatment of zoonoses.   We also established international collaboration research networks for zoonosis control in this program.   Based on these achievements and the international collaboration research networks so far created, an extended Global COE Program entitled *Establishment of International Collaboration Centers for Zoonosis Control* (supported by a grant from Japanese Ministry of Education, Culture, Sports, Science and Technology) was launched in 2008 at Hokkaido University in Japan.   Under this program, we aim to strengthen work toward clarifying the mechanisms of perpetuation and routes of transmission for zoonotic pathogens, the molecular basis of the host range and the pathogenicity of zoonotic agents, establish prophylactic and therapeutic measures, and participate in an extensive search for potential zoonotic pathogens through the

international collaboration research networks formed. In addition to these research activities, we are also committed to the development of human resources to play pivotal roles in the control of zoonoses at the locations of outbreaks.

To achieve these goals, the Global COE Program is promoting the *Advanced Training Course for Zoonosis Control 2010* for personnel who will be involved in control management at the locations of disease outbreaks.

The objective of the *Advanced Training Course for Zoonosis Control 2010* is:

To contribute to the development of human resources for engagement in education, research and diagnosis toward the control of zoonoses by providing basic and advanced knowledge and introducing related technologies.

Through this training program, we hope to strengthen the diagnostic capabilities and research activities of participants.

1. Essential Information

Course Title: Advanced Training Course for Zoonosis Control 2010

Duration：   From the end of August, 2010 to the middle of December, 2010

Number of Participants: about 4
                    (from our international collaboration research network and other universities/institutes)

Language: English

Course objectives:

The Advanced Training Course for Zoonosis Control (ATCZC) 2010 aims to:
   (1) Contribute to the development of human resources engaged in education, research and diagnosis at international collaboration centers.
   (2) Provide the basic and advanced knowledge and introduce the technologies required for zoonosis control.

Training Institutes:

Graduate School of Veterinary Medicine
Graduate School of Medicine
Research Center for Zoonosis Control
Institute for Genetic Medicine
(at Hokkaido University, Sapporo, 060-0818 Japan)
URL: http://www.vetmed.hokudai.ac.jp/gcoe/

Allowances & Expenses:

The Global COE Program covers the following expenses through the Global COE office in accordance with the relevant laws and regulations:

   (1) Round-trip air ticket between an international airport designated by the Global COE Program administration and Sapporo
   (2) Accommodation allowance
   (3) Daily allowance

Accommodation: Accommodation for the whole period of the training will be arranged by the COE Program administration.

The accommodation provided in Sapporo is as follows:

Weekly Inn   Tel 011-708-1717   (www.weekinn-iris.com)
(From Sapporo station, take the subway to Kita-18-jou station. The inn is a one-minute walk from this station)

   ・A coin-operated washing machine is available.
   ・Bath towels, face towels, hand towels and nightwear are not provided.
   ・Toothbrushes, razors, soap, shampoo and rinse are not provided.

・Meals are not provided.
　　　　　　　　　　　　　　　・Kitchen facilities are available.
　　　　　　　　　　　　　　　・Wireless LAN access is available in rooms.

Contact:

　　　　　　　　　Global COE Office:
　　　　　　　　　Graduate School of Veterinary Medicine,
　　　　　　　　　Hokkaido University,
　　　　　　　　　Kita-18, Nishi-9, Kita-ku,
　　　　　　　　　Sapporo, 060-0818 Japan
　　　　　　　　　E-mail: **gcoe@vetmed.hokudai.ac.jp**
　　　　　　　　　Tel. & Fax: +81-11-706-5294

　　　　　　　　　URL: http://**www.vetmed.hokudai.ac.jp/gcoe/**

2. Course Schedule (Tentative)

2010	Events	ATCZC 2010
the middle of August	・Opening ceremony	Participation in actual research activities at the laboratories of host researchers
Aug.24 – Aug.27	・Lecture "Epidemiology"	
Aug. 31– Sept. 24	・Core curriculum for Zoonosis Control	
Sept. 13-14	・Young Researcher Seminar	
the middle of December	・Final report ・Closing ceremony	

Participants will gain advanced knowledge and learn about the technologies required for zoonosis control through actual research activities. Each person will join one laboratory (optionally, it may be possible to join several laboratories) and participate in its daily activities. The areas of training to be provided in ATCZC 2010 are listed in the *Areas of Training* section.

Participants will be required to attend the core curriculum of the Training Course for Zoonosis Control to be held on from August 31 to September 24, 2010.   This will consist of an intensive three-week session, and will provide lectures on general and specific aspects of zoonosis as well as outlining practices for basic experimental skills related to zoonotic research.

Participants will be asked to present their work in progress by way of reports and their results through a final report.

3. Areas of Training

ATCZC 2010 will provide the areas of training outlined below. Participants will select one area of training on application, and will basically be associated with the laboratory of the corresponding host researcher (indicated in parentheses) throughout the period. Under the supervision of the host researcher, participants will gain advanced knowledge and learn about technologies related to zoonosis control through actual research activities.

Epidemiological Research

E-1) Epidemiological study of hantavirus infection among human and reservoir animals
(*Dr. J. Arikawa, Dept. of Microbiology, Graduate School of Medicine*)

Hantaviruses are RNA viruses belonging to the *Hantivirus* genus of the *Bunyaviridae* family. They are a causative agent of hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS), which are typical rodent-borne viral zoonoses. Firstly, participants will learn the principles of diagnostic methods for hantavirus infection, such as those that detect hantavirus-specific antibodies, antigens and genomes. Secondly, participants will gain knowledge and learn about related technologies by joining research groups for the development of diagnostic methods or for epidemiological (in human infection) and epizootiological (in reservoir animals) study by applying the previously acquired diagnostic methods.

E-2) Molecular epidemiology of viral infection in wild birds and domestic chickens
(*Dr. K. Ohashi, Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine*)

In this series of lectures, participants will learn the basic information on viral infection in birds that is important in the fields of public health, veterinary medicine and conservation medicine for wild birds, and the basic methodology of surveying viral infection in wild birds and domestic chickens using molecular biological techniques including conventional PCR (also RT-PCR), real-time PCR and others. The lectures also include an introduction to some new techniques such as loop-mediated isothermal amplification (LAMP) of viral genomes. In addition, reference work on a molecular epidemiological survey of viral infection in wild waterfowl will be shown in the second part of the lectures.

E-3) Diagnosis of protozoan diseases
(*Dr. C. Sugimoto, Dept. of Education and Collaboration, Research Center for Zoonosis Control*)

Training in immunological and DNA-based diagnoses (PCR, LAMP (loop-mediated isothermal amplification) of protozoan/rickettsial diseases is offered here. Parasites used on the course include *Theileria parva, T. orientalis, Trypanosoma brucei, T. congolense, T. evansi*, and *Ehrlichia ruminantium*. Culture methods and the preparation of biological materials, including the production and purification of recombinant proteins, are also taught. Those who pursue further advanced subjects can acquire knowledge of advanced molecular biological techniques including

molecular cloning, DNA sequence analysis and genotyping of the above-mentioned parasites.

E-4) Molecular basis of ecology and evolution of influenza viruses
(Dr. H. Kida, Lab. of Microbiology, Dept. of Disease Control, Graduate School of Veterinary Medicine)

The genes of highly pathogenic avian and human pandemic influenza viruses originate from those of viruses circulating in their natural hosts – water birds. Previous pandemic influenza viruses have arisen via genetic reassortment between humans and avian viruses in pigs. Since it has been shown that pigs are susceptible to infection with avian influenza viruses of each of the known subtypes, none of the 16 HA and 9 NA subtypes can be ruled out as potential candidates for pandemic strains. A highly pathogenic avian influenza virus is generated when a nonpathogenic virus is brought in by migratory birds to chickens via terrestrial poultry and acquires pathogenicity for chickens through repeated multiple infection in the chicken population. To prepare for the emergence of pandemic influenza virus strains in poultry and humans, it is essential to have information on the dominant strains in nature, birds and mammals including humans. The aim of this course is to elucidate the molecular basis of the ecology and evolution of influenza viruses to enable prediction and prevention of the emergence of pandemic influenza in the future.

E-5) Ecology of zoonotic viruses
(Dr. A. Takada, Dept. of Global Epidemiology, Research Center for Zoonosis Control)

Participants will learn the principles of standard methods in virology, including isolation and identification of viruses, detection and sequencing of viral genomes, and cloning and expression of viral protein genes. In addition, antibody assays such as ELISA, neutralization testing, immunofluorescence systems and immunoblotting analysis will also be covered on this course. Depending on the completion of activities during the course period, participants will also have the chance to learn how to generate monoclonal antibodies specific to viral proteins.

E-6) Epidemiological study of viral zoonoses
(Dr. H. Kariwa, Lab. of Public Health, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine)

Using hantavirus infection as a model, participants will learn the procedures and methods involved in conducting an epidemiological study of a viral zoonosis. Those taking this course will capture rodents in the field and collect materials such as blood and tissue. Sera will be observed through serological examination methods such as immunofluorescent testing and ELISA for the presence of antibodies. Tissue samples will be analyzed using PCR to detect viral RNA, and amplified RNA will be further examined to determine nucleotide sequences.

E-7) Epidemiological studies of rabies and leptospirosis in Sri Lanka
(Dr. H. Tamashiro, Dept. of Global Health and Epidemiology, Graduate School of Medicine)

The department will provide participants with an overview of epidemiological methods, research protocol development, survey methodologies and analytical exercises with actual data using SPSS and Epi Info. Those taking the course will learn how to prepare epidemiological abstracts in the form of tables and figures, and to present these data in an appropriate scientific manner. In addition, they will share information on rabies and leptospirosis in Sri Lanka, which will become a

basis for epidemiological discussion among our staff and graduate students.

E-8) Studies on the relationship between wildlife ecology and infectious diseases
(Dr. T. Tsubota, Lab. of Wildlife Biology, Dept. of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine)

Participants will learn about the ecology and physiology of wildlife such as bears, deer and seals, and about the relationship between wildlife ecology and infectious diseases in Hokkaido.   In particular, with Lyme disease as a model, participants will study infection rates in animals and insect vectors as well as the relationship between these parameters on Lyme disease infection and animal movement and/or interaction among mammals, particularly those such as bears and deer. Participants can also join research surveys for capturing wildlife, taking blood and tissue samples, handling animals and attaching radio transmitters, followed by the release and radio tracking of these animals.   Furthermore, those on the course can take part in surveys for capturing and handling to obtain samples from wild birds in Hokkaido.

E-9) Bioinformatics for zoonosis control
(Dr. K. Ito, Dept. of Global Epidemiology, Research Center for Zoonosis Control)

The rapid development of molecular biology has accelerated the accumulation of gene sequences on zoonotic pathogens. As larger data sets on individual zoonotic pathogens are accumulated in databases, the importance of the role of bioinformatics may well increase in comprehensive studies to develop effective strategies for the control of disease. On this training course, participants will learn bioinformatics techniques used to analyze sequence data on zoonotic pathogens. Using the influenza A virus as a model, participants will conduct phylogenetic analysis on gene sequences for a large number of isolates. They will also learn the underlying theory behind phylogenetic analysis to determine the evolutionary relationship among strains and to trace the evolutionary history of genes.

Immunological and Pathobiological Research

I-1) Examination of interferon-induced anti-virus factors Mx (for orthomyxovirus) and Oas1b (for flavivirus) in various animals
(Dr. T. Agui, Lab. of Laboratory Animal Science and Medicine, Dept. of Disease Control, Graduate School of Veterinary Medicine)

Mx- or Oas-congenic mice will be used to study the induction of the interferon-induced anti-virus factors Mx and Oas1b. In this project, participants will inject interferons into mice and detect the expression of the anti-virus factors Mx and Oas1b in splenocytes. Through this procedure, participants will learn about RT-PCR and sequencing the cDNA of Mx and Oas1b. Next, those on the course will prepare macrophages or fibroblasts from Mx- or Oas-congenic mice, and will perform infection experiments with vesicular stomatitis virus replicons or West Nile virus replicons to learn about the protective effects of Mx or Oas1b against these viruses. Through this procedure, participants will learn how to culture cells and observe replicons as fluorescence under a confocal microscope.

I-2) Studies on ERAD (endoplasmic reticulum-associated degradation) and

trafficking of viral membrane proteins

(*Dr. M. Inaba, Lab. of Molecular Medicine, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine*)

The essential components of virus membranes such as HA and NA in an influenza virus are synthesized, quality-controlled and transported to the plasma membrane in infected cells to generate virus particles. The purpose of this session is to learn the basic theories and techniques needed to examine intracellular localization and the trafficking of membrane proteins including viral transmembrane proteins by immunofluorescence microscopy, real-time imaging and cell-surface labeling. The effects of several agents that influence vesicular transport and the proteolytic degradation of viral membrane proteins in the endoplasmic reticulum through the proteasomal pathway will also be examined.

I-3)   Analysis of immune response in mice and its application to the immunological monitoring of infectious diseases

(Dr. K. Iwabuchi, Division of Immunobiology, Research Section of Pathophysiology, Institute for Genetic Medicine)

Participants will learn the principles and actual techniques needed to probe immunological status in mice by analyzing lymphocyte subsets, quantifying cytokines in culture supernatants or sera, and integrating these variables with functional outcomes to understand immune response against infectious agents.   Multi-color flow cytometric analysis will be the main method used on this course to detect various surface markers of lymphocytes and to quantify multiple cytokines.   In addition, participants will learn how to detect NKT cells with α-galactosylceramide-loaded CD1d multimers, and how to detect peptide-Ag-specific CD8$^+$ T cells with peptide-loaded class I MHC tetramers, which are applicable in visualizing the dynamics of NKT cells and Ag-specific T cells, respectively.

I-4)   Diagnosis of viral infectious diseases and basic techniques for cell biology research

(*Dr. H. Sawa, Dept. of Molecular Pathobiology, Research Center for Zoonosis Control*)

We in the Department of Molecular Pathobiology at Hokkaido University's Research Center for Zoonosis Control are investigating the relationship between viral proteins and host proteins to clarify the molecular mechanisms of virus-induced pathogenesis. Participants will learn a range of methods for diagnosing infectious diseases, including cell culture techniques, isolation of nucleic acids (RNA and DNA) from animal tissues and cells, PCR techniques, immunohistochemical staining, and sequencing of target genes. They will also learn how to transfect plasmids to mammalian cells and prepare proteins from the resulting cells, as well as studying sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting as basic techniques for cell biology research. The department also holds a research meeting every Friday morning and runs a Journal Club session every Monday evening.

I-5)   Necropsy and pathological diagnosis of infectious diseases

(*Dr. T. Umemura, Lab. of Comparative Pathology, Dept. of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine*)

Practice and lectures regarding the diagnosis of infectious diseases will be provided to trainees. The practice part includes necropsy of animals and training/consultation using histopathology

slides. Lectures will be focused on diagnostic and experimental pathology of infectious and zoonotic diseases in animals.

I-6) Vaccine-induced immune response against infectious diseases in poultry
(*Dr. K. Ohashi, Lab. of Infectious Diseases, Dept. of Disease Control, Graduate School of Veterinary Medicine*)

Some viral infections can be prevented and controlled through the use of effective vaccines. Vaccines are divided into live and inactivated types, which differ in terms of the immune response induction mechanism(s) in their hosts. In this series of lectures, a brief introduction to the various immune responses induced by different kinds of vaccines will be given. This will include the advantages of different vaccine types and the basic molecular mechanism(s) for the induction of immune responses effective against viral infection. In addition, problems related to the control of viral infection by vaccines along with possible methodologies to improve protection will also be discussed using virus infections as models.

I-7) Study of the molecular mechanism of bacterial infection-mediated gastric carcinogenesis
(Dr. *H. Higashi, Division of Molecular Oncology, Institute for Genetic Medicine*)

This program focuses on the oncogenic mechanism of the bacterial oncoprotein *Helicobacter pylori* CagA. To study the effects of CagA on cellular mechanisms, which involve the signaling pathway and cell structure, participants mainly learn techniques of cell culture, ectopic expression of proteins of interest and DNA cloning. Using cultured cells that ectopically express proteins, the lectures cover the separation of proteins in gel, immunoprecipitation and immunoblotting for the analysis of protein interaction within the cells. The immunohistochemistry method is also used to determine the localization of proteins in cells.

Diagnostic and Therapeutic Research

D-1) Diagnosis and clarification of the pathogenesis of influenza
(*Dr. T. Miyazaki, Dept. of Bioresources, Research Center for Zoonosis Control*)

We identify the host factors involved in the infection and replication of influenza viruses by antibody array and the yeast two-hybrid system. We also analyze the functions of these factors in the infection or replication of influenza viruses by plaque assay, PCR and Western blot analysis. In addition, we clarify the molecules that are critical for apoptosis induction, cytokine production and regulation of the function of immune cells after viral infection using cell lines and gene knockout mice. Finally, we aim to develop diagnostic methods and effective drugs for the prevention and treatment of influenza.

D-2) Investigation of signal transduction and its application to therapy
(*Dr. M. Noguchi, Division of Cancer Biology, Institute for Genetic Medicine*)

Our laboratory focuses its attention on therapeutic intervention for H5N1 influenza through the molecular activation of PI3K-AKT signaling pathways. The PI3K-AKT network regulates a wide

variety of cellular responses, including anti-apoptosis, proliferation, cell cycling, protein synthesis and glucose metabolism. Updated knowledge of the PI3K-AKT network will certainly aid understanding of the various pathological conditions of viral infection. Our laboratory will provide an understanding of intracellular signaling through this program, including the assay system of phosphorylation-dephosphorylation and cell death pathways. Basic knowledge and experimental skills in molecular biology are required for involvement in the program.

D-3) Molecular diagnosis of zoonoses
      (*Dr. Y. Suzuki, Dept. of Global Epidemiology, Research Center for Zoonosis Control*)

Using mycobacteriosis as a model, participants will learn the principles of methods for the molecular diagnosis of zoonoses. They will learn techniques such as the purification of high-molecular-weight DNA from pathogens or specimens, the amplification of target genes by PCR, and the determination of nucleotide sequences in targeted genes. They will also learn about loop-mediated isothermal amplification – an isothermal nucleic acid amplification technique to be widely used in the diagnosis of a number of infectious diseases. Molecular methods for drug susceptibility testing will also be taught.

D-4) Diagnosis and immunological studies of emerging respiratory viruses
      (*Dr. T. Ariga, Dept. of Pediatrics, Graduate School of Medicine*)

Participants will learn the following techniques for the diagnosis of respiratory tract infection due to emerging infectious pathogens such as human metapneumovirus (hMPV) and human bocavirus (HBoV): (1) Detection of virus genomes by PCR (RT-PCR) or quantitative real-time PCR (RT-PCR). (2) Formation of recombinant baculoviruses that express the VP1 and VP2 proteins of HBoV. (3) Isolation and amplification of hMPV using cell culture techniques. (4) Detection of antibodies against the VP1 and VP2 proteins of HBoV in serum samples using immunofluorescence assay (IFA) or ELISA. (5) Formation of monoclonal antibodies against the VP1 and VP2 proteins of HBoV.

D-5) Diagnosis and therapeutic research of parasitic diseases
      (*Dr. K. Katakura, Lab. of Parasitology, Dept. of Disease Control, Graduate School of Veterinary Medicine*)

Participants will learn various methods of diagnosis for cutaneous and visceral leishmaniasis, including smear preparation, PCR amplification of target genes, microsatellite analysis of parasites and Western blotting. Cultured parasites and biopsy materials from humans and dogs with leishmaniasis will be used as materials. Those on the course will learn how to maintain leishmanial or trypanosomal parasites in culture and how to assess the in vivo parasite burden of different organs in mice by directly counting the number of parasites and by using the real-time PCR method. In addition, participants will learn in vitro tests for antileishmanial or antitrypanosomal activity of different drugs and chemical compounds.

D-6) Diagnosis and pathogenesis of prion diseases
      (Dr. M. Horiuchi, Dept. of Prion Diseases, Graduate School of Veterinary Medicine)

Using prion diseases as a model, participants will learn the principles of methods for the diagnosis of infectious diseases, including ELISA, immunofluorescence assay, immunoblotting and

immunohistochemistry. They will also learn how to manipulate polyclonal and monoclonal antibodies, including the production and purification of antibodies as well as the labeling of antibodies with fluorescent dyes or enzymes. In addition, participants will learn the molecular genetic techniques needed to conduct molecular epidemiological studies such as the purification of high-molecular-weight DNA from tissue, the purification of DNA/RNA from microorganisms, the amplification of target genes by PCR, and the determination of nucleotide sequences of targeted genes.

4. Requirements for Application

How to Apply

1) Application Form (attached) clearly stating your areas of research interest and the primary and secondary areas you wish to study (please select items from E-1 to 14, I-1 to 6, and D-1 to 4 listed in *Areas of Training*)
2) Publication/presentation lists
3) Report on the following subjects in 1,200 words or less.
☆ Select one zoonotic disease in which you are interested, and describe current prevalence and countermeasures against the disease in your country and/or world-wide.
4) Describe the purpose of your application to ATCZC2010 (500 words or less)
5) Study program in Japan in detail and concreteness (1,500 words or less)
6) The names of more than two references and their contact information
7) Recommendation letters if possible

Submission details:
Application materials should be submitted by e-mail to the Global COE Office (**gcoe@vetmed.hokudai.ac.jp**) or directly to the relevant host researcher (who should be a member of Global COE Program).

Application deadline:
March 20, 2010

Points to Note

Applicants should:
1) Have at least a B.Sc. in a field related to life science (e.g., veterinary science, agriculture, biology, etc.)
2) Be proficient in spoken and written English
3) Be in good enough health physically, mentally and socially to participate in the Advanced Training Course for Zoonosis Control
4) Not be serving in the military
5) Be nominated by their institute
6) Preferably be under 35 years of age

Participants are required to:
1) Respect and follow the course schedule, and not change course subjects or the course period
2) Refrain from bringing family members to the course
3) Return to their home countries at the end of the course according to the international travel schedule designated by the program
4) Refrain from engaging in political activities or any form of employment for

profit or gain

5) Observe the rules and regulations of their accommodation providers and refrain from changing from the accommodation designated by the program

Information on Sapporo and its climate is available on the following sites:

    http://www.hokudai.ac.jp/en/pickup/usefullinks.html

    http://www.welcome.city.sapporo.jp/english/

    http://english.wunderground.com/global/stations/47412.html+weather,+Sapporo&hl=ja