Activities of Hokkaido University Research Center for Zoonosis Control (CZC)
Hokkaido University, Sapporo, Japan
Hokkaido is Japan's second largest island and the largest, northernmost of its 47 prefectoral-level subdivisions. The largest city and prefectural capital of Hokkaido is Sapporo, the fifth largest city in Japan. 1.9 million people are lived in the city.

Sapporo is best known outside Japan for hosting the 1972 Winter Olympics, and the annual Snow Festival in the city, which draws more than 2 million tourists from around the world.

The distance between Jakarta and Sapporo is 6486 km.
History of Hokkaido University

July, 1876:
Dr. William S. Clark, the President of the Massachusetts Agricultural College in the United States was invited to be the Vice President of the Sapporo Agricultural College.

April, 1918
Hokkaido Imperial University was established.

October, 1947
Hokkaido Imperial University was renamed as Hokkaido University.
Nobel Prize in Chemistry 2010 was awarded jointly to Akira Suzuki (emeritus professor of Hokkaido Univ.), Richard Heck (USA), and Ei-ichi Negishi (Purdue Univ., USA) and for palladium-catalyzed cross couplings in organic synthesis.

The number of under graduate students: 12,000
The number of graduate students: 6,400
The number of staff: 3,900
The number of graduate/under graduate schools and facilities: 30
The number of Institutes and Research Centers : 40
Students from Abroad in Hokkaido Univ. (as of Nov. 1, 2010)

1412 international students from 85 countries and regions

Europe/ NIS 67

Middle East 17

Asia 1229

Africa 51

North America 17

South America 27

Oceania 4
Hokkaido University
Research Center for Zoonosis Control

WHO Collaborating Centre for Zoonoses Control
Backgrounds for establishment
**Outbreaks of infectious disease**

- **1918, All over the world**
  - Spanish Flu
  - Over 30 million death all over the world

- **1919, All over the world**
  - Spanish Flu
  - Over 30 million death all over the world

- **1940, Asia**
  - Hemorrhagic fever with renal syndrome (HFRS)
  - 3,000 death

- **1951, Asia**
  - Influenza
  - Over 600 death

- **1968, Asia**
  - Influenza
  - Over 50,000 death

- **1976, DRC Congo**
  - Ebola hemorrhagic fever
  - 185 death

- **1977, China**
  - SARS
  - Over 800 death

- **1978, Malaysia**
  - Nipah Virus Encephalitis
  - 105 death

- **1980, USA, Canada**
  - West Nile Fever
  - Over 1,000 death

- **1991, Venezuela**
  - Venezuelan hemorrhagic fever
  - Over 30 death

- **1993, USA, Canada**
  - Hantavirus pulmonary syndrome (HPF)
  - Over 100 death

- **1998, Malaysia**
  - Nipah Virus Encephalitis
  - 105 death

- **2001, Uganda**
  - Ebola hemorrhagic fever
  - 22 death

- **2001, USA, Canada**
  - West Nile Fever
  - Over 600 death

- **2003, China, Hong Kong**
  - SARS
  - 813 death

- **2003, Asia and Africa**
  - H5N1 Influenza
  - Over 18,000 death

- **2007, Uganda**
  - Ebola
  - 279 death

- **2009, Asia and Africa**
  - Swine-origin H1N1 Influenza
  - Over 18,000 death

- **2009, From Mexico**
  - Swine-origin H1N1 Influenza
  - Over 18,000 death

- **2010, South Africa**
  - Norovirus
  - Over 200 death

- **2010, USA, Canada**
  - Influenza
  - Over 800 death

- **2011, Thailand**
  - H5N1 Influenza
  - Over 100 death

- **2014, USA, Canada**
  - H1N1 Influenza
  - Over 800 death

- **2015, Africa**
  - Rift Valley Fever
  - Over 1,000 death

- **2015, Malaysia**
  - Nipah Virus Encephalitis
  - 105 death

- **2018, USA, Canada**
  - Hantavirus pulmonary syndrome (HPF)
  - Over 100 death

- **2018, Russia**
  - Hemorrhagic fever with renal syndrome (HFRS)
  - Over 3,000 death

- **2019, USA, Canada**
  - Influenza
  - Over 800 death

- **2020, USA, Canada**
  - Influenza
  - Over 800 death

- **2021, USA, Canada**
  - Influenza
  - Over 800 death

- **2021, Africa**
  - Rift Valley Fever
  - Over 1,000 death

- **2022, Asia**
  - Influenza
  - Over 800 death

- **2022, Africa**
  - Rift Valley Fever
  - Over 1,000 death

- **2023, Africa**
  - Influenza
  - Over 800 death
The simplest definition of a zoonosis is a disease that can be transmitted from other animals to humans. Research and education in medicine is aimed at maintaining and improving human and public health, while that in veterinary medicine is designed for infectious-diseases prevention and clinical treatment of livestock and pet animals. There was no research, educational, and administrative basis for the control of zoonoses, because it falls between the two sciences of human and veterinary medicine.
• Hokkaido University established the “Research Center for Zoonosis Control” on 1st April, 2005.
• This Research Center will accomplish unique scientific and educational activities by bringing together experts in bacteriology, virology, parasitology, immunology, pathology, and computer science.
• In addition, the Research Center addresses the diagnosis of field materials collected in other countries.
Organization
Department of Global Epidemiology
- Identification of natural host animals of zoonotic pathogens
- Genetic analysis of pathogens
- Database development of genome information
- Prevention and control of zoonoses

Department of Molecular Pathobiology
- Diagnosis of zoonotic diseases
- Identification of determinant for host specificity
- Molecular basis of pathogenicity
- Development of rapid and highly sensitive detection methods of zoonotic pathogens

Department of Bioresources
- Preservation and supply of zoonotic pathogens, cells, genes, antibodies and animal strains
- Development of prevention and treatment methods for zoonoses

Department of Collaboration and Education
- Coordination of collaboration programs with international and domestic organizations
- Training of experts for the control of zoonoses
- Improvement of IT infrastructure for the international collaboration for research and education

Department of Infection and Immunity
- Investigation of host response against zoonotic pathogens
- Structural analysis of molecules of pathogens and cellular factors
- Development of novel strategies for therapeutic and prophylactic methods

Department of Bioinformatics
- Prediction of outbreaks and epidemics of the zoonoses
- Computational analysis of genetic information and protein structure of zoonotic pathogens
- Database development of genetic information of zoonotic pathogens

Hokudai Center for Zoonosis Control in Zambia
- Identification of natural host animals and transmission routes of zoonotic pathogens in Africa
- Comprehensive screening of unknown pathogens in Africa
- Prevention and control of zoonoses

Hokkaido University Research Center for Zoonosis Control
## Faculty members (currently 20 full-time)

<table>
<thead>
<tr>
<th>Division</th>
<th>Position</th>
<th>Name</th>
<th>Degree</th>
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<tbody>
<tr>
<td><strong>Division of Global Epidemiology</strong></td>
<td>Director</td>
<td>Hiroshi KIDA</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td></td>
<td>Vice-Director</td>
<td>Hirofumi SAWA</td>
<td>MD, PhD</td>
</tr>
<tr>
<td></td>
<td>Professor</td>
<td>Ayato TAKADA</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td></td>
<td>Professor</td>
<td>Yasuhiko SUZUKI</td>
<td>PhD</td>
</tr>
<tr>
<td></td>
<td>Specially Appointed Assistant Professor</td>
<td>Reiko YOSHIDA</td>
<td>PhD</td>
</tr>
<tr>
<td></td>
<td>Specially Appointed Assistant Professor</td>
<td>Chie NAKAJIMA</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td><strong>Division of Molecular Pathobiology</strong></td>
<td>Professor</td>
<td>Hirofumi SAWA</td>
<td>MD, PhD</td>
</tr>
<tr>
<td></td>
<td>Associate Professor</td>
<td>Takashi KIMURA</td>
<td>DVM, PhD</td>
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<tr>
<td></td>
<td>Specially Appointed Assistant Professor</td>
<td>Yasuko ORBA</td>
<td>PhD</td>
</tr>
<tr>
<td><strong>Division of Bioresources</strong></td>
<td>Professor</td>
<td>Chihiro SUGIMOTO</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td><strong>Division of Collaboration and Education</strong></td>
<td>Associate Professor</td>
<td>Kiichi KAJINO</td>
<td>MD, PhD</td>
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<tr>
<td></td>
<td>Lecturer</td>
<td>Ichiro NAKAMURA</td>
<td>PhD</td>
</tr>
<tr>
<td><strong>Division of Bioinformatics</strong></td>
<td>Associate Professor</td>
<td>Kimihito ITO</td>
<td>PhD</td>
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<tr>
<td></td>
<td>Specially Appointed Assistant Professor</td>
<td>Manabu IGARASHI</td>
<td>PhD</td>
</tr>
<tr>
<td><strong>Division of Infection and Immunity</strong></td>
<td>Professor</td>
<td>Hideaki HIGASHI</td>
<td>PhD</td>
</tr>
<tr>
<td></td>
<td>Assistant Professor</td>
<td>Naomi OHNISHI</td>
<td>PhD</td>
</tr>
<tr>
<td><strong>Hokudai Center for Zoonosis Control in Zambia (HUCZCZ)</strong></td>
<td>Head</td>
<td>Chihiro SUGIMOTO</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td></td>
<td>Assistant Professor</td>
<td>Akihiro ISHI</td>
<td>PhD</td>
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<tr>
<td></td>
<td>Specially Appointed Professor</td>
<td>Yoshizo ASANO</td>
<td>MD, PhD</td>
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<td>Specially Appointed Assistant Professor</td>
<td>Yuka THOMAS</td>
<td>PhD</td>
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<td>Specially Appointed Assistant Professor</td>
<td>Hirohito OGAWA</td>
<td>DVM, PhD</td>
</tr>
<tr>
<td></td>
<td>Specialist</td>
<td>Emiko NAKAGAWA</td>
<td>PhD</td>
</tr>
</tbody>
</table>

7 profs, 4 associate profs, 1 lecturer, 8 assistant profs; 7 DVM, 3 MD, 10 PhD
Facility

- 1st floor
  - BSL3 laboratories
  - SPF animal rooms
  - BSL3 animal rooms
  - BSL2 animal rooms

- 2nd floor
  - BSL2 laboratories
  - WHO・OIE reference laboratory

- Effluent treatment facility

BSL3 11 rooms 484m²
Facility

BSL3 animal rooms

- Rodent
- Chicken
- Dog/Pig
- Sheep/Calf
1. 3 Japanese Research staff

2. BSL-3 and BSL-2 Laboratory from Aug. 2008

3. Capacity
   Serology-ELISA
   DNA and/or RNA-based diagnosis (PCR)
   Isolation of virus/bacteria
   Cell culture equipment
   Storage (-80 °C and Dry ice)
   Animal accommodation
   DNA sequencer

To be installed:

Ultracentrifuge

BSL-3 (negative pressure)  BSL-2 (normal pressure)
Funds from the Ministry of Education, Culture, Sports, Science & Technology in Japan

- Joint Usage / Research Center
  \$368 million/year (2010-2015) *one million JPY=114.5 million IDR

- Japan Initiative for Global Research Network of Infectious Diseases (J-GRID)
  \$170 million/year (2010-2014)

- Global COE program: Establishment of International Collaboration Centers for Zoonosis Control
  \$180 million/year (2010-2014)

- Leading-edge Research Infrastructure Program: Establishment of frontline research environment for the control of emerging and reemerging infectious diseases
  \$570 million/3 years (2010-2012)
Research activities
Control of Zoonoses

- Identification of natural host & elucidation of the route of transmission
- Development of measures for diagnosis and prevention
- Clarification of the molecular basis of pathogenesis
Field surveillance
International Collaboration Research Network

- **Hantavirus (Russia)**
- **Avian influenza (Mongolia)**
- **Hantavirus (Mexico)**
- **Hantavirus (Thailand, Viet Nam, Indonesia)**
- **Leishmaniasis (Pakistan)**
- **Bacteria (TB, plague etc.), Viruses (Filoviruses, Influenza viruses, retroviruses, Polyomaviruses, Arenaviruses, Rabies viruses etc.), Parasites (Trypanosoma, Theileria etc.) (Zambia)**

**The World Health Organization Regional Office for the Western Pacific (WPRO)**

- **WHO Collaborating Centre for Zoonosis Control (From Nov. 2011)**
- **OIE-FAO Reference Laboratories for Avian Influenza**

**Establishment of laboratory and epidemiological research and exchange of students**
Epidemiological study for Human African Trypanosomiasis

Study area: Mbambanda Sanctuary, Chama, Luangwa valley

by Prof. Sugimoto’s team
Human-infective Trypanosoma
*(T. brucei rhodesiense)*

Goats  0/36
Cattle  1/105

2009. 5
Tick-borne diseases

by Prof. Sugimoto’s team
Tick-borne rickettsial diseases

Genotyping Marker development

E. ruminantium
Welgevonden (Erwe)
genome
1.52 Mbp
Surveillance of Avian Influenza Virus in 2010 by Prof. Kida’s team

Mongolia (36 isolates)
- H1N1 (1)
- H3N3 (1)
- H3N6 (7)
- H3N8 (14)
- H4N6 (8)
- H7N9 (1)
- H10N8 (4)

Hokkaido (20 isolates)
- H2N3 (1)
- H3N8 (3)
- H5N1 (2)
- H5N2 (1)
- H6N2 (2)
- H7N7 (10)
- H8N4 (1)

Vietnam (26 isolates)
- H6N2 (25)
- H9N6 (1)

Hong Kong (3 isolates)
- H3N2 (1)
- H5N1 (2)

Laos (0 isolates)

Total number of obtained samples: 5,642
Influenza A virus (+): 85
Surveillance of Avian Influenza in Zambia

Materials: Bird feces

1. MWERU WANTIPA NP
2. SUMBU NP
3. BANGWEULU SWAMPS
9. LOCHINVAR NP

by Prof. Takada’s team
Characterization of H3N6 avian influenza virus isolated from a wild white pelican in Zambia

Edgar Simulundu · Aaron S. Mweene · Daisuke Tomabechi · Bernard M. Hang'ombe · Akihiro Ishii · Yuka Suzuki · Ichiro Nakamura · Hirofumi Sawa · Chihiro Sugimoto · Kimihito Ito · Hiroshi Kida · Lewis Saiwana · Ayato Takada

Received: 31 March 2009 / Accepted: 8 July 2009
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Characterization of influenza A viruses isolated from wild waterfowl in Zambia

Edgar Simulundu,1 Akihiro Ishii,1,2 Manabu Igarashi,1 Aaron S. Mweene,3 Yuka Suzuki,1,2 Bernard M. Hang'ombe,4 Boniface Namangala,4 Ladislav Moonga,4 Rashid Manzoor,1 Kimihito Ito,1 Ichiro Nakamura,1,3 Hirofumi Sawa,1,3 Chihiro Sugimoto,1,3 Hiroshi Kida,1,5,6 Chuma Simukonda,7 Wilbroad Chansa,7 Jack Chulu7 and Ayato Takada1,2
Surveillance of anthrax in Zambia

About 400 cases of human anthrax were found with 5 people dying in 2011.

Patients contacted with *Bacillus anthracis* contaminated hippopotamus meat.

Over hundred hippopotamuses as well as other wildlife died from suspected anthrax.

by Prof. Higashi’s team
Sensitivity of PCR test

Amplification plots for different samples (PA5/8, MO, BA813, PA6/7, CAP1/2, and CAP1234/1303)

- 10^7 copies
- 10^6 copies
- 10^5 copies
- 10^4 copies
- 10^3 copies
- 10^2 copies
- Non-template control

Amplification plot and Dissociation Curve showing the detection of 10 and 100 copies.
## Results of PCR test for *B. anthracis*

### Chama

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<tr>
<th>Sample Type</th>
<th>Number of Samples (positive/total)</th>
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<tr>
<td>human</td>
<td>0 / 14</td>
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<tr>
<td>hippopotamus</td>
<td>2 / 7</td>
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<tr>
<td>soil</td>
<td>4 / 24</td>
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### Lower Zambezi

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<tr>
<td>soil</td>
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<tr>
<td>elephant</td>
<td>4 / 6</td>
</tr>
<tr>
<td>buffalo</td>
<td>2 / 6</td>
</tr>
<tr>
<td>cattle</td>
<td>1 / 2</td>
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</tbody>
</table>
Identification of natural reservoir of viruses

Endemic area of Filovirus

Natural reservoir

Bat?
Rodents?

- Most of the zoonotic pathogens are introduced from microbial flora that exist in the wild
- To predict and prevent their outbreak, it is important to identify viral natural reservoir (preemptive measure for zoonosis outbreak)

by many members
Surveillance of rodent-transmitted pathogens in Zambia
Isolation of novel arenavirus

Co-cultivation with Vero E6 cells and kidney homogenate
- Medium change in every 7 days
- Cultivation for 28 days
- Harvest the culture supernatant
- Re-infect to Vero E6 cells
- Medium change at day 6
- Harvest the supernatant in every 2 days
- RT-PCR to detect viral RNA in culture supernatant

Electron microscopy of Luna virus LSK-1 with negative staining

Until now, we have isolated 6 Luna virus strains from Mastomys natalensis and 1 Lymphocytic choriomeningitis virus-Related virus (LCRV) from Mus minutoides.

by Assistant Prof. Ishii
Novel Arenavirus, Zambia

Akihiro Ishii, Yuka Thomas, Ladislav Moonga,
Ichiro Nakamura, Aiko Ohnuma,
Bernard Hang’ombe, Ayato Takada,
Aaron Mweene, and Hirofumi Sawa

To investigate arenavirus in Zambia, we characterized virus from the kidneys of 5 arenavirus RNA-positive rodents (*Mastomys natalensis*) among 263 captured. Full-genome sequences of the viruses suggested that they were new strains similar to Lassa virus–related arenaviruses. Analyzing samples from additional rodents and other species can elucidate epizootiologic aspects of arenaviruses.
Surveillance of fruit bat-transmitted pathogens in Zambia
Surveillance of insectivore bat-transmitted pathogens
Surveillance of fruit bat-transmitted pathogens in Indonesia by Associate Prof. Kimura’s team
Why fruit bat? Because…
Fruit bat has been recognized as a reservoir for zoonotic pathogens

- Henipa (Hendra and Nipah) virus
- SARS corona virus
- Ebora virus
- Bat lyssavirus

→ Potentially be a reservoir of currently unrecognized viruses
Why Indonesia?

- More than 50 species of fruit bat are distributed nationwide
- Lots of wild and domestic animals
- Human-animal contact may occur easily
- Lots of zoonotic diseases
- Located between peninsular Malaysia (where Nipah virus emerged) and Australia (where Hendra virus emerged)
- Recent satellite telemetry studies showed regular bat movement from Malaysia to Sumatra and vice versa
Detection of Novel paramyxovirus RNA in fruits bats from Indonesia

Samples were collected from 4 locations
Case positivity: 5/73 (6.8%)
Next-generation Sequencers has been installed

Roche GS Junior (50 Mb, \[150,000- / Run\) LT SOLiD5500xl (40 Gb, \[1,000,000- / Run) x2

Test run (E. coli genome)

estimatedGenomeSize = "5.4 MB";
numAlignedReads = 130155, 97.94%;
umAlignedBases = 58771763, 98.16%;

largeContigMetrics
numberOfContigs = 170;
numberOfBases = 4638568;
avgContigSize = 27285;
N50ContigSize = 54753;
largestContigSize = 154107;

allContigMetrics
numberOfContigs = 206;
numberOfBases = 4646634;

→ 58.7 Mb

Test run (16strains Leptospira interrogans genome)

1 lane / 16 tag (6 lane / Slide)
1 strain = 40Gb / 6 / 16 ≈ 400 Mb

<table>
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<tr>
<th>Type</th>
<th>Reference mapping</th>
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<td>GC contents in %</td>
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<td>Total read count</td>
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<td>Mean read length</td>
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<tr>
<td>Total read length</td>
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Summary of our activities

- Hokkaido University Research Center for Zoonosis Control (CZC) will conduct global surveillance to identify natural host animals and transmission routes of zoonotic pathogens, and will reveal determinants for the pathogenicity and the host range of the pathogens.
- The outcomes of the research will be pooled as a database for preservation and utilization of biological resources, and the materials will be supplied for diagnosis technology and vaccine production.
- At the same time, our educational program will provide lectures and training courses for researchers, technicians and graduate students, and will have a mission to bring up “Zoonosis Control Experts” who are responsible for the control of zoonotic diseases worldwide.
Thank you very much for your attention!

“BIMASENA” in Sapporo Snow Festival 2011