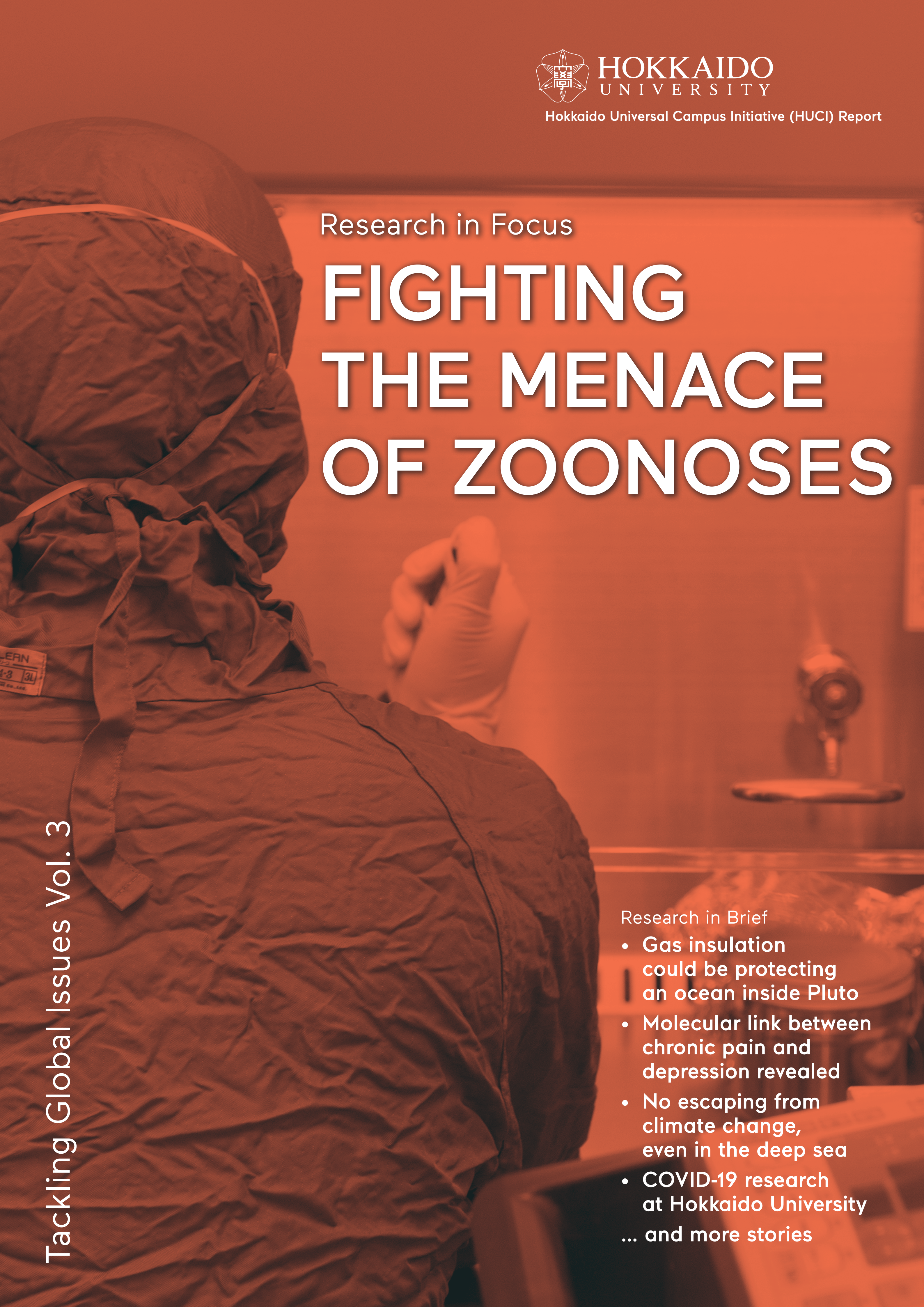

HOKKAIDO UNIVERSITY

Hokkaido Universal Campus Initiative (HUCI) Report

Research in Focus

FIGHTING THE MENACE OF ZOONOSES

Research in Brief

- Gas insulation could be protecting an ocean inside Pluto
- Molecular link between chronic pain and depression revealed
- No escaping from climate change, even in the deep sea
- COVID-19 research at Hokkaido University

... and more stories

Tackling Global Issues Vol. 3

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Preface

The outbreak of the new coronavirus SARS-CoV-2 at the end of 2019 quickly spread across the globalized world; as 2021 dawns, it is still afflicting people around the world and changing our daily lives. It has changed the way we work, travel, eat, and even communicate. Healthcare workers tirelessly continue to battle the virus and help patients under the great pressure of increasing cases and limited resources.

Many people did not expect a virus to change the world as much as it has, but it was not entirely unexpected. In 2005, in the wake of 2003 SARS, Hokkaido University established the Research Center for Zoonoses Control (CZC) to conduct comprehensive research on zoonotic diseases. It aims to prevent outbreaks of zoonotic diseases by identifying host animals and transmission routes of potential pathogens, as well as to promote basic research and developing diagnostic and therapeutic measures. The center has made a number of contributions in the research field, as exemplified in this magazine.

Hokkaido University will celebrate the 150th anniversary of its founding in 2026. Towards this milestone, the university formulated an action strategy under the slogan of "contributing towards the resolution of global issues." One of the applied strategies was the establishment of the Global Institution for Collaborative Research and Education (GI-CoRE). The organization conducts top-level international research in collaboration with world-leading researchers from around the globe. It has launched seven programs, including the Global Station for Zoonosis Control, which was established in 2014, jointly with researchers from the University of Melbourne, University College Dublin, and King Abdullah University of Science and Technology. In 2020, the program was integrated into CZC to further promote and develop international collaborative research and education in cooperation with GI-CoRE.

This magazine, following the second issue featuring quantum medical science and engineering, will highlight the university's effort to help control zoonotic and other infectious diseases, and showcase various research activities at the university. We hope readers of this magazine will gain insight into how Hokkaido University strives to contribute to the resolution of global issues through research.

Atsushi Yokota, Ph.D.
Executive Vice President
Executive Director, Institute for International Collaboration
Hokkaido University

introduction

Fighting the menace of zoonoses

The world has witnessed outbreaks of zoonoses – any disease or infection naturally transmissible from vertebrate animals to humans – as our environment has drastically changed over the years. An explosive increase in the global population has required more farmland to produce food, while the expansion of economic activities has caused deforestation and desertification. Such radical environmental changes have disrupted the ecology and home range of natural reservoirs, eliminating some boundaries between wildlife and human society. Indeed, zoonoses present an existing and future menace to mankind.

Animals are natural hosts to bacterial, viral or parasitic agents that do not cause any harm to the reservoirs themselves, but transmit infections to other species of wild and domestic animals – and eventually to humans.

About 60 percent of infectious diseases and almost all emerging ones are zoonoses, according to Hiroshi Kida, one of the world's top zoonosis experts and Head of Hokkaido University's Research Center for Zoonosis Control (CZC). Zoonoses include pandemic and avian influenzas, Ebola hemorrhagic fever, acquired immunodeficiency syndrome (AIDS), bovine spongiform encephalopathy (BSE), Lassa fever and yellow fever. About 1 billion cases of illness and millions of deaths occur every year from zoonoses, according to an estimate by the World Health Organization (WHO). In one high-profile case, the 2014-16 Ebola pandemic in Guinea, Liberia and Sierra Leone resulted in about 28,000 people becoming infected and more than 11,000 deaths.

In the most recent outbreak, the novel coronavirus (SARS-CoV-2) , which was first reported from Wuhan, China, on December 31, 2019, and causes Coronavirus Disease 2019 (COVID-19), has infected more than 63 million people around the globe causing nearly 1.5 million deaths as of December 3, 2020, according to the WHO. Chinese officials say the virus first transmitted from animals to humans at a fresh fish market and human-to-human transmission was soon reported, suggesting it is a zoonosis.

"It is impossible to eradicate zoonoses," Kida said. "Therefore, our mission is to control zoonotic diseases as much as possible through preemptive measures and effective treatments. That's why it is crucial to identify the natural reservoirs as well as the transmission route of diseases."

> "Everything we do at the center must help us achieve the goal of controlling zoonoses under the concept of One Health"

Spearheading comprehensive research on zoonoses

The researchers' endeavors are in line with the "One Health" concept, which recognizes that human health and animal health are interdependent and bound to the health of our ecosystem. This concept, established in the 2000s, is now embraced by such international organizations as the WHO, the World Organization for Animal Health (OIE), the World Bank, UNICEF and the Food and Agriculture Organization (FAO).

Just as the concept was starting to become accepted, Hokkaido University established the CZC in 2005 as a unique research and educational institution. The center has adopted a unique interdisciplinary approach and brings together experts in wide-ranging fields such as microbiology, virology, immunology, pathology and information science to conduct comprehensive research. "Zoonoses cannot be studied solely with expertise in medicine or veterinary medicine," Kida said. "It is a new academic area that does not fit in any conventional field." Indeed, the center's research topics include isolating and detecting new viruses; developing easy-to-use, rapid and accurate diagnosis kits; developing antiviral drugs to treat zoonoses; and even playing a key role as the OIE Reference Laboratory for Avian Influenza. "Everything we do at the center must help us achieve the goal of controlling zoonoses under the concept of One Health," Kida said. ●

Influenza expert with unbending faith in research

Hiroshi Kida, DVM, Ph.D.

+ University Professor, Hokkaido University
+ Head, Specially Invited Professor of the Research Center for Zoonosis Control (CZC)
+ Head, Collaborating Research Center for the Control and Prevention of Infectious Diseases, Nagasaki University
+ Head of the WHO Collaborating Centre for Zoonoses Control

Hiroshi Kida, a top authority on zoonoses, has frequently been challenged by naysayers who dispute virtually everything he has asserted about avian, pandemic and seasonal influenzas.

But every time he faced such a challenge, Kida simply concentrated on his pursuit of scientific truths involving influenza – one of the zoonoses – rather than engaging in verbal arguments. "In some cases, it was me against 100 opponents," Kida recalled. "But influenza can be tackled only by steadily doing careful research. In 10 years, we all will know where the truth lies."

> "In 10 years, we all will know where the truth lies"

One recent assertion Kida made is that inactivated whole virus particle vaccines, which are now undergoing clinical studies, should replace conventional split vaccines made of viruses that are disrupted by ether or detergent.

Kida is a long-time proponent of using whole virus particle vaccines after he succeeded in making one as a researcher at Takeda Pharmaceutical Co., Ltd. in the 1970s. Whole virus particle vaccines are more immunogenic than split vaccines, he claims.

"In current clinical trials, whole virus particle vaccines have shown they are effective," Kida said. "They are also much cheaper to produce because there is no need to disrupt the viruses."

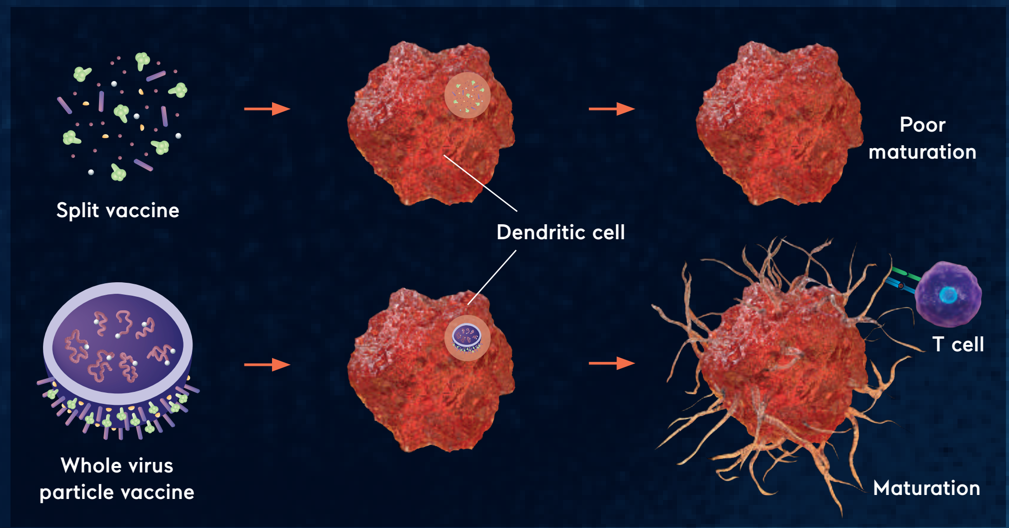
Since 2015, Kida has been working with all five influenza vaccine producers in Japan to study the introduction of inactivated whole virus particle vaccines. The National Institute of Infectious Diseases in Japan has joined this industry-academia-government joint project, while the Japan Agency for Medical Research and Development has provided much-needed funds for the research. "Failure is not an option," Kida said, expressing his resolve to make the vaccines available to society.

Identifying natural reservoirs and transmission routes of avian influenza

After a seven-year stint at Takeda, Kida joined Hokkaido University in 1976 to pursue his research on influenza. Kida later established that ducks are the natural host for Type A influenza viruses and identified the transmission route to humans for the first time.

The migratory bird is orally infected with the virus with varying hemagglutinin (HA; a glycoprotein) and neuraminidase (NA;

Antigen presentation: When exposed to a split vaccine (SV), a dendric cell (DC) – a special type of immune cell – displays a poor maturation and antigen presentation (top) while it exhibits a strong maturation and efficient antigen presentation when exposed to whole virus particle vaccines (bottom).



Adobe stock © Kateryna_Kon, © 7activestudio enzyme) subtypes as they nest in lakes and marshes in Siberia, Canada or Alaska near the Arctic in summer. The virus proliferates in the duck's colon crypts and is discharged with feces, but does not do any harm to the duck. When the duck migrates south in autumn, it infects poultry and livestock on the way to or in its wintering places, such as southern China, Southeast Asia, the southern United States and Mexico. The virus is transmitted by water to domestic ducks and geese and then to terrestrial poultry such as quails, which serve as intermediate hosts that transmit the virus to chickens. While water birds have the receptor to viruses in their intestine, land birds such as chickens have the receptor in their respiratory organs. The infection of quails makes it easier for the virus to spread to chickens.

As the virus is repeatedly transmitted between chickens over more than six months, it can acquire pathogenicity in chickens. Only H5 and H7 subtype viruses become highly pathogenic avian influenza viruses and highly transmittable among chickens. These subtype viruses cause a high fatality rate.

> "Transmissibility and pathogenicity are totally different things"

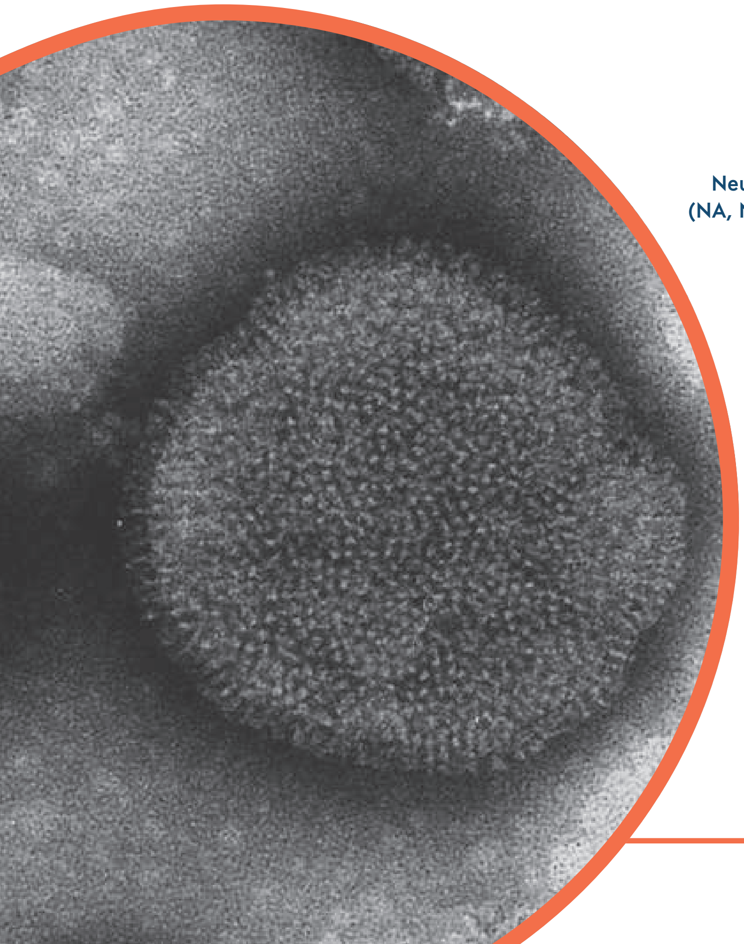
A genetic reassortant virus can cause a pandemic in humans

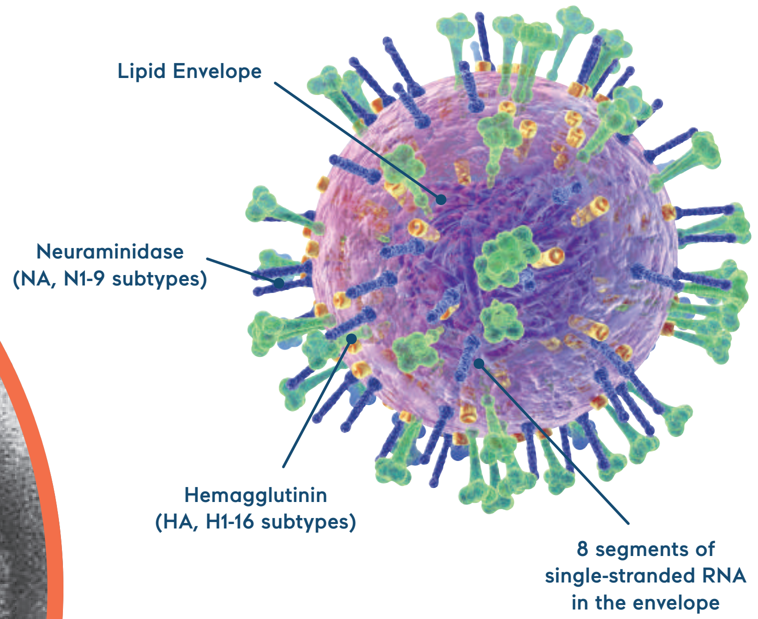
Humans can rarely be infected with avian influenza viruses. The virus has not been transmitted between humans to date, so it has never caused a pandemic. Then, what caused the pandemics in the past: the Spanish flu in 1918; Asian flu in 1957; Hong Kong flu in 1968; and the 2009 pandemic flu?

Kida strongly suspected influenza was a zoonosis after reading academic articles by overseas researchers pointing to similarities between a viral protein found in ducks and horses, and that of the 1968 Hong Kong pandemic influenza virus strain. He examined feces of migratory birds and finally, in 1977, he isolated a virus (A/duck/Hokkaido/5/77) found in the intestine of a duck that had flown from Siberia to Hokkaido. This virus had HA identical to that of the 1968 Hong Kong influenza virus and NA undistinguishable from that of the 1957 Asian flu virus.

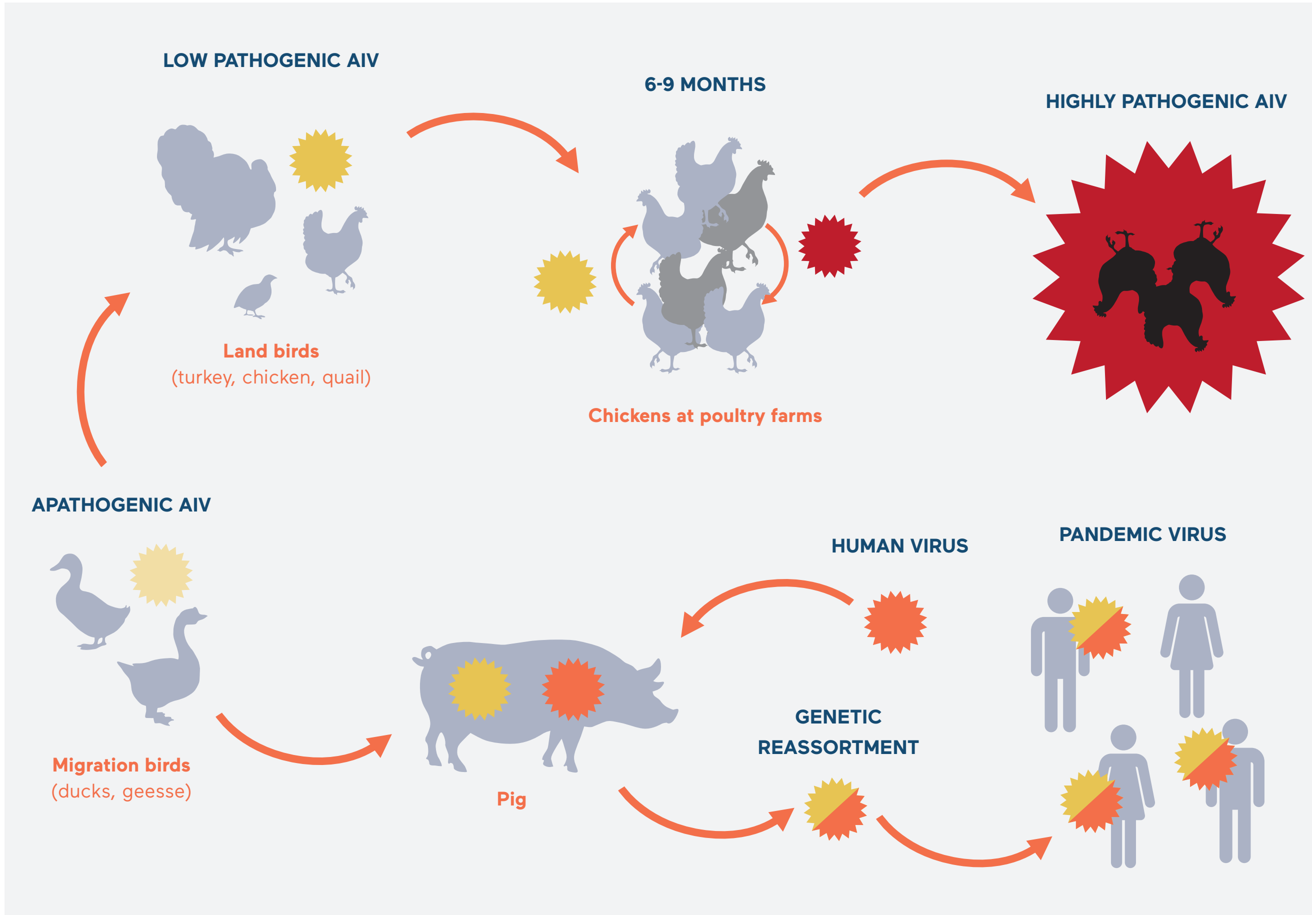
"This duck taught me that the emergence of a new virus is not a result of antigenic variation, but genetic reassortment between different strains," Kida said. Genetic reassortment is the process by which influenza viruses swap gene segments. Ensuing experiments showed that two of the eight viral genes originated in ducks and the six others were from the Asian flu virus.

"We proved pigs, which have receptors both to the avian and human viruses, serve as a mixing vessel," Kida said. "When pigs are simultaneously infected with both influenza viruses, the genetic reassortment will take place, producing an 'avian-human hybrid virus' that can infect and transmit between humans. Among them, those with HA genes that originated in ducks are pandemic influenza viruses."

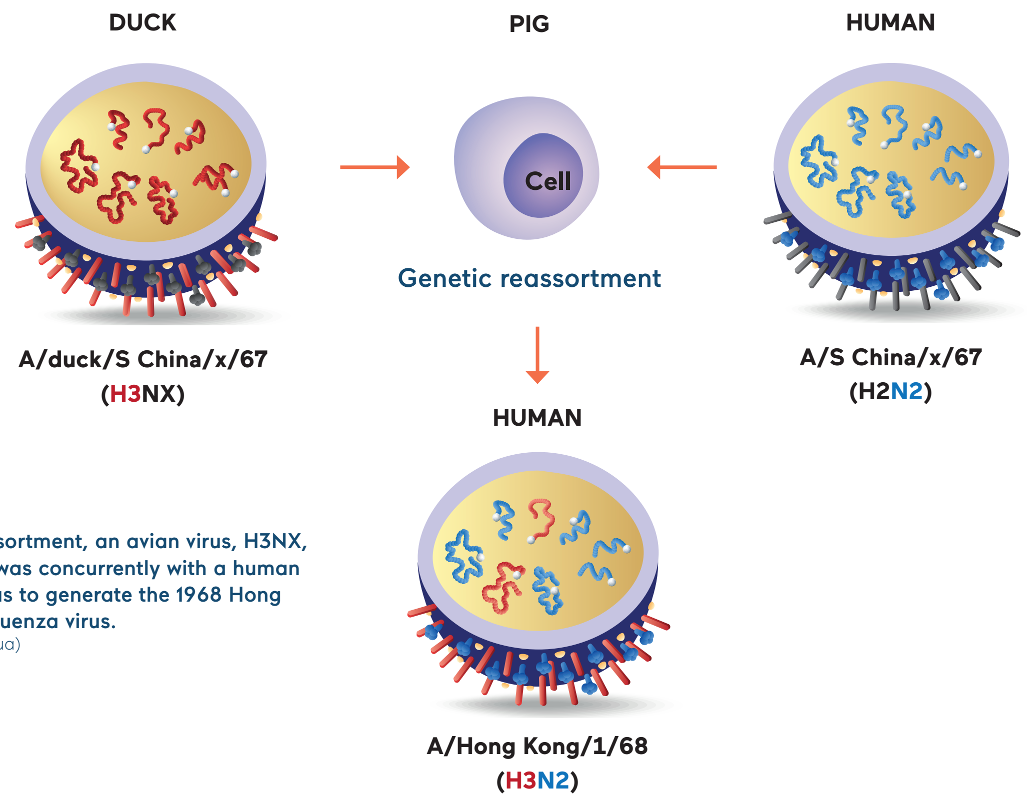




The electron microscopic image of a Type A influenza virus particle (left). The virus has hemagglutinin (HA) and neuraminidase (NA) proteins on its surface (above). HA has sixteen subtypes and NA has nine.
(Illustration Adobe Stock © Axel Kock)



How avian and human pandemic virus strains emerge: An apathogenic avian influenza virus (AIV) carried by wild ducks is transmitted to poultry such as geese, quails and turkeys and becomes low pathogenic. After the virus is transmitted among chickens over six months, the virus becomes highly pathogenic (HPAIV). A human pandemic virus emerges after a pig contracts both avian and human influenza viruses, and produces hybrid viruses through genetic reassortment.
(Adobe Stock © marinavorona, © ssstocker)



In this genetic reassortment, an avian virus, H3NX, carried by a duck, was concurrently with a human H2N2 influenza virus to generate the 1968 Hong Kong pandemic influenza virus.
(Adobe Stock © designua)

Pandemic flu virus highly transmittable, but not so pathogenic

At least the past four pandemic influenza viruses were proven to have come from pigs. During the 2009 pandemic, there were ungrounded fears that more than 500,000 people would die from the flu in Japan alone, which led to the enactment of special legislation to deal with pandemic influenza.

"I strongly opposed the government's move because the virus that proliferates in pigs will not propagate as much in the human body," Kida said. "But since humans do not have immunity against the pandemic virus, it is highly transmissible, albeit not so pathogenic. Transmissibility and pathogenicity are totally different things."

The 1918 Spanish flu was allegedly so pathogenic that it caused the deaths of 40 million people, but later studies showed many of these deaths were caused by simultaneous infection with bacteria and poor living conditions, Kida said.

Kida laments the wide spread of what he considers to be a gross misunderstanding of pandemic influenza. "The media still incorrectly report that a new type of influenza emerges as a result of mutations acquired by avian influenza viruses."

Kida said a flu virus strain is the collection of mutant viruses in the first place. While they transmit from human to human, some mutant viruses that are more proliferative than others will be dominant. A pandemic flu then becomes a seasonal one next year and beyond, becoming more pathogenic in humans.

Trying to dispel misunderstanding over influenza

Kida said understanding how people get sick from influenza is crucial. There is a widespread misperception that the virus itself causes sickness in the human body. "Sickness occurs as innate immune responses when the amount of a virus increases in the body," Kida said. "When excessive responses occur, a person runs a fever or develops serious symptoms. In the most acute cases, blood vessels can be damaged by clots and vascular hyperpermeability, resulting in multiple organ failure, including encephalopathy in children."

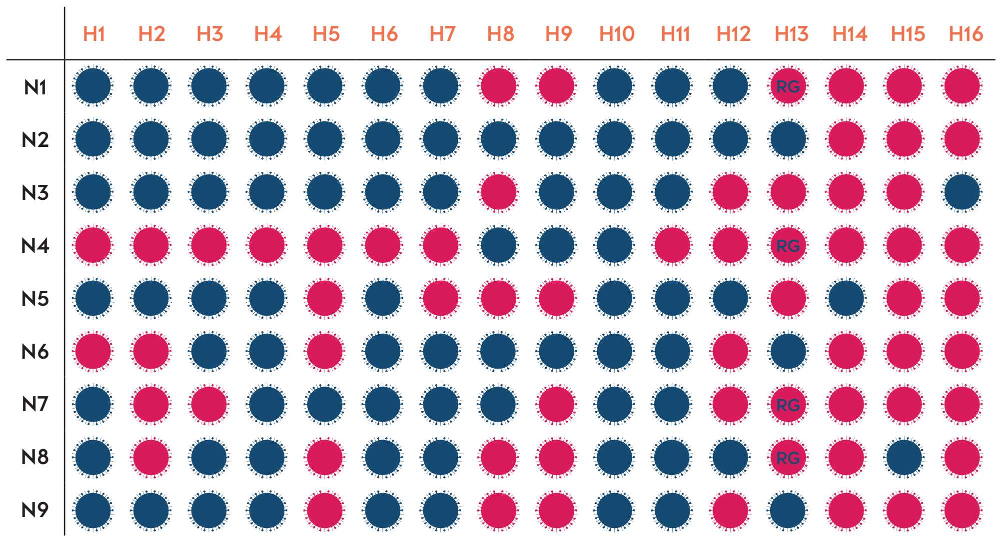
It is vital to control pandemic influenza based on countermeasures against seasonal influenza, Kida stressed. Accordingly, improving seasonal flu vaccines is urgently needed. Kida is determined to realize the introduction of whole virus particle vaccines to replace the weak immunogenic split vaccines that are currently used.

> "It is my responsibility to make the database available to whoever needs it"

One of Kida's many contributions is accumulating influenza virus data from duck feces to make a library of pandemic influenza vaccine strain candidates. The library stores more than 4,600 avian influenza virus strains of all 144 combinations of HA and NA subtypes as vaccine strain candidates. Their pathogenicity, antigenicity, genetic information and yield in chicken embryo have been analyzed, put in a database and made available on a website (https://virusdb.czc.hokudai.ac.jp/).

"I declined suggestions that the database be patented," Kida said. "It is my responsibility to make the database available to whoever needs it, because I cannot achieve much by myself as a researcher." ●

The library of pandemic influenza vaccine strain candidates: Black viruses were isolated from a waterfowl (81 combinations), while red ones were generated in the laboratory (63 combinations). More than 4,600 avian influenza virus strains of 144 combinations of HA and NA subtypes are stored in the library.



Virus isolated from a waterfowl (81 combinations)

Virus generated in the laboratory (63 combinations)

VOICES: Dr. Lesa Thompson

Hokkaido University alumna Dr. Lesa Thompson is a Regional Project Officer at the World Organisation for Animal Health (OIE), Regional Representation for Asia and the Pacific (RRAP) office in Tokyo. Here, she talks about the OIE, Hokkaido University's Research Center for Zoonosis Control and her days as a student at the Graduate School of Veterinary Science.

Dr. Lesa Thompson

I first met some students and professors from Hokkaido University's Faculty of Veterinary Medicine who were visiting the Royal (Dick) School of Veterinary Studies at the University of Edinburgh. When I found out that their One Health PhD course was open to international applicants, I applied. During my course, I did two internships. One of them was with the OIE RRAP office, where I now work full-time.

The OIE is an intergovernmental organization responsible for improving animal health worldwide. It recognizes the growing importance of zoonotic animal pathogens because, often, animals are biosensors for human disease and zoonoses. The OIE also works with the Food and Agricultural Organization of the United Nations (FAO) and World Health Organization (WHO) against jointly identified priorities: rabies, zoonotic influenza and antimicrobial resistance.

> "It is an excellent opportunity to experience something new to consider for a future career"

As a member of the One Health team at the OIE RRAP, my main role is implementing various activities to support OIE Members under the One Health concept, and to support them in collaboration with our partner organizations. These activities include regional and in-country workshops and seminars, for example, hands-on training of laboratory procedures in collaboration with our Reference Laboratory experts, or workshops to establish multi-sectoral coordination mechanisms.

The OIE has a global network of 263 Reference Laboratories covering 110 diseases or scientific topics in 37 countries. Hokkaido University's Research Center for Zoonosis Control (CZC) is an OIE Reference Laboratory for highly pathogenic avian influenza and low pathogenic avian influenza (poultry); it is also a WHO Collaborating Centre for Zoonoses Control. The center is responsible for providing high quality disease diagnostic services as global references to all Member countries of the OIE. The OIE expert at CZC should provide expert advice on topics linked to diagnosis and control of the disease for which the Reference Laboratory is responsible.

Regarding veterinary education, I am also involved in the OIE support of Twinning Projects between Veterinary Education Establishments to improve the quality of veterinary graduates. Recently, a project was completed between Hokkaido University and the Mongolian University of Life Sciences, and there is an ongoing project between the University of Tokyo and the Royal University of Agriculture in Cambodia.

For current students at Hokkaido University, I would recommend that if you have the opportunity to do an internship, think carefully about your placement. It's an excellent opportunity to experience something new to consider for a future career, and it might also help you gain the experience you need to get the job!

Participants of the "OIE Regional Expert Group Meeting for diseases of poultry in Asia and the Pacific Region" held at CZC, Hokkaido University, 2-4 October 2019.

Working toward eradication of avian influenza

Yoshihiro Sakoda, DVM, Ph.D.

+ Professor at the Faculty of Veterinary Medicine and Research Center for Zoonosis Control (CZC)
+ Research areas: Avian influenza, microbiology, disease control
+ Responsible Official of the OIE Reference Laboratory at Hokkaido University

From April to June in 2013, 43 cases of a low pathogenic avian influenza were reported among poultry and wild pigeons in nine provinces, one city and two autonomous regions of China. This influenza, caused by the H7N9 virus strain, took many specialists by surprise because few of them had expected such a virus subtype would emerge to infect birds.

As the virus infection was repeated among poultry, the virus became highly pathogenic, killing birds and then humans. According to the United Nations' Food and Agriculture Organization (FAO), 1,568 cases of human infection have been reported, including 616 fatal cases.

There was, however, one institution that had stockpiled the H7N9 virus just in case such a virus began to infect birds: the Research Center for Zoonosis Control (CZC) of Hokkaido University. "Our strategy of having all 144 avian influenza virus subtypes ready and stored in a library was worthwhile," said Yoshihiro Sakoda, who is a key architect of the avian influenza virus library at CZC, which stores all 144 combinations of hemagglutinin (HA; a glycoprotein) and neuraminidase (NA; an enzyme) subtypes. Eighty-one combinations of HA and NA subtypes have been isolated from fecal samples of waterfowls in surveillance studies. The 63 other combinations were generated by the genetic reassortment procedure in embryonated chicken eggs.

"Because of our preparation, we were able to write a report about human vaccination against the H7N9 influenza right after its outbreak," Sakoda said. "It is crucial to have preemptive measures in place."

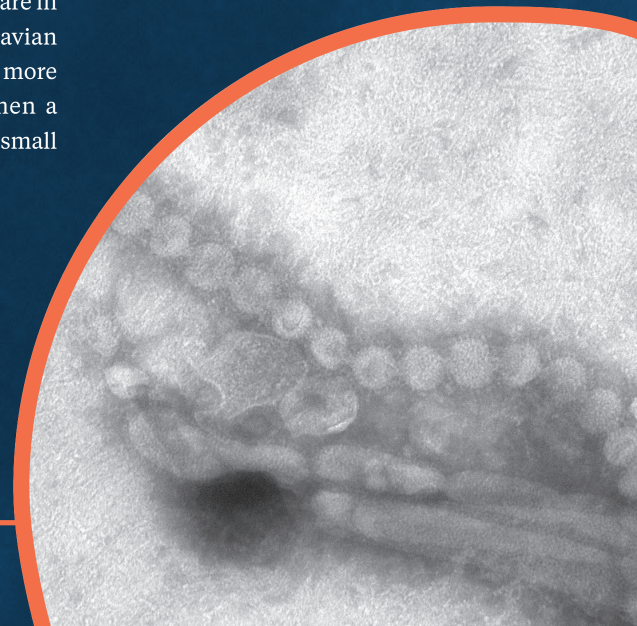
> "It is crucial to have preemptive measures in place"

Researchers monitor a fresh poultry market in Vietnam.

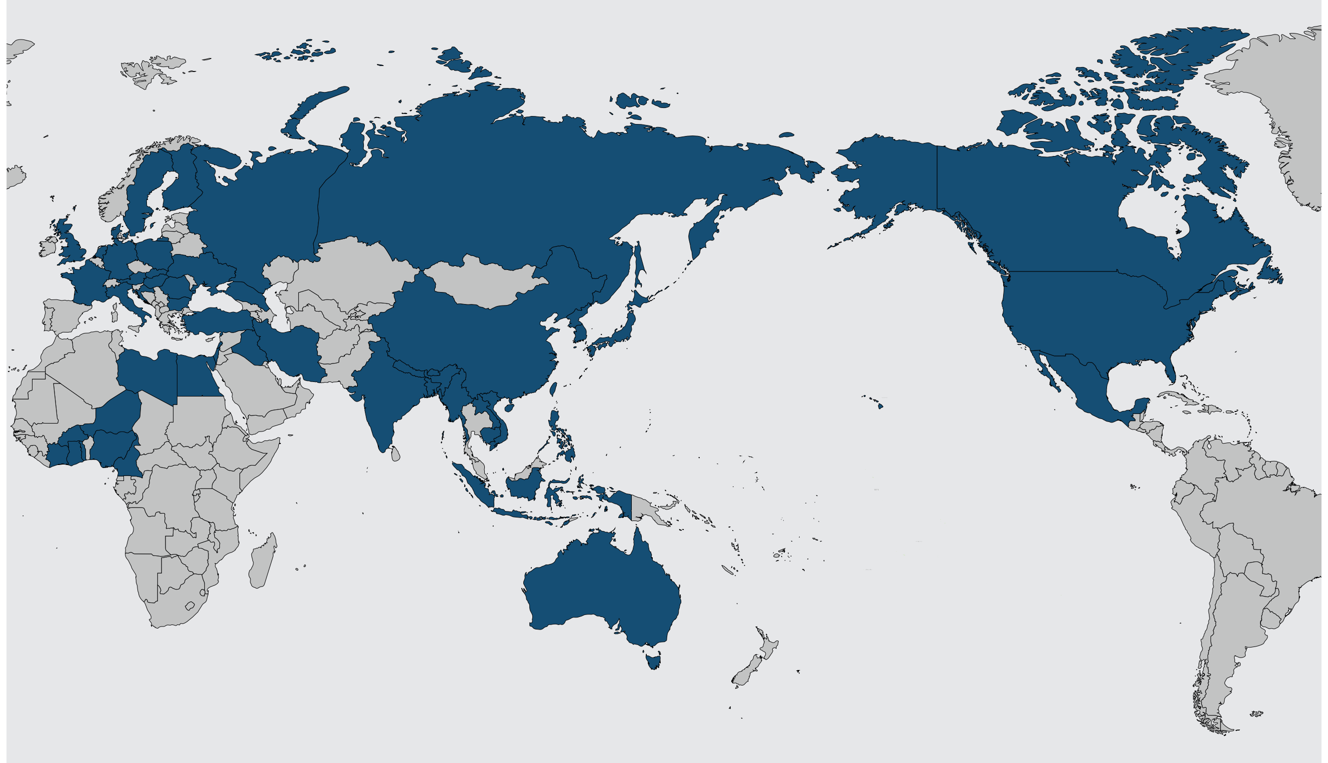
Spread of highly pathogenic avian influenza

The most prominent emergence of highly pathogenic avian influenza virus dates back to 1996, when the H5N1 virus strain was first detected in geese in China. It is highly contagious among birds and can be deadly for poultry. Human infection was first reported in 1997 during a poultry outbreak in Hong Kong and has since been detected in poultry and wild birds in more than 60 countries in Africa, Asia, Europe and the Middle East. Four countries – China, Egypt, Vietnam and Indonesia – are considered to be endemic for H5N1 in poultry. According to the World Health Organization (WHO), 861 cases of humans infected by the H5N1 strain, including 455 fatal ones, were reported as of January 2, 2020.

According to Sakoda, the spread of highly pathogenic avian influenza across the world can be blamed on the explosive increase in the global population, which has driven up demand for more sources of protein. "Humans are in fact accountable for the spread of avian flu viruses because of the need for more poultry farms," Sakoda said. When a large number of birds are kept in a small space, a virus can spread easily and quickly, gaining higher pathogenicity in the process. "The lack of hygiene measures at such farms has helped preserve influenza viruses of various subtypes in the environment and spread



Electron microscopic image of H7N9 influenza virus.
Photo courtesy of CDC/Cynthia S. Goldsmith and Thomas Rowe



The countries that have experienced a highly pathogenic avian influenza incident since 2014.
(Adobe Stock © pyty)

highly pathogenic influenza around the world via migratory birds," Sakoda said.

Although no human infection in Japan has been reported, people have been infected in China and Southeast Asian nations, where live poultry markets are thriving. Sakoda said people working at live bird markets are exposed to a huge amount of viruses and some accidentally contract the viral infection.

So far, there has been no reported human-to-human transmission of avian influenza. However, the possibility of human-to-human transmission cannot be completely ruled out. Sakoda said eradicating avian influenza is essential under the One Health concept, which recognizes that the health of animals and humans are interrelated.

Obstacles in containing avian influenza

"The best way today to contain avian influenza is to cull all chickens at contaminated poultry farms, disinfect the farms and take all possible measures to stop the viruses spreading from them," Sakoda said. They had researched how to control avian influenza based on scientific evidence long before the 2004 outbreak in Japan, and that is why it was handled very effectively, according to Sakoda. Since then, Japan has detected influenza viruses in wild birds and limited contamination of poultry farms, but its control of the avian disease is considered one of the best in the world. "Some people oppose culling, but it is really necessary for helping farmers return to their business as soon as possible," Sakoda explained.

However, culling is not an option for such countries as China, Vietnam, Indonesia and Egypt, where avian influenza is endemic. Instead, they vaccinate poultry.

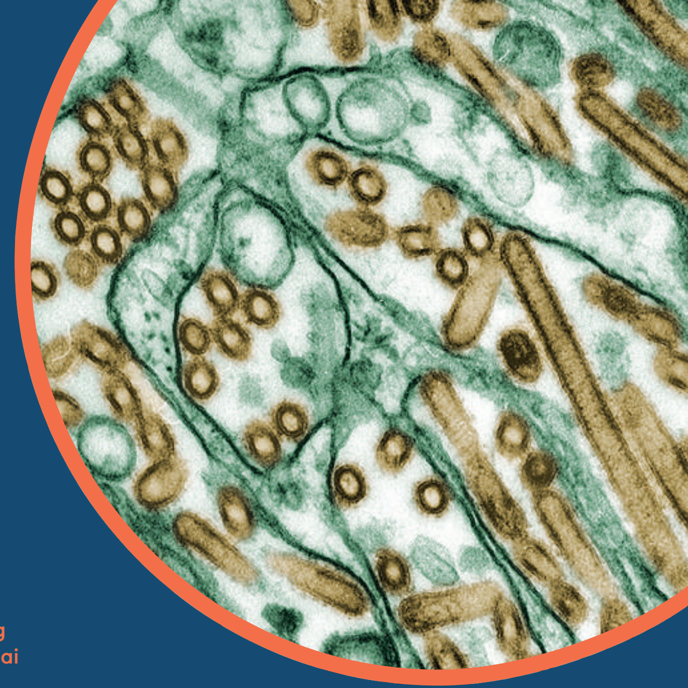
This approach, Sakoda said, is problematic in that it will spread "hidden infections" because vaccinated chickens develop less serious symptoms than unvaccinated ones, but can still spread the viruses.

Improving hygiene at live poultry markets in Asia is a daunting task. Many developing nations lack cold-chain logistic systems to transport fresh meat safely from farms to consumers. "Crafting such a system is important for preventing people from getting exposed to avian viruses at live poultry markets and for preventing the viruses from proliferating," Sakoda said.

Furthermore, poultry farmers in those countries are often unwilling to cull their birds because there is no subsidy program to cover the resulting financial damage. Some developed nations give handsome subsidies to affected farmers.

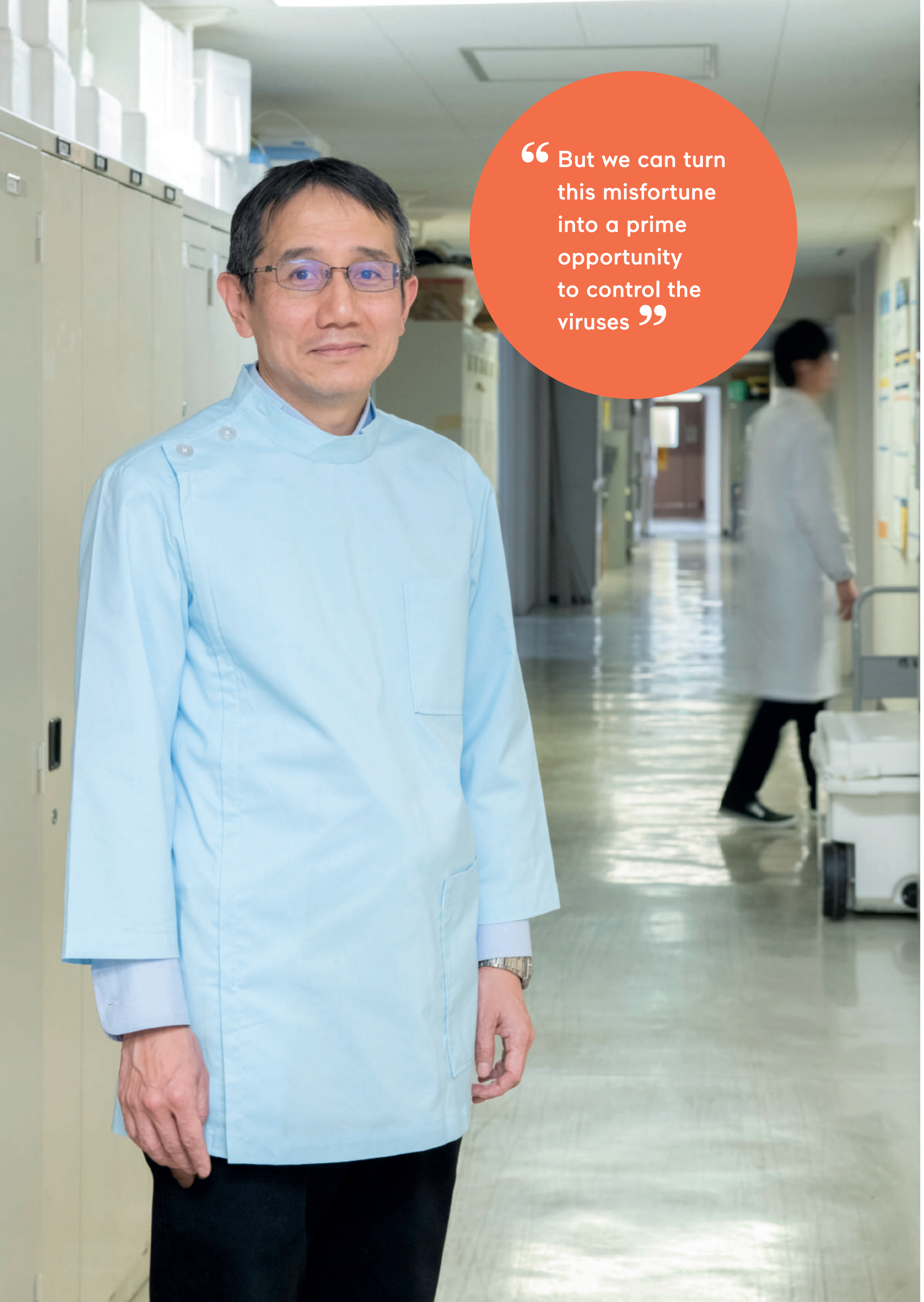
> "Humans are in fact accountable for the spread of avian flu viruses"

> "Some people oppose culling, but it is really necessary for helping farmers return to their business as soon as possible"



Colorized transmission electron micrograph of avian influenza A H5N1 viruses (brown) grown in cultured cells (green). Avian influenza A viruses do not usually infect humans, but several instances of human infections and outbreaks have been reported since 1997.
Photo courtesy of CDC/Cynthia Goldsmith; Jacqueline Katz; Sherif R. Zaki

Some obstacles in containing avian influenza are difficulties in improving hygiene at live poultry markets in Asia. (Chicken market in Xining, Qinghai province, China, *below*.) © M M (Padmanaba01).



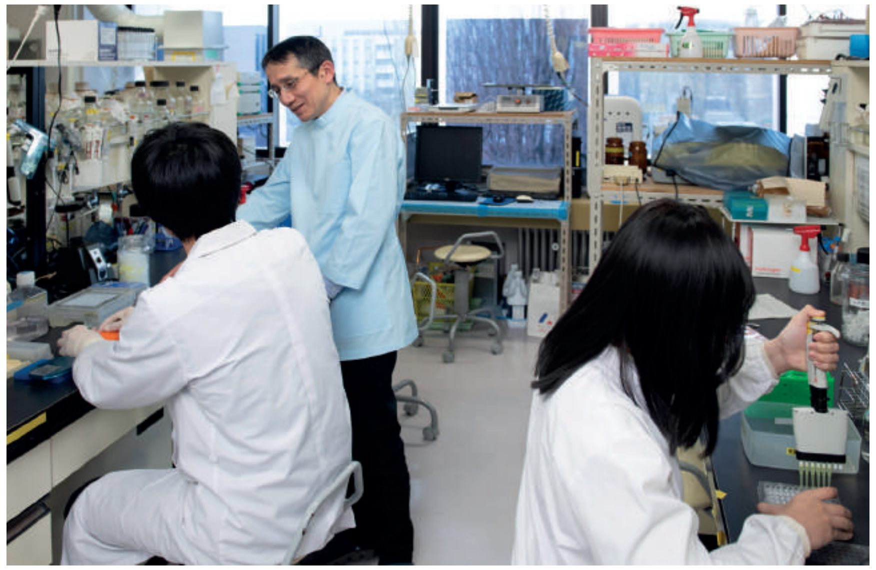
Global surveillance is essential

Hokkaido University is one of the nine World Organization for Animal Health (OIE) Reference Laboratories tasked with supporting the diagnosis of avian influenza, developing necessary diagnostic methods, and providing scientific and technical advice to other Asian countries.

Hokkaido University has already developed an innovative, easy-to-use diagnosis kit capable of rapidly identifying virus subtypes in the field or on farms. “We are doing it for the benefit of Asia,” Sakoda said. “If the spread of the viruses is contained elsewhere in Asia, there will be a smaller chance that migratory birds will infect poultry in other countries. We have to take a leading role in helping eradicate or contain avian influenza viruses in China and Southeast Asian nations.”

Global surveillance of avian influenza is also essential. Wild waterfowls such as ducks are natural hosts to influenza viruses, which are not harmful to them. But they can spread the viruses around the world as they migrate, which could later become highly pathogenic. “By monitoring viruses those migratory birds bring, we can warn poultry farmers to take protective measures when the risk grows,” says Sakoda.

Hokkaido University conducts surveillance in Japan, Mongolia and Vietnam. “If the viruses kill migratory birds or poultry, it will be unfortunate,” Sakoda said. “But we can turn this misfortune into a prime opportunity to control the viruses if we thoroughly examine the viruses and make the results available to countries that have not experienced an outbreak.”



Dr. Sakoda in his research lab at Hokkaido University.

Working toward the eradication of avian influenza

Sakoda is a graduate of the School of Veterinary Medicine. He returned to Hokkaido University in 2001 as a research assistant under Dr. Hiroshi Kida, now CZC head, after a seven-year-stint at the National Institute of Animal Health, where he conducted extensive research on swine fever.

When Sakoda returned to the university, Kida told him to continue researching swine fever while also studying avian influenza. Kida said, “Each researcher should have their own research topics.” Sakoda’s years of research on swine fever, which made him one of the world’s foremost experts on the subject, has helped Japan deal with the swine fever outbreak that began in 2018.

Nonetheless, Sakoda remains committed to working toward the eradication of avian influenza. “If we can properly control avian influenza viruses, I think their eradication is possible,” he said. “I want to see that before I retire from the university 15 years from now.”

“If we can properly control avian influenza viruses, I think their eradication is possible”

Sakoda collecting feces of ducks and swans that flew from Siberia during a survey in Wakkanai, Hokkaido, the northernmost part of Japan.

Industry-academia collaboration to fight flu and emerging infections

Akihiko Sato, DVM, Ph.D.

+ Visiting Professor at the Research Center for Zoonosis Control (CZC)
+ Team Leader of Pharmaceutical Research Division, Shionogi & Co., Ltd.
+ Research areas: Virology, anti-virus drug development

After he began a joint research project with Hokkaido University around 2005 as a drug discovery specialist from Shionogi & Co., Ltd., Akihiko Sato made his childhood dream come true.

"I was keenly interested in yellow fever ever since I read a biography about Hideyo Noguchi as an elementary school student," Sato said, referring to the prominent Japanese bacteriologist who conducted research on yellow fever until his 1928 death due to the disease of his research topic. "I had a chance to examine the flavivirus that causes yellow fever, for the first time at Hokkaido University. I was really excited to be able to learn what kind of virus it was. I had longed to see this virus for such a long time."

Sato – a visiting professor at the university since 2013 (while retaining his position at Shionogi) – is now following the footsteps of Noguchi by researching infectious diseases that afflict people in developing countries. Sato's research centers on drugs that can treat such infectious diseases as Lassa fever and dengue fever. In particular, he has made headway in discovering a new drug candidate effective in treating Lassa fever, an acute viral hemorrhagic illness caused by Lassa virus, a member of the arenavirus family. The deadly disease causes shock, seizures, tremors, disorientation and coma in the late stages, and is endemic in West African nations including Benin, Togo, Liberia, Sierra Leone and Nigeria.

HIDEYO NOGUCHI

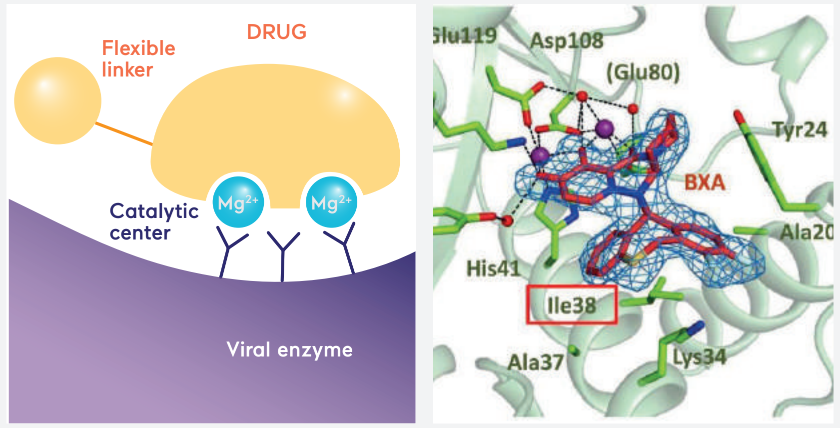
Hideyo Noguchi, a Japanese bacteriologist who discovered *Treponema pallidum*, the causative agent of syphilis, in the brains of patients suffering from paresis. He conducted research on yellow fever until he died in 1928 due to the disease.
Photo courtesy of Noguchi Hideyo Memorial Hall

Development of anti-influenza drugs

Sato is a virus specialist with more than 40 years of experience. When he first took up the subject upon joining Shionogi, however, research on drugs to treat viral diseases was not receiving the kind of attention it deserved. In 1974, the world's first anti-viral drug, Acyclovir, was developed in the United States, but it was not until the discovery of human immunodeficiency virus (HIV) and the development of genetic engineering in the 1980s that research on anti-viral drugs accelerated.

After devoting much of his time at Shionogi to researching HIV/AIDS (acquired immune deficiency syndrome) drugs, Sato was assigned to the development of drugs to treat influenza, which is estimated by the World Health Organization (WHO) to cause about 3 million to 5 million cases of severe illness, and about 290,000 to 650,000 respiratory deaths annually worldwide. Hokkaido University was an ideal institution for Shionogi to collaborate with, Sato said, given the presence of Dr. Hiroshi Kida, Dr. Yoshihiro Sakoda and other world-class influenza specialists. Also, the Research Center for Zoonosis Control (CZC), established in 2005, has an excellent library on influenza viruses. In 2008, Shionogi Innovation Center for Drug Discovery was established in Hokkaido University, the first research institute of a private company on a Japanese national university campus. "Thanks to the rich talent and resources at the university, the collaboration broadened the scope of my research," said Sato.

"Through the collaboration to develop the anti-influenza drug Peramivir, one important thing I learned from Dr. Kida is to accurately measure the amount of viruses in animals after a drug is administered," Sato said. The method is still unconventional because researchers tend to focus on changes in the symptoms, which are often difficult to measure quantitatively, he said.



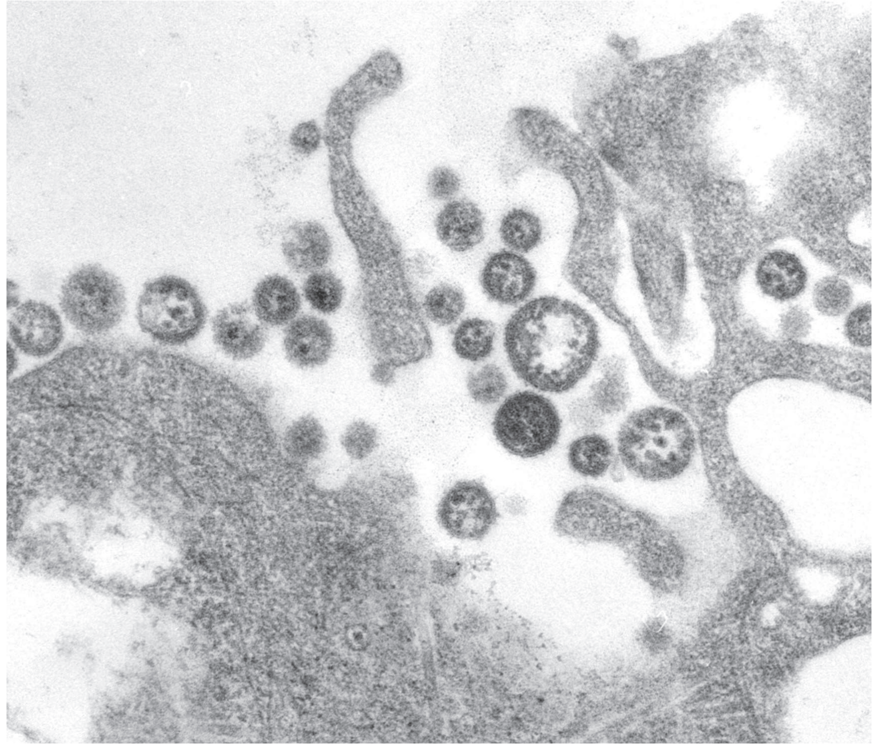
Some key viral enzymes, including the CEN of the flu virus, have two metals (magnesium) at their active center. The anti-flu drug Xofluza is designed to bind to the two metals to suppress the viral enzyme (*Scientific Reports* (2018) 8:9633). In the right diagram, the drug compound is shown in red at the center with two metals in purple.

> "I had longed to see this virus for such a long time"

> “The 2-metal model is a robust design principle for developing other anti-viral drugs”

“The virus counts drastically dropped after Peramivir was administered, and that was the result we had wanted,” Sato explained.

Based on results from the joint pre-clinical research with Hokkaido University, Shionogi has introduced Peramivir to the market. Peramivir was developed by BioCryst Pharmaceuticals and put on the market as Rapiacta in intravenous formulation by Shionogi. BioCryst was looking for a drugmaker that could put Peramivir on the market after a failed attempt by another drugmaker. Rapiacta turned out to be effective, especially for children who do not like to take oral medications, with fewer side effects.



Transmission electron micrograph (TEM) depicting a number of Lassa virus virions adjacent to cell debris. Photo courtesy of CDC/C. S. Goldsmith

Pursuing a robust drug design principle — 2-metal binding model

Sato was involved in the development of another anti-flu drug Xofluza, which prevents virus replication in the infected cells. The drug, which went on the market in 2018, was developed by using the same design principle as anti-HIV/AIDS drug Dolutegravir (marketed as Tivicay by ViiV Healthcare) that Shionogi developed earlier.

Dolutegravir is an HIV integrase inhibitor, which blocks the functioning of integrase, an enzyme found in HIV and other retroviruses. HIV uses integrase to insert its viral DNA into the DNA of the host cell, making the HIV integrase essential for viral replication.

To develop this drug, Shionogi used the “2-metal binding model” that can yield safe (low toxicity), effective drugs. Integrase has two metals, or magnesium, at its active center where the reaction occurs, and the drug is designed to bind to the two metals to suppress the enzyme’s activity, thereby inhibiting viral replication. Sato and his colleagues noticed that influenza viruses also have a 2-metal enzyme called cap-dependent endonuclease (CEN), which plays an important role in viral replication. “It meant a drug with a similar chemical skeleton to Dolutegravir could bind to the active site of CEN. After repeated chemical fine-tuning, we finally found such a compound, which was later developed into Xofluza,” Sato said.

Xofluza works fundamentally differently from other anti-influenza drugs. While the former is designed to suppress an increase in the amount of viruses in the body, the latter, such as Tamiflu and Inavir, are neuraminidase inhibitors, which stop the release of viruses from the infected host cell, thus preventing new host cells from becoming infected.

“As exemplified by Dolutegravir and Xofluza, the 2-metal model is a robust design principle for developing other anti-viral drugs,” Sato said. “Based on this understanding, Shionogi has developed a large chemical library of 2-metal compounds.”

Fight against diseases in developing nations

According to Sato, using Hokkaido University’s library of viruses and Shionogi’s library of 2-metal chemical compounds can boost discovery of new drug candidates in his current research. “It takes a long time to discover drugs from scratch by making a chemical skeleton tailored to a certain disease,” Sato said. “But it is very efficient to screen 2-metal compounds from Shionogi’s library against viruses held by Hokkaido University.” The university has a wide range of viruses, which a pharmaceutical company alone cannot possibly store and utilize, Sato added.

Through this method, Sato found 2-metal compounds that are effective in treating Lassa fever and South American hemorrhagic fevers. “Drugs suppressing CEN will be able to treat three of the four major viral hemorrhagic fevers, except

> “Now I want to help society by developing new drugs for infectious diseases afflicting the poor in developing nations”

continued from previous page

Ebola," Sato said, referring to Lassa, Crimean-Congo, and South American fevers. He has been tirelessly working to develop such drugs using viruses isolated by Hirofumi Sawa's group at Hokkaido University.

The challenge now is how to have the drugs undergo clinical trials. Drugmakers are generally not interested in developing medicines to treat a limited number of people in a limited area because doing so is not economically viable. Sato, therefore, is turning to international organizations or philanthropists willing to finance a clinical trial project. But even if he is able to find a sponsor, the challenge does not stop there. Clinical trials have to be conducted in Africa and other distant places, so he needs to find local partners to help this project.

Making effective drugs available is an urgent task, given the deadly nature of viral hemorrhagic fevers. Developing one drug generally takes 10 years to 20 years due to the steps that must be followed to ensure it is safe to use, but regulators tend to hasten the approval of drugs to treat emerging infections because of the pressing need to save as many lives as possible.

"I have been engaged in making drugs to treat a large number of seriously ill patients," Sato said. "But now I want to help society by developing new drugs for infectious diseases afflicting the poor in developing nations. I am determined to protect as many children as possible from infections." ●

VOICES: Dr. Kazuaki Miyagishima

Former World Health Organization (WHO) Director for Food Safety and Zoonoses, Dr. Kazuaki Miyagishima, talks about zoonoses, the role WHO has played in combatting them, and the challenges surrounding controlling zoonoses today and in the future.

Dr. Kazuaki Miyagishima

Human history has seen the rise and fall of epidemics, including ones caused by zoonotic diseases. In the past decades, we have faced problems related to Ebola, SARS, MERS and, currently, COVID-19, the causal agents of which are believed to have originated in animals. On top of causing sickness and death to human beings, they also have a profound impact on the world economy; not a single country is spared. When developing strategies to tackle zoonotic diseases, it is of utmost importance that we utilize international and inter-disciplinary collaborations.

> "It is of utmost importance that we utilize international and inter-disciplinary collaborations"

WHO is committed to reducing the public health burden of all zoonoses. One of the challenges is that, under its current administrative arrangements, various zoonotic diseases are tackled by separate departments which do not operate under a single, unified strategy. This means that the points of contact as well as the strategies for different vectors, or transmission types, aren't directly connected. During my service at WHO, from which I recently retired, I exerted myself to strengthen the ties between the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE), and WHO. Even though they all share the idea of so-called "One Health," their interpretations are quite different, and so are the objectives that come with the actions taken. Priorities may indeed diverge between livestock industry, public health and other sectors from time to time. Constant efforts to maintain dialogues between sectors at all levels, alone, can prevent misunderstanding, lower barriers and foster collaborations.

From WHO's perspective, the Research Center for Zoonosis Control at Hokkaido University acts as an important hub in Asia where practical research on zoonoses and inter-country exchange of experiences take place, bringing together professionals coming from different horizons. I anticipate the center will train talented personnel who can be an influence worldwide.

For professionals in the field, there remain a number of challenges in fighting zoonoses. For example, the areas where people and wild animals come into contact are often geographically remote or in places of conflict not under governmental control. It is also difficult to change the relationship between people and animals due to cultural traditions, making zoonoses not only a major topic in the public health domain but also a sociological and political one. I strongly hope WHO, the research center at Hokkaido University, and other parties will continue to work together to address those issues and towards controlling zoonoses.

Research Center for Zoonosis Control (CZC)

The CZC was established at Hokkaido University in 2005, in the wake of the 2003 SARS outbreak. The mission of the CZC is to preemptively prevent outbreaks of zoonotic diseases by identifying host animals and transmission routes of potential pathogens, promoting basic research into diseases; developing diagnostic, prophylactic and therapeutic measures and spreading technology and training experts in the field of zoonoses. In 2011, the CZC was designated a WHO Collaborating Centre for Zoonoses Control; this status was renewed in 2019.

Currently, the CZC includes seven divisions, three units, and one overseas center in Zambia. The seven divisions aim to accomplish unique research and educational activities by bringing together experts in bacteriology, virology, parasitology, immunology, pathology, statistics, and computer science, while the three units focus on areas that complement the work being done by the divisions.

Hokudai Center for Zoonosis Control in Zambia (HUCZCZ)

The HUCZCZ was established in 2007 at the University of Zambia School of Veterinary Medicine (UNZA-Vet Med) to conduct research on zoonoses in sub-Saharan Africa. Collaborative research between the UNZA-Vet Med and Hokkaido University has been on-going for more than 35 years. In the HUCZCZ facility, BSL-2 and 3 laboratories were set up with state-of-the-art equipment such as the next-generation sequencer, ultracentrifuge, mobile laboratory with BSL-3-equivalent equipment, etc. Collaborative research and education have so far been very successful in contributing to the public health sector in Zambia and the surrounding region. At the HUCZCZ facility, they have carried out scientific activities which include; the identification of natural host and transmission routes of zoonotic pathogens, search for known and unknown pathogens, and the prevention and control of zoonoses under the strong umbrella of collaboration with the UNZA-Vet Med.

Researchers working at the Research Center for Zoonosis Control, Hokkaido University

A mission to conquer tuberculosis and sleeping sickness in developing nations

Chie Nakajima, DVM, Ph.D.

+ Professor at the Research Center for Zoonosis Control (CZC)
+ Research areas: Tuberculosis and drug-resistant bacteria

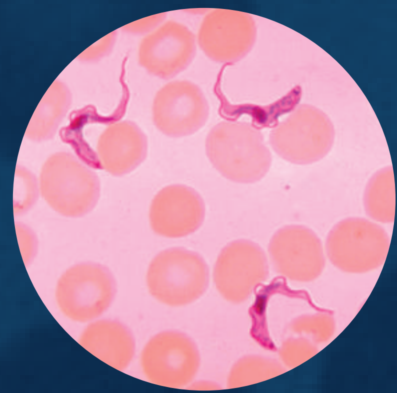
Kyoko Hayashida, DVM, Ph.D.

+ Assistant Professor at the Research Center for Zoonosis Control (CZC)
+ Research area: African trypanosomiasis

Developing reasonably priced, easy-to-use diagnosis kits that can swiftly and accurately detect infectious diseases in developing countries has become the main mission of Chie Nakajima and Kyoko Hayashida, as they seek to alleviate some of the hardship endured by poverty-stricken people.

"We have developed such kits for patients in remote regions where there is no electricity or running water, let alone expensive medical equipment needed to make a diagnosis," said Nakajima, whose research focuses on tuberculosis (TB). "Poverty provides the conditions for TB to spread. If developing countries become more affluent and can provide better nutrition for people, this disease can be eradicated. We are simply helping patients until their economic circumstances improve."

Nakajima is developing diagnosis kits to detect TB, which causes over 4,000 deaths daily worldwide. Her main target now is multidrug-resistant (MDR) TB that does not respond to at least isoniazid and rifampin, two powerful anti-TB medications. Multidrug-resistance emerges due to the mismanagement of TB treatment and person-to-person transmission. MDR-TB accounts for 3.3 percent of all new cases and has been reported around the world.



Trypanosoma among red blood cells (above, Photo courtesy of CDC/Dr. Myron G. Schultz) that causes sleeping sickness, and its vector tsetse fly (right).

Hayashida, meanwhile, is developing a diagnosis kit to detect human African trypanosomiasis (HAT), also known as sleeping sickness. This disease, which is endemic in 36 sub-Saharan African countries, is caused by protozoan parasites belonging to the genus *Trypanosoma* and transmitted by the tsetse fly. The fly acquires its infection from infected humans or animals.

In the first stage of the disease, patients develop symptoms such as a fever, headache and joint pains one to three weeks after being bitten by the tsetse fly. Once the parasite crosses

> "We only need a drop of blood to test for the infection"

The mobile diagnostic system includes all necessary components — small test tubes, a battery, a heating device and an LED illuminator — and does not require any sophisticated equipment or infrastructure, enabling on-site diagnoses.

the blood-brain barrier and infects the central nervous system, the disease enters the second stage, triggering mental disorientation, neurologic problems, and eventually death if not treated.

"This is a critical moment if we want to eradicate HAT," Hayashida said. "It is important to identify and treat hidden cases as early as possible because the treatment method for the initial stage has been established."

Modifying LAMP system for developing countries

Hayashida often visits Zambia to conduct research on HAT.

Patients tend to wait until their symptoms worsen before seeking medical help. Molecular diagnosis has been developed to detect early-stage infection, but this is not easily affordable for impoverished communities, Hayashida said. Such a system also requires complicated sample preparation steps at a well-equipped laboratory, which developing countries often lack. As a consequence, most HAT patients are misdiagnosed as having malaria and receive treatment for the wrong disease.

To address these problems, Hayashida and Nakajima have developed a new diagnostic kit based on a loop-mediated isothermal amplification (LAMP) system, a nucleic acid amplification test method originally developed by Eiken Chemical Co., Ltd. "With our new system called CZC-LAMP, we only need a drop of blood to test for the infection. It costs less than one U.S. dollar per test and the result can be obtained within one hour without the use of sophisticated equipment," says Hayashida.

They substantially modified the LAMP system to allow on-site diagnosis even in remote areas. The first major improvement was to dry and stabilize reagents in a single tube by incorporating trehalose, which locks up necessary enzymes and molecules in "glass" when it dries. This prolongs the kit's shelf life at ambient temperature, so it does not require a cold chain to stay in a functional state. Hayashida also utilized a bio inkjet printer to semi-automatically produce the dry LAMP.

To enable a HAT diagnosis in local clinics or villages in endemic areas, the researchers simplified the diagnostic procedure. Now, DNA or RNA can be amplified directly from detergent-lysed blood samples. The sample is transferred into the dried CZC-LAMP tube and incubated for 45 minutes at 60°C using a portable battery-supplied heat block. The sample is then checked with a portable reaction detector, or a handmade LED illuminator, which is suitable for field use.

Braving risk of infection to conduct research in Zambia

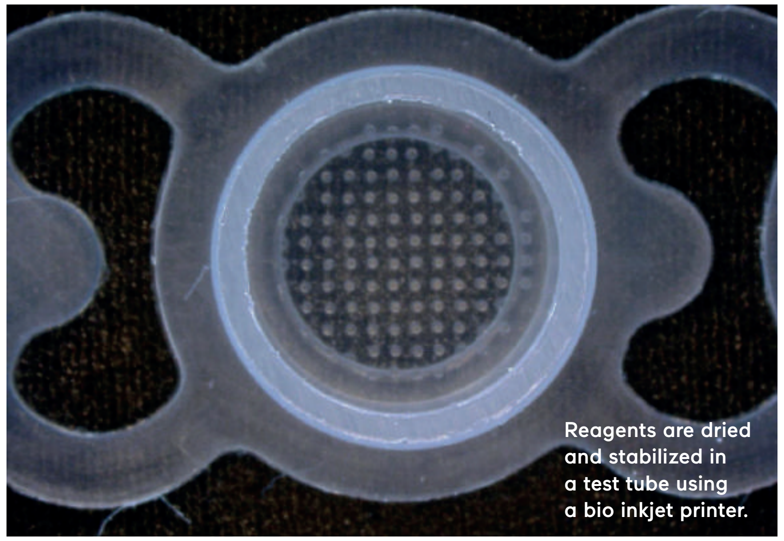
The kits developed by researchers at Hokkaido University's Research Center for Zoonosis Control (CZC) can be tailored to various emerging and reemerging infections caused by viruses, bacteria and parasites. The kits can be made disease-specific by changing the LAMP primers. A primer is a short nucleic acid sequence that provides a starting point for DNA synthesis. "It is, however, difficult to find optimal primers, so repeated experiments are required to find the most suitable ones," Hayashida explains.

She continued, "While conducting research on people with the disease, we also conduct vector and animal surveillance. By doing molecular testing in the veterinary domain, we are trying to elucidate how the parasite *Trypanosoma* spreads in the environment, and which wild animals are infected with the parasite." The parasite does not harm wild animals, but afflicts some domestic animals that came from Europe.

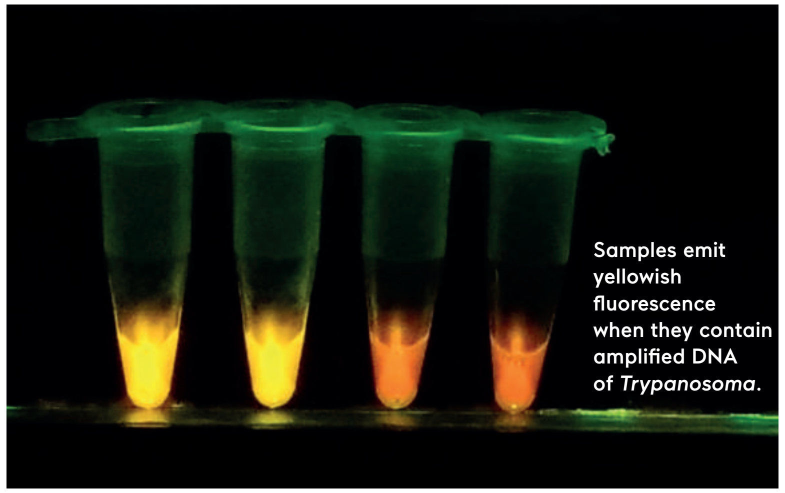
Hayashida often goes to remote areas to collect thousands of tsetse flies. Her aim is to compile maps and locate risk areas where tsetse flies are infected with the parasite. Hayashida is often bitten by the flies, but remains undaunted. "Not all flies are infected, but even if I get bitten by one with the parasite, I can use our kit to make a prompt diagnosis," she said.

Halting spread of multidrug-resistant tuberculosis essential

According to the World Health Organization (WHO), there are 10 million new cases of TB every year,



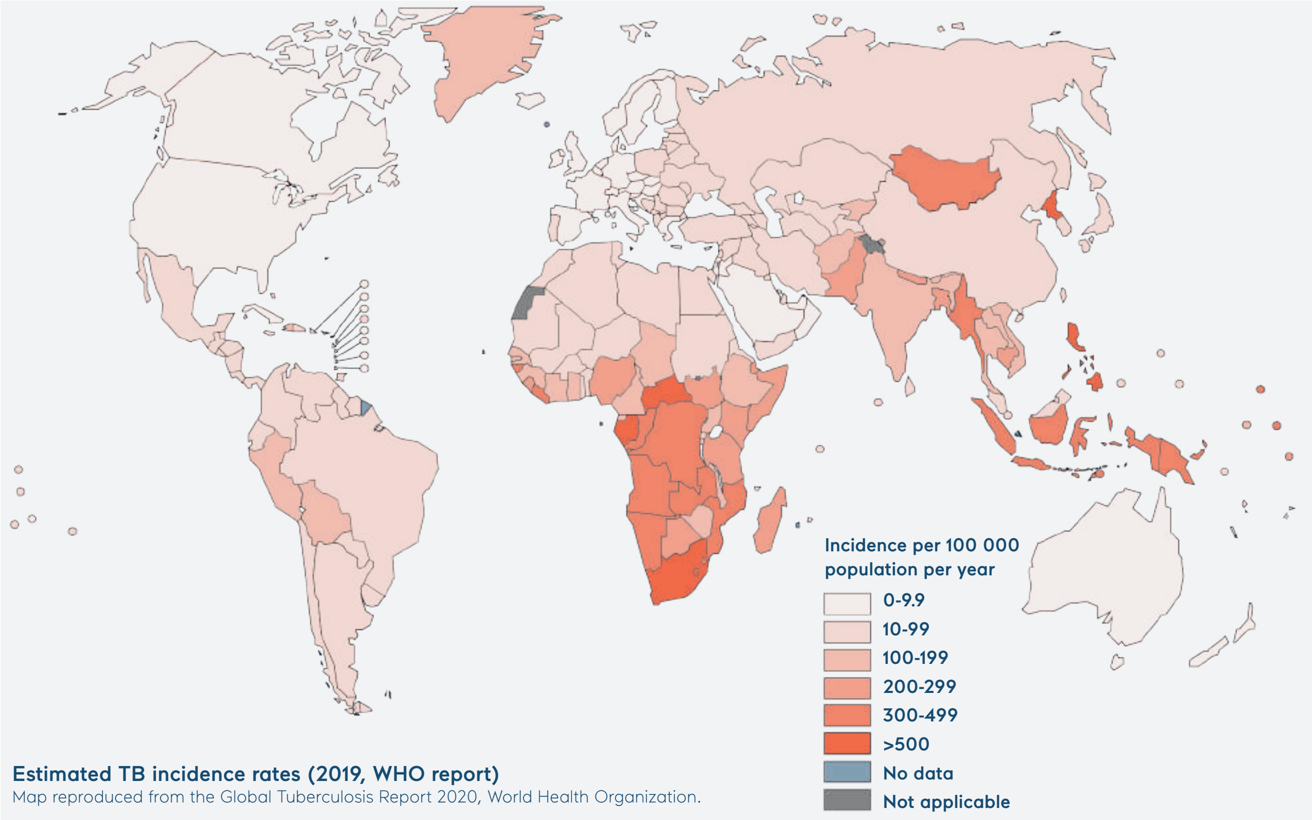
Reagents are dried and stabilized in a test tube using a bio inkjet printer.



Samples emit yellowish fluorescence when they contain amplified DNA of *Trypanosoma*.

“To eradicate HAT, it is important to identify and treat hidden cases”

Hayashida and a colleague examine local residents in Zambia, where advanced medical equipment is often unavailable.



Estimated TB incidence rates (2019, WHO report)
Map reproduced from the Global Tuberculosis Report 2020, World Health Organization.

claiming the lives of 1.4 million people. High TB incidence rates are found in African and Asian nations. TB is essentially a human disease, but animals such as elephants and monkeys can be infected with strains that originated in humans.

"The bacteria proliferate very slowly," Nakajima said. "TB can be cured with the long-term administration of medicines, but impoverished people tend to quit taking the drugs midway through the six months required. That enables the bacteria to develop drug resistance."

There is no gene exchange between bacterial cells in *Mycobacterium tuberculosis* (MTB), so MTB obtains drug resistance when a point mutation in the genes causes a structural change in the target molecule. Bacteria with "low fitness cost" mutations will survive, Nakajima said.

It is, therefore, important to accurately diagnose patients at an early stage to prevent further person-to-person infection.

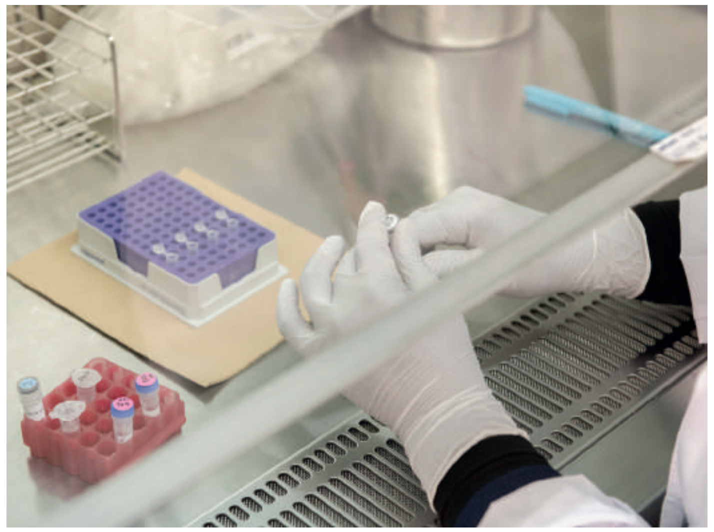
This drove Nakajima to develop a LAMP system to detect MTB and an affordable, easy-to-use DNA array for spoligotyping, a polymerase chain reaction (PCR)-based method for simultaneous detection and typing of MTB strains. While conventional spoligotyping methods are costly and complicated, the kit she developed costs only three to four U.S. dollars and is simple to use. Moreover, Nakajima developed a method to detect isoniazid-resistant MTB using an easy-to-use printed-array strip (PAS) through a joint research project with Tohoku Bio Array Corporation, as well as a PCR method to distinguish "Modern Beijing" MTB, a multi-drug resistant strain spreading rapidly in some Asian countries.

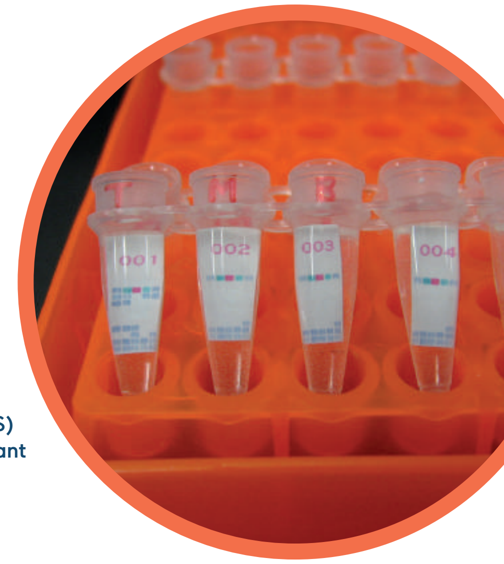
"By distinguishing dangerous strains from less serious strains, we will be able to determine which patient should be treated first, as the capacity of healthcare facilities are usually limited in high TB-burden areas." Nakajima said, stressing the importance of controlling person-to-person transmission.

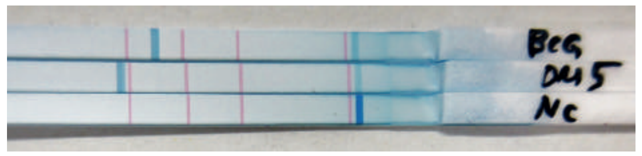
As an educator, Nakajima has another pressing issue that worries her. Some of her international students hesitate to return to their native country because there are few jobs available for such specialists. "It is our responsibility to help their governments to properly deal with these infectious diseases," Nakajima said. "My research, which involves science and nature, opened my eyes to various social issues in these nations." ●

"My research opened my eyes to various social issues in these nations"

Pulmonary Tuberculosis. Chest X-ray : Interstitial infiltration at left upper lung due to *Mycobacterium Tuberculosis* infection. © istock







Nakajima and colleagues develop spoligotyping, a PCR and hybridization-based method, to simultaneously detect MTB strains (right), and a printed-array strip (PAS) to detect isoniazid-resistant MTB (below).

VOICES: Dr. Bernard Mudenda Hang'ombe

The University of Zambia and Hokkaido University are long term collaborators in veterinary medicine and zoonoses control. Professor Bernard Mudenda Hang'ombe from the Department of Para-Clinical Studies at the University of Zambia, who is also the Centre Leader of the Africa Centre of Excellence for Infectious Diseases of Humans and Animals, shares his thoughts about the situation in Zambia and collaborations with Hokkaido University.

Dr. Bernard Mudenda Hang'ombe

Zambia is a country endowed with natural resources and wildlife. And so, the challenge in controlling zoonosis here is that hosts of zoonotic diseases move naturally without boundaries. Wildlife can harbor infections which are transmitted when they come into contact with humans. Communities living in rural areas can easily interact with hosts that transmit zoonotic diseases. As new pathogens are being identified in Zambia, the major challenge in the future is the detection of novel pathogens that have not been documented and could cause serious national and international issues.

The collaboration between the University of Zambia and Hokkaido University dates back to 1983, when the School of Veterinary Medicine was established in Zambia, and it has brought a lot of benefits to our university. Our country did not have much capacity in the veterinary field, but now, there are veterinarians even in the most remote parts of the country. This has been augmented by highly trained and specialized manpower who are able to work in a multicultural setting and well-equipped laboratories. Here at our university, we have a high turnover of Ph.D. level staff trained at Hokkaido University in various fields. On the research front, the collaboration has also strengthened the capacity of research, with advanced laboratory support in the diagnosis of dangerous diseases like anthrax, ebola and currently coronavirus.

> "The younger generation should continue to work together and do well for the good of mankind"

There are direct benefits for both parties. Zambia, for example, has a diverse environment which Hokkaido University undergraduate and postgraduate students can use for practical training. Collaborations have also allowed for joint research funding opportunities with national and international agencies and societies. Government ministries are able to tap into the support offered by our joint laboratories, the capacity of which has been strengthened through our joint efforts in research and education.

Hokkaido University has shown true friendship and mutual respect to the University of Zambia. The younger generation should not be discouraged as every society has good and bad people; they should continue to work together and do well for the good of mankind. Africa has a lot of challenges emanating from society itself. The presence of collaborators from Hokkaido University brings hope in this area of research despite the challenges. They have uplifted us and we will continue cherishing every moment. I really hope that the relationship and fruits from our collaborations will be passed on to younger generations in both Japan and Africa.

Researchers setting up mosquito traps in Zambia (left); at a collaboration meeting between the University of Zambia and Hokkaido University (below)

Working in the wild to help protect animals and humans from zoonoses

Hirofumi Sawa, MD, Ph.D.

+ Professor and Deputy Director of the Research Center for Zoonosis Control (CZC)
+ Research Area: Molecular pathobiology, virology, epidemiology, animal model

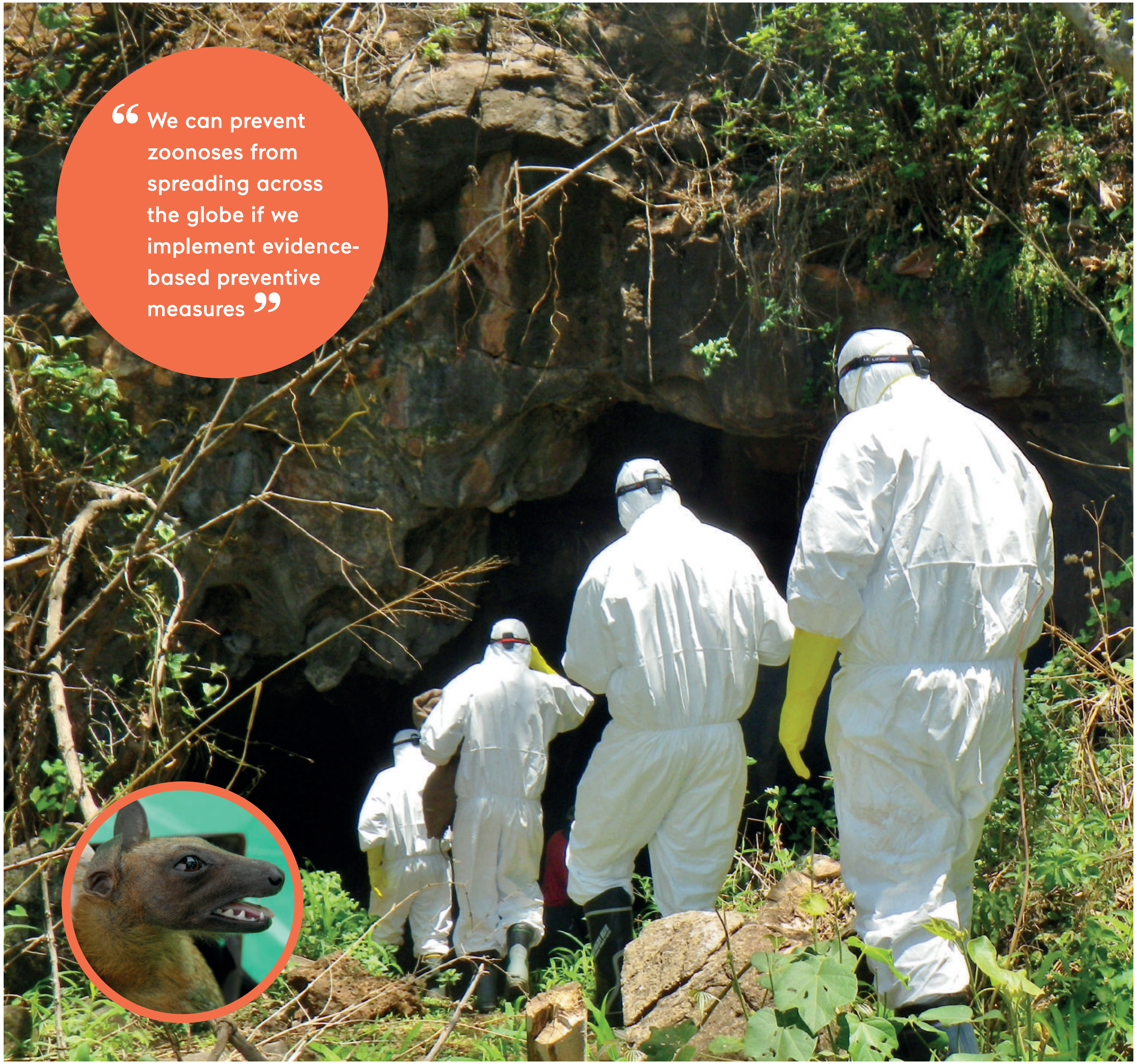
> "I've been able to keep up because I like Zambian people for their kindness and humility"

Hirofumi Sawa started his career three decades ago working as a clinical cardiologist conducting basic research at Hokkaido University's Graduate School of Medicine. He soon acquired a keen interest in viruses, leading him to start research on JC polyomavirus, which causes fatal demyelinating disease of the central nervous system, under Dr. Kazuo Nagashima. Therefore, in 2005, it was a natural progression for him to apply for a professorship at the university's Research Center for Zoonosis Control (CZC).

Sawa leaves his CZC lab three to four times a year to conduct research in the field in Zambia, catching mosquitos and collecting wildlife and athropods in caves. Indeed, his work style fluctuates from being a researcher at state-of-the-art medical institutions at Hokkaido University and Washington University in St. Louis to working in the wild where there is no electricity or other modern comforts.

"I have always navigated my career path to a direction that has interested me," Sawa said. "I aspired to be a medical doctor because I wanted to cure patients. Now I am devising preemptive measures against zoonoses. Broadly speaking, this has the same goal. My strategy is just a bit different from many of my colleagues."

RESEARCH IN FOCUS



Discovering new zoonosis viruses

Sawa's research base in Zambia is the Hokudai Center for Zoonosis Control in Zambia (HUCZCZ), which was established in 2007 by Hokkaido University's CZC as a hub for epidemiological studies in sub-Saharan Africa. HUCZCZ is located at the University of Zambia's School of Veterinary Medicine.

Pathogens that pose a huge threat to public health, such as the human immunodeficiency virus (HIV), Ebola virus and Zika virus, are believed to have originated in Africa, where yellow fever and malaria are also endemic. This makes Africa a key continent for conducting research on infectious diseases.

"If we want to control zoonoses, it is important to conduct research in developing countries where outbreaks of known and unknown infectious diseases are reported," Sawa said. "It is difficult to eradicate infectious diseases, but we can prevent zoonoses from spreading across the globe if we implement evidence-based preventive measures."

To that end, Sawa said, "In addition to unravelling the diversity of viruses carried by animals, we also need to search for new viruses, and determine their host range, pathogenicity and possible transmission routes. This task deserves proper attention considering that over 70 percent of emerging infectious diseases originated from wildlife."

Sawa's research team has been credited with detecting and isolating more than 20 new viruses carried by wild animals and arthropods.

For example, Sawa's colleague Dr. Michihito Sasaki, who is a lecturer at Hokkaido University, detected a virus belonging to the *paramyxoviridae* family from feces of wild baboons in Zambia. Other members of this family include two well-known viruses — measles virus and mumps virus, which are mainly associated with human respiratory disorders. Detection of *paramyxoviruses* in wild baboons was made possible by employing a method called reverse transcriptase-polymerase chain reaction (RT-PCR) with the use of degenerate primers, which is simply a mixture of primers that are similar, but not identical. Using this method, his research team identified one of the viruses as human parainfluenza type 3 (HPIV3), which is most closely related to HPIV3 strain Riyadh 149/2009, isolated from a hospitalized child in Saudi Arabia in 2009.

"We believe that the virus is transmitted from baboons to humans and

(Left) Sawa and his colleagues enter a cave in Zambia to collect bat feces. The inset shows a fruit bat (*Eidolon helvum*).
(Below) Investigating viruses carried by rodents

from humans to other animals," Sawa said. "Baboons tend to come close to humans in search of food. Because of this close contact, the virus can be transmitted from baboons to humans and vice versa."

Next-generation sequencers for comprehensive genomic profiling

According to Sawa, next-generation sequencing (NGS), which enables sequencing of DNA and RNA much more quickly and comprehensively than previous techniques, has revolutionized genomic and molecular biology. RT-PCR (used for analyzing RNA viruses) and PCR (used for analyzing DNA viruses) are instrumental when the targeted virus is known, but NGS becomes a powerful tool when researchers do not know exactly what kind of virus they are dealing with. NGS allows for a comprehensive and cost-effective sequencing of known and unknown pathogens, such as DNA and RNA viruses.

Thanks to these new technologies, the number of virus species acknowledged by the International Committee on Taxonomy of Viruses increased from 1,898 in 2005 to 6,590 as of May 2020.

Specifically, Sasaki employs shotgun metagenomics, an approach that involves shearing DNA or RNA extracted from samples and subsequent sequencing of the small fragments using NGS. The generated information is analyzed using bioinformatics tools. Now, the team has a convenient, portable sequencing device capable of detecting viruses in the field.

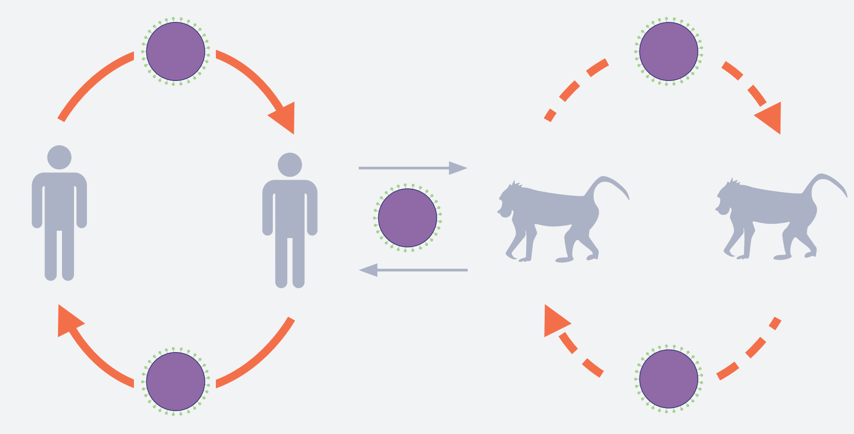
Next Generation Sequencer (NGS)

Using the latest technologies, the team detected Group A rotavirus in insect-eating bat species from Zambian caves. Rotaviruses have a double-stranded RNA genome consisting of eleven segments. Rotaviruses detected by Sasaki had six genome segments highly similar (96 percent to 99 percent) to the human rotaviruses, while the remaining five segments were similar to those of rotaviruses found in livestock and wild animals. "The result suggests that rotaviruses carried by bats have a similar genetic profile to those found in humans. This is also evidence of cross-species transmission and probable genetic reassortment being behind the high genetic diversity seen in rotaviruses," said Sawa.

Furthermore, Sasaki detected several new viruses in insect-eating white-toothed shrews, which inhabit some rural residential areas in Zambia. A new picornavirus found in shrews was similar to human picornaviruses, which cause gastroenteritis. Additionally, his team detected a new cyclovirus that was markedly different from other known cycloviruses found in animal feces. This new cyclovirus was very similar to viruses detected in the cerebrospinal fluid of encephalomyelitis patients.

"These findings suggest that white-toothed shrews, which are found widely around the globe, carry viruses unique to them, and can, therefore, be a potential host to zoonotic viruses," Sawa warned.

"Collecting samples from wildlife and isolating viruses from them are time-consuming tasks, but they are integral parts in the fight against zoonoses," Sawa said. To shorten the process, Sawa and his collaborators are developing a system to effectively isolate viruses by making optimal cells needed for replication of viruses and then rotary-culturing cells and viruses together to boost viral replication.



The suggested transmission cycle of HPIV3 viruses between humans and baboons.

A total of 17,826 female mosquitos were gathered between 2012 and 2019 in Zambia. Traps that produce odor attractant and a common mosquito *Culex quinquefasciatus* (inset) that was found to be a vector of West Nile virus.

> "Collecting samples in the wildlife and isolating viruses from them are integral parts in the fight against zoonosis"

Mosquitos the "most dangerous insect"

Dr. Yasuko Orba, who works with Sawa, focuses on virus surveillance in mosquitos, which are widely considered to be the world's most dangerous insect because they are a vector for many diseases. From 2012 to 2019, Orba collected more than 17,800 female mosquitos from different places in Zambia in search of known and unknown viruses. This effort culminated in the first isolation of West Nile virus in Zambia. Orba also surveyed birds, horses and humans based on the West Nile virus transmission route: from mosquitos to birds; birds to mosquitos; and finally to horses and humans.

"Preemptive measures can be taken based on research on the diversity of viruses wild animals and arthropods carry, as well as studies on the natural hosts and pathogenicity of viruses," Sawa said. For instance, Sawa is helping one of his colleagues, Dr. Akihiko Sato from Shionogi & Co., Ltd., to develop an antiviral drug to treat arthropod-borne viruses (arboviruses) and coronaviruses.

"As a doctor conducting basic research on human cardiovascular diseases, I never imagined that I would visit Zambia to research infectious diseases," Sawa said. "It's not easy, but I've been able to keep up because I like Zambian people for their kindness and humility." ●

The One Health Frontier Graduate School of Excellence

The One Health Frontier Graduate School of Excellence is a four-year doctoral program offered by Hokkaido University's Graduate School of Veterinary Medicine and Graduate School of Infectious Diseases. To help protect humans and animals from infectious diseases and hazardous chemicals and build a sustainable healthy environment, the program aims to foster outstanding experts who have clear vision of disease control and prevention, and are able to design and implement solutions towards One Health.

To ensure its goal, the program facilitates interdisciplinary studies including veterinary medicine, infectious disease, general medicine, dentistry, pharmacology, health science, and environmental science. Students will gain a variety of experience in collaborative research and cooperative activities with international organizations such as the World Health Organization (WHO), World Organisation for Animal Health (OIE) and Japan International Cooperation Agency (JICA), as well as private companies.

The program consists of four modules that allow the students to practice theoretical and practical skill-building through a series of training. The course also opens part of modules to students with a background in humanities and social sciences to promote interdisciplinary interactions.

The Global Station for Zoonoses Control (GSZ)

The GSZ was established in 2014 as part of the Global Institution for Collaborative Research and Education (GI-CoRE). Operating in tandem with the Research Center for Zoonosis Control (CZC), the GSZ promotes scientific research for the control of zoonoses through collaboration with leading international researchers. The collaborating institutions are The University of Melbourne, University College Dublin, and King Abdullah University of Science and Technology. The GSZ has organised the Consortium for the Control of Zoonoses, whose members meet yearly to further collaborative efforts.

As of April 1, 2020, the GSZ has transitioned into the International Collaboration Unit of CZC, and was certified as a GI-CoRE Cooperating Hub.

Hokkaido University
GI-CoRE
Global Institution for Collaborative Research and Education

Research in brief 2019-2020

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WHY ROBOTS ARE PIONEERS IN THE FUTURE OF AGRICULTURE?

Agriculture in Japan is gravely suffering because of aging population and a shortage of experienced leaders. On a global level, there are also concerns about food shortages as the population explodes on the planet. In response, scientists are seeking ways to solve these social problems by utilizing the technology of robotic farming.

Professor Noboru Noguchi of Hokkaido University, one of the world's leading experts of robotic agriculture, says, "Our vehicle robotics lab is developing robots that perform unmanned agricultural work. For example, they are running tractors, rice planters, harvesters, boats that sprinkle chemicals in paddy fields, and drones that observe farmlands from the sky." From 2018, three major companies started selling the unmanned tractors. They enabled automatic driving with an error of 5 cm or less by utilizing GPS and the Quasi-Zenith Satellite or "MICHIBIKI," which allows highly precise navigation.

"The underlying technology was developed in our laboratory. To implement our machinery for the awaiting farmers, we opened up the research results without acquiring patents. Thereby, many manufacturers became interested in commercializing products based on our technology," elaborated Noguchi. The Japanese government is also enthusiastic about supporting the "smart agriculture" utilizing robotics and information and communication technologies.

Noguchi's lab is now developing new technologies to allow unmanned tractors to work together, to select and harvest ripe pumpkins, or to evaluate the growth state of crops. In the future, Noguchi plans to make a small agricultural robot that can navigate narrow hills and mountainous areas. "In the times to come, my dream is to develop a robot that works on the rice terraces, an agricultural landscape unique to Japan. I would also like to make smarter agricultural robots that can make judgments based on gathered information as if they were skilled farmers," said Noguchi, "I am very particular about developing technology for the people who can make use of it."

In the implementation of smart agriculture, Noguchi believes that its incorporation into social systems is as important as achieving technological development. His team is conducting tests on Hokkaido University's experimental farms as well as fields in Hokkaido Prefecture. They listen to the opinions of farmers and request feedback on their needs and problems. Further, it is necessary to collaborate with both researchers and the private sector which has a good management strategy, to disseminate technology that leads to industrialization and regional revitalization. They also discuss the deregulation of related laws with the relevant authorities.

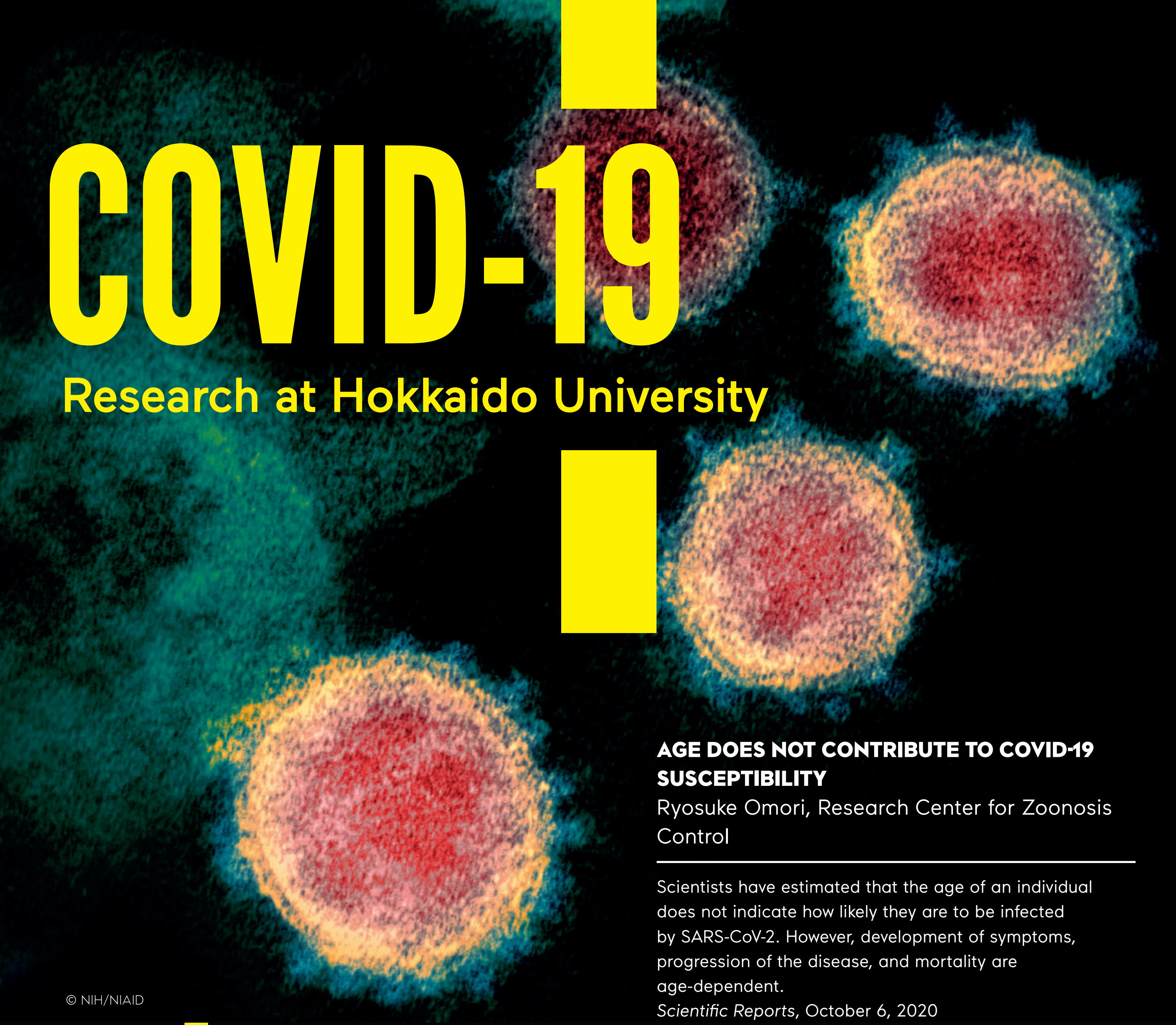
"Hokkaido is known for its accessibility to agricultural sites, and it is blessed with an environment that is more than suitable for pursuing agricultural research. There are challenges right before our eyes, many farmers are cooperative in our research, and local governments are also interested. So, I advise young people to have great ambitions! Make the best use of the large fields and go heart and soul into this dynamic research," commented Noguchi. ●

Photos and video provided by the Vehicle Robotics Laboratory

Watch the video of multiple unmanned tractors working in cooperation as a unit

Professor Noboru Noguchi in front of an unmanned tractor.

Equipment to harvest pumpkins attached to an unmanned tractor.



COVID-19

Research at Hokkaido University

© NIH/NIAID

AGE DOES NOT CONTRIBUTE TO COVID-19 SUSCEPTIBILITY

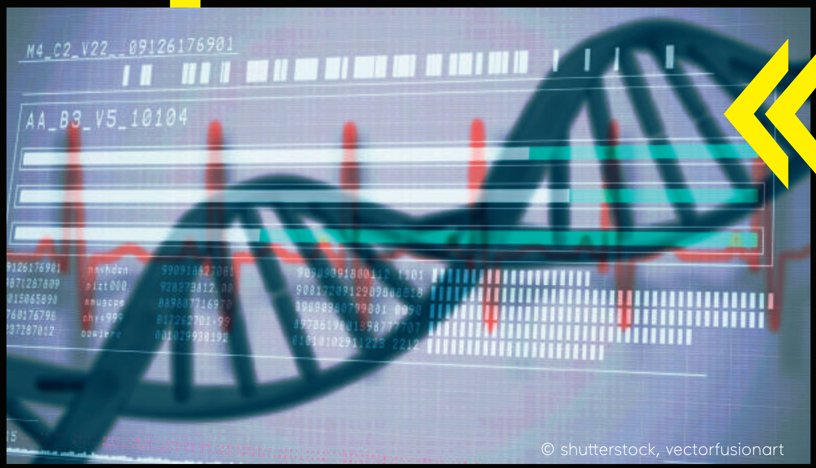
Ryosuke Omori, Research Center for Zoonosis Control

Scientists have estimated that the age of an individual does not indicate how likely they are to be infected by SARS-CoV-2. However, development of symptoms, progression of the disease, and mortality are age-dependent.
Scientific Reports, October 6, 2020

CELLULAR-LEVEL INTERACTIONS THAT LEAD TO THE CYTOKINE STORM IN COVID-19

Ryo Otsuka and Ken-ichiro Seino, Institute for Genetic Medicine

Scientists address the role that macrophage activation syndrome (MAS) plays in severe cases of COVID-19, and highlight how existing therapies for MAS have shown initial success in ameliorating the disease.
Inflammation and Regeneration, August 6, 2020



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GENETIC VARIATION UNLIKELY TO INFLUENCE COVID-19 MORBIDITY AND MORTALITY

Ji-Won Lee, Graduate School of Dental Medicine

A comprehensive search of genetic variation databases has revealed no significant differences across populations and ethnic groups in seven genes associated with viral entry of SARS-CoV-2.
Infection, Genetics and Evolution, August 25, 2020

USING WASTEWATER TO MONITOR COVID-19

Masaaki Kitajima, Faculty of Engineering

Kitajima and colleagues suggested that wastewater could be used as a surveillance tool to monitor the spread of COVID-19 in communities. Later, they demonstrated that an adsorption-extraction technique can most efficiently detect SARS-CoV-2 in untreated wastewater.
Science of The Total Environment, June 5 and April 30, 2020.

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COVID-19 CYTOKINE STORM: POSSIBLE MECHANISM FOR THE DEADLY RESPIRATORY SYNDROME

Masaaki Murakami, Institute for Genetic Medicine

Leading immunologists proposed a possible molecular mechanism that causes massive release of proinflammatory cytokines, or a cytokine storm, leading to the acute respiratory distress syndrome (ARDS) in COVID-19 patients as well as its potential treatments.
Immunity, April 22, 2020

COVID-19: SALIVA TESTS COULD DETECT THE SILENT CARRIERS

Takanori Teshima, Faculty of Medicine

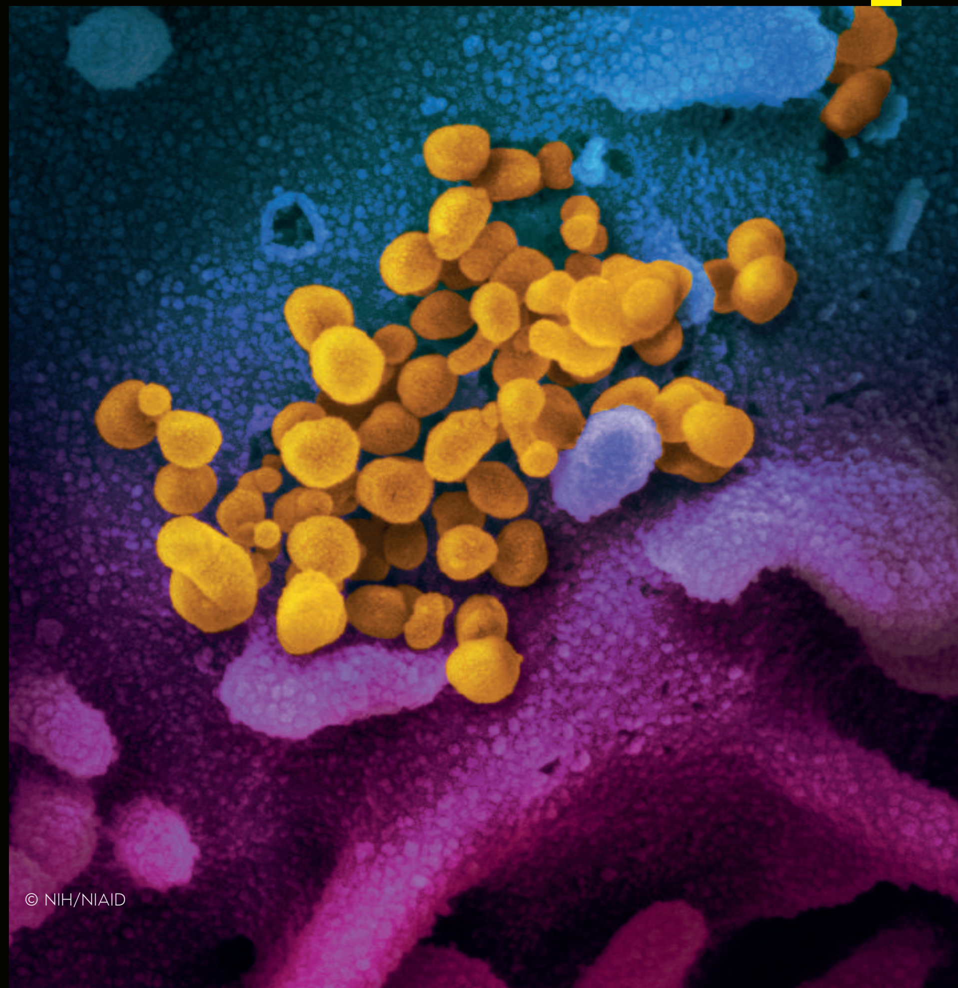
Scientists have demonstrated a quick and effective mass testing approach using saliva samples to detect individuals who have been infected with COVID-19 but are still not showing symptoms.
Clinical Infectious Diseases, September 25, 2020

GROWTH RATE OF THE COVID-19 PANDEMIC MAY BE OBSCURED DUE TO CHANGES IN TESTING RATES

Ryosuke Omori, Research Center for Zoonosis Control

Reviewing reported cases of COVID-19 in Italy, Japan, and California, USA, scientists argue that changes in the testing rate may be masking the true growth rate and extent of the pandemic.
International Journal of Infectious Diseases, April 19, 2020

Check out the most recent COVID-19 Research at Hokkaido University



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A new duck-billed dinosaur, *Kamuysaurus japonicus*, identified

The dinosaur, whose nearly complete skeleton was unearthed from 72 million year old marine deposits in Mukawa Town in northern Japan, belongs to a new genus and species of a herbivorous hadrosaurid dinosaur, according to the study published in *Scientific Reports*.
The scientists named the dinosaur *Kamuysaurus japonicus*.

ORIGINAL ARTICLE

Kobayashi Y., et al, A new hadrosaurine (Dinosauria: Hadrosauridae) from the marine deposits of the late Cretaceous Habouchi Formation, Yezo Group, Japan, *Scientific Reports*, September 5, 2019.

CONTACT

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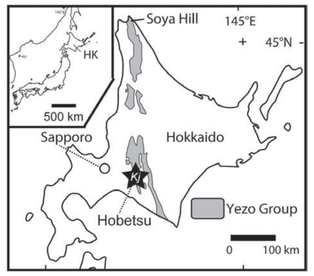
A partial tail of the dinosaur was first discovered in the outer shelf deposits of the Upper Cretaceous Hakobuchi Formation in the Hobetsu district of Mukawa Town, Hokkaido, in 2013. Ensuing excavations found a nearly complete skeleton that is the largest dinosaur skeleton ever found in Japan. It's been known as "Mukawaryu," nicknamed after the excavation site.

In the current study, a group of researchers led by Professor Yoshitsugu Kobayashi of the Hokkaido University Museum conducted comparative and phylogenetic analyses on 350 bones and 70 taxa of hadrosaurids, which led to the discovery that the dinosaur belongs to the Edmontosaurini clade, and is closely related to *Kerberosaurus* unearthed in Russia and *Laiyangosaurus* found in China.

The research team also found that *Kamuysaurus japonicus*, or the deity of Japanese dinosaurs, has three unique characteristics that are not shared by other dinosaurs in the Edmontosaurini clade: the low position of the cranial bone notch, the short ascending process of the jaw bone, and the anterior inclination of the neural spines of the sixth to twelfth dorsal vertebrae.

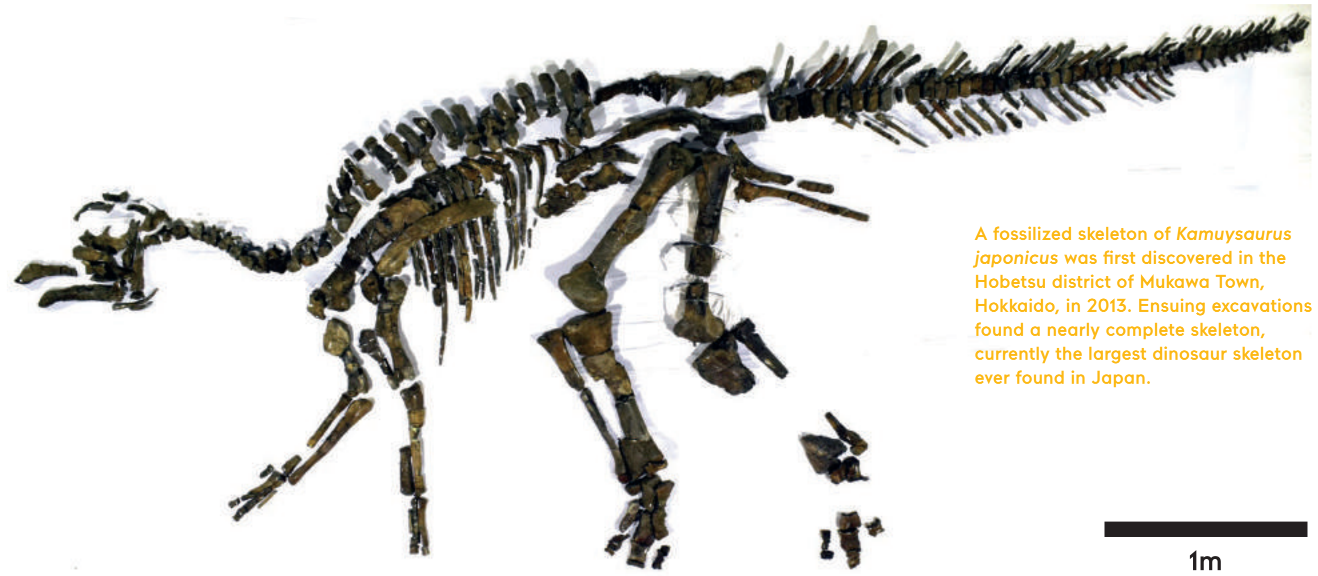
According to the team's histological study, the dinosaur was an adult aged 9 or older, measured 8 meters long and weighed 4 tons or 5.3 tons (depending on whether it was walking on two or four legs respectively) when it was alive. The frontal bone, a part of its skull, has a big articular facet connecting to the nasal bone, suggesting the dinosaur may have had a crest. The crest, if it existed, is believed to resemble the thin, flat crest of *Brachylophosaurus* subadults, whose fossils have been unearthed in North America.



Map of Hokkaido showing the location of Hobetsu district where *Kamuysaurus* (black star labeled "Kj") was excavated. (Kobayashi Y., et al, *Scientific Reports*, September 5, 2019)

The study also shed light on the origin of the Edmontosaurini clade and how it might have migrated. Its latest common ancestors spread widely across Asia and North America, which were connected by what is now Alaska, allowing them to travel between the two continents. Among them, the clade of *Kamuysaurus*, *Kerberosaurus* and *Laiyangosaurus* inhabited the Far East during the Campanian, the fifth of six ages of the Late Cretaceous epoch, before evolving independently.

The research team's analyses pointed to the possibility that ancestors of hadrosaurids and its subfamilies, Hadrosaurinae and Lambeosaurinae, preferred to inhabit areas near the ocean, suggesting the coastline environment was an important factor in the diversification of the hadrosaurids in its early evolution, especially in North America. ●



A fossilized skeleton of *Kamuysaurus japonicus* was first discovered in the Hobetsu district of Mukawa Town, Hokkaido, in 2013. Ensuing excavations found a nearly complete skeleton, currently the largest dinosaur skeleton ever found in Japan.

ARCHAEOLOGY

Selected skull elements of Kamuysaurus japonicus. Its unique characteristics include the low position of the cranial bone notch (quadratojugal notch, qjn) and the short ascending process of the jaw bone (surangular, acp). (Kobayashi Y., et al, *Scientific Reports*, September 5, 2019)

Gas insulation could be protecting an ocean inside Pluto

A gassy insulating layer beneath the icy surfaces of distant celestial objects could mean there are more oceans in the universe than previously thought.

Computer simulations provide compelling evidence that an insulating layer of gas hydrates could keep a subsurface ocean from freezing beneath Pluto's icy exterior, according to a study published in the journal *Nature Geoscience*.

In July 2015, NASA's New Horizons spacecraft flew through Pluto's system, providing the first-ever close-up images of this distant dwarf planet and its moons. The images showed Pluto's unexpected topography, including a white-colored ellipsoidal basin named Sputnik Planitia, located near the equator and roughly the size of Texas.

Because of its location and topography, scientists believe a subsurface ocean exists beneath the ice shell which is thinned at Sputnik Planitia. However, these observations are contradictory to the age of the dwarf planet because the ocean should have frozen a long time ago and the inner surface of the ice shell facing the ocean should have also been flattened.

Researchers at Japan's Hokkaido University, the Tokyo Institute of Technology, Tokushima University, Osaka University, Kobe University, and at the University of California, Santa Cruz, considered what could keep the subsurface ocean warm while keeping the ice shell's inner surface frozen and uneven on Pluto. The team hypothesized that an "insulating layer" of gas hydrates exists beneath the icy surface of Sputnik Planitia. Gas hydrates are crystalline ice-like solids formed of gas trapped within molecular water cages. They are highly viscous, have low thermal conductivity, and could therefore provide insulating properties.

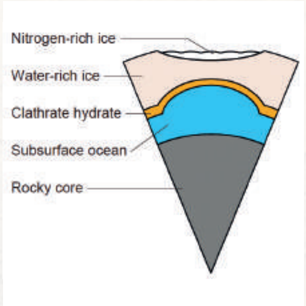
The researchers conducted computer simulations covering a timescale of 4.6 billion years, when the solar system began to form. The simulations showed the thermal and structural evolution of Pluto's interior and the time required for a subsurface ocean to freeze and for the icy shell covering it to become uniformly thick. They simulated two scenarios: one where an insulating layer of gas hydrates existed between the ocean and the icy shell, and one where it did not.

The simulations showed that, without a gas hydrate insulating layer, the subsurface sea would have frozen completely hundreds of millions of years ago; but with one, it hardly freezes at all. Also, it takes about one million years for a uniformly thick ice crust to completely form over the ocean, but with a gas hydrate insulating layer, it takes more than one billion years.

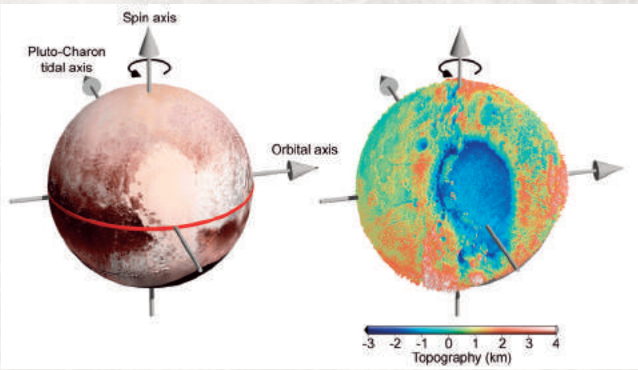
The simulation's results support the possibility of a long-lived liquid ocean existing beneath the icy crust of Sputnik Planitia.

The team believes that the most likely gas within the hypothesized insulating layer is methane originating from Pluto's rocky core. This theory, in which methane is trapped as a gas hydrate, is consistent with the unusual composition of Pluto's atmosphere — methane-poor and nitrogen-rich.

Similar gas hydrate insulating layers could be maintaining long-lived subsurface oceans in other relatively large but minimally heated icy moons and distant celestial objects, the researchers conclude. "This could mean there are more oceans in the universe than previously thought, making the existence of extraterrestrial life more plausible," says Shunichi Kamata of Hokkaido University who led the team. ●



The proposed interior structure of Pluto. A thin clathrate (gas) hydrate layer works as a thermal insulator between the subsurface ocean and the ice shell, keeping the ocean from freezing. (Kamata S. et al., *Nature Geosciences*, May 20, 2019)



The bright "heart" on Pluto is located near the equator. Its left half is a big basin dubbed Sputnik Planitia. Figures created using images by NASA/Johns Hopkins University Applied Physics Laboratory/Southwest Research Institute.

(From the left) Atsushi Tani of Kobe University, and Shunichi Kamata and Kiyoshi Kuramoto of Hokkaido University from the research team. Photo taken by Yu Kikuchi.

ORIGINAL ARTICLE
Kamata S. et al., Pluto's ocean is capped and insulated by gas hydrates. *Nature Geosciences*, May 20, 2019.

FUNDING
The Japan Society for the Promotion of Science (JSPS)'s KAKENHI (JP16K17787, JP17H06456, JP17H06457) and the Astrobiology Center Program of the National Institutes of Natural Sciences (NINS).

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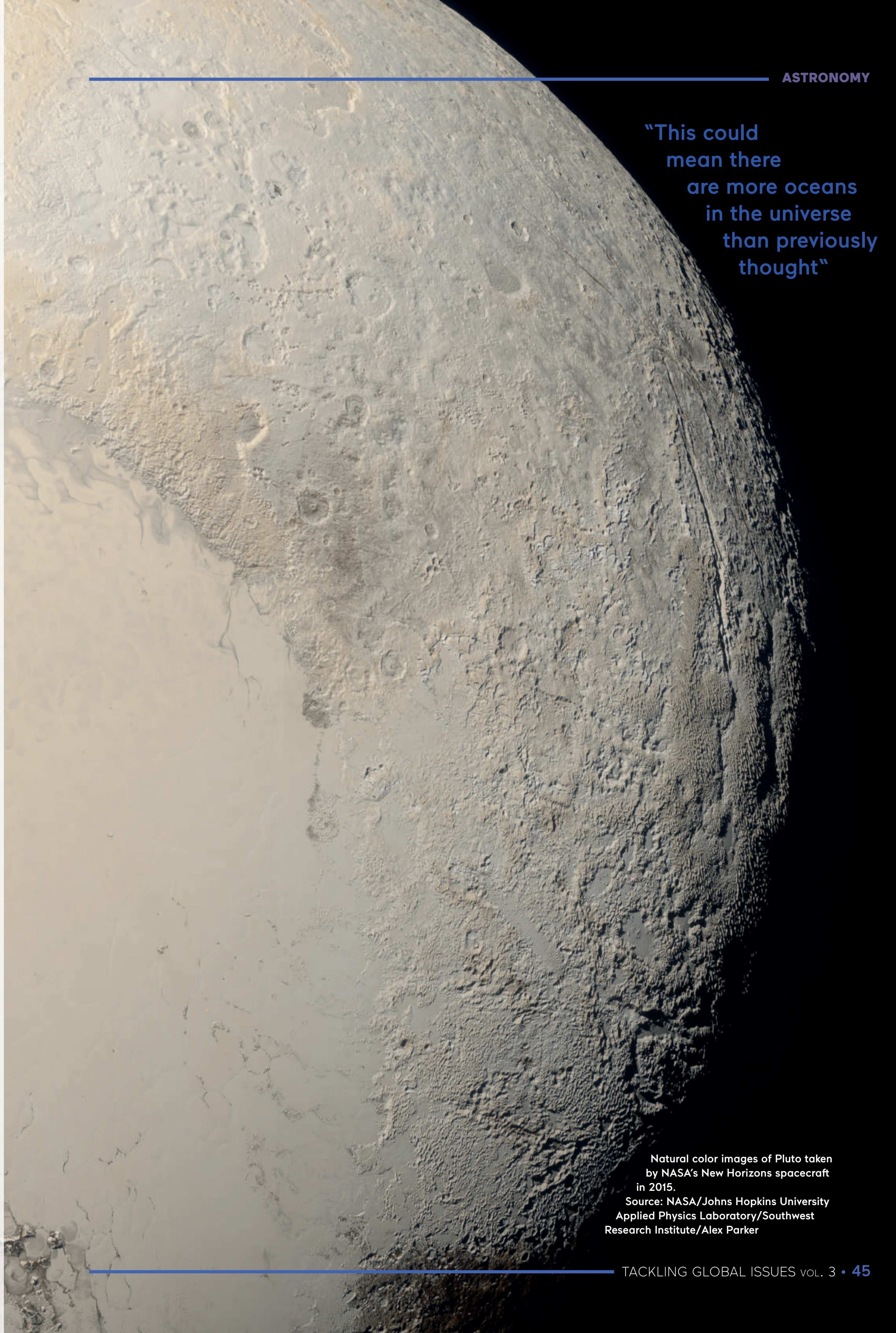
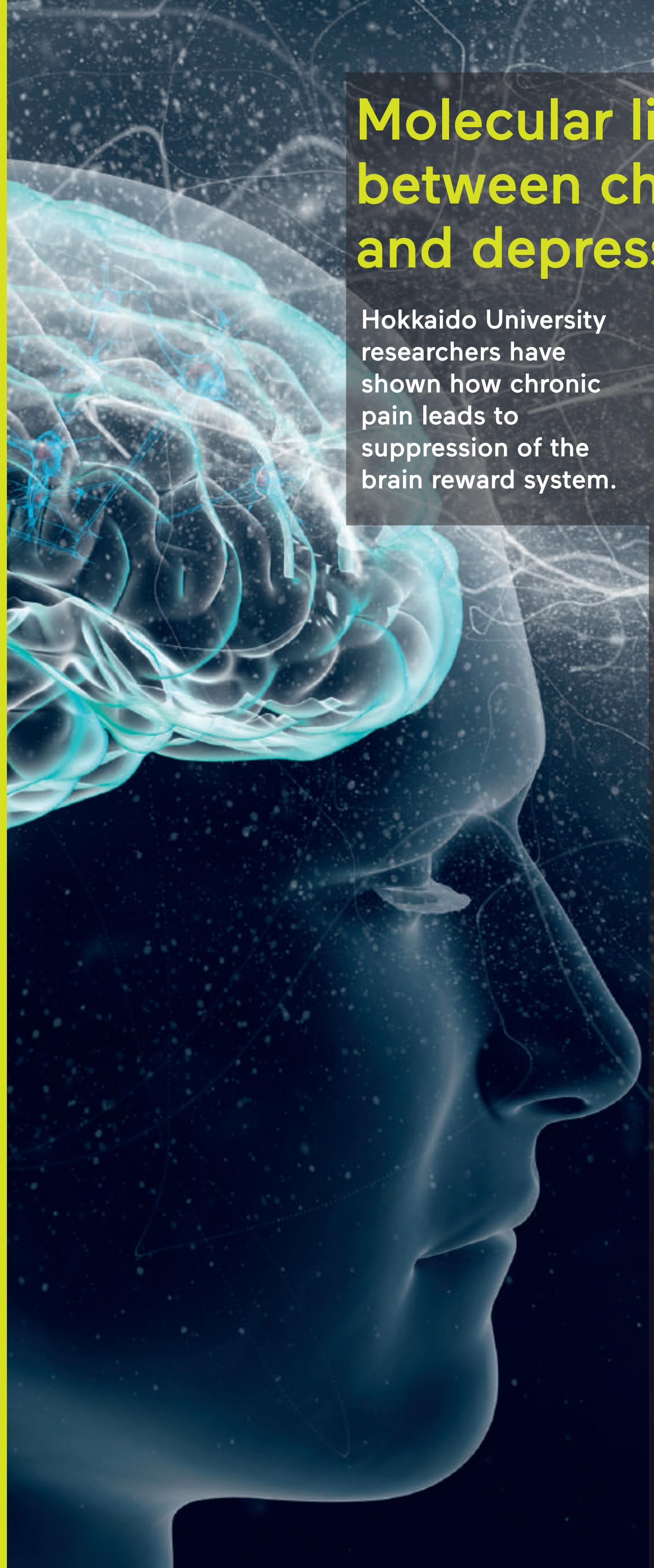
"This could mean there are more oceans in the universe than previously thought"



Natural color images of Pluto taken by NASA's New Horizons spacecraft in 2015.
Source: NASA/Johns Hopkins University Applied Physics Laboratory/Southwest Research Institute/Alex Parker



Molecular link between chronic pain and depression revealed

Hokkaido University researchers have shown how chronic pain leads to suppression of the brain reward system.

Researchers at Hokkaido University have identified the brain mechanism linking chronic pain and depression in rats. Their research, published in *The Journal of Neuroscience*, could lead to the development of new treatments for chronic pain and depression.

"Clinicians have known for a long time that chronic pain often leads to depression, however the brain mechanism for this was unclear," said Professor Masabumi Minami at Hokkaido University, one of the authors of the paper.

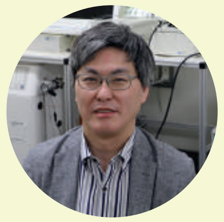
The researchers looked at how neuronal pathways were affected by chronic pain in rats. They used an electrophysiological technique to measure the activities of neurons after four weeks of chronic pain. They found that persistent pain caused changes in the neuronal pathway projecting from the brain region called bed nucleus of the stria terminalis (BNST) to the region called ventral tegmental area (VTA). Specifically, they found enhanced signaling mediated by corticotropin-releasing factor (CRF), which is a neuropeptide known to be involved in the negative emotions such as anxiety and fear, in the BNST of chronic pain animals.

Crucially, they showed that this enhanced CRF signaling leads to suppression of the brain reward system, the nervous system that is activated by rewards and related to production of pleasure and motivation. Suppression of the reward system is considered to be an underlying mechanism of depression, which leads to decreased pleasure and motivation.

"By clarifying the mechanism by which the brain reward system is continuously suppressed, we found the missing link between chronic pain and depression," says Masabumi Minami.

The researchers found that when they treated the rats with a drug that blocked the excessive CRF signals, the activity of dopamine neurons, which play an important role in the brain reward system, was increased. This suggests that drugs targeting neuropeptides such as CRF could be developed in order to treat chronic pain and depression in the future.

"These findings could not only lead to improved treatment of emotional aspect of chronic pain, but also to new therapeutics for depressive disorders," says Masabumi Minami. ●



ORIGINAL ARTICLE
Takahashi D., Asaoka Y., Kimura K. et al., Tonic suppression of the mesolimbic dopaminergic system by enhanced corticotropin-releasing factor signaling within the bed nucleus of the stria terminalis in chronic pain model rats. *The Journal of Neuroscience*, August 26, 2019.

FUNDING
A Grant-in-Aid for Scientific Research (23300130, 26290020, 17H03556) and Challenging Research (17K19469) from the Japan Society for the Promotion of Science (JSPS), a Grant-in-Aid for Scientific Research (25116501, 15H01273) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), and the Japan Agency for Medical Research and Development (JP19gm0910012).

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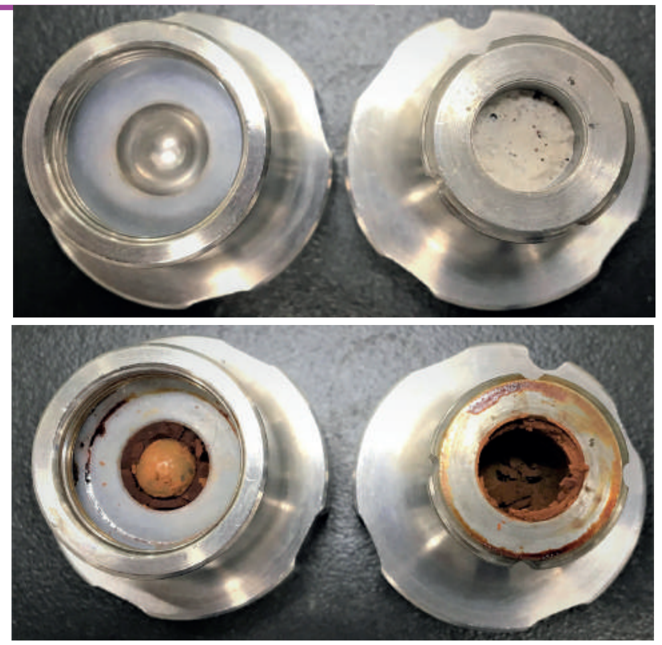
Mechanical force as a new way of starting chemical reactions

Researchers have shown mechanical force can start chemical reactions, making them cheaper, more broadly applicable, and more environmentally friendly than conventional methods.



Reaction mixtures before and after grinding in a ball mill. Mechanoredox arylation reactions occurred with aryl diazonium salts and furan in the presence of a piezoelectric material ($BaTiO_3$). (Kubota K. et al. *Science*, December 19, 2019)

Chemical reactions are most conventionally prompted by heating up the reaction mixtures. Within the last ten years, there has been extensive research on "photoredox catalysts" that can be activated by visible light and enable highly specific and efficient chemical reactions. However, these reactions often require a large amount of harmful organic solvents, making them applicable only to soluble reactants.

"Piezoelectric materials" such as barium titanate are known to generate electric potentials when a mechanical pressure is applied to them, which is why they are used in microphones and lighters. In the current study published in *Science*, the research team led by Hajime Ito and Koji Kubota of the Institute for Chemical Reaction Design and Discovery (WPI-ICReDD) at Hokkaido University proved this electric potential can also be used to activate chemical reactions. "In our system, we use the mechanical force provided by a ball mill to activate a piezoelectric material for redox reactions, while eliminating the use of organic solvent," says Koji Kubota. They call it a mechanoredox reaction as opposed to a photoredox reaction.

The team demonstrated that electric potentials derived from piezoelectric material ($BaTiO_3$) activate a compound called aryl diazonium salts generating highly reactive radicals. The radicals undergo bond-forming reactions such as arylation and borylation reactions — both of which are important in synthetic chemistry — with high efficiency. The team also showed that the borylation reaction could occur by striking the mixture in a plastic bag with a hammer.

"This is the first example of arylation and borylation reactions using mechanically induced piezoelectricity," says Koji Kubota. "Our solvent-free system using a ball mill has enabled us to eliminate organic solvents, making the reactions easier to handle, more environmentally friendly, and applicable even to reactants that cannot be dissolved in the reaction solvent." They could also recycle the barium titanate and achieve better yields than photoredox reactions, even further increasing the attractiveness of this approach.

"We are now exploring the tunability of the mechanically generated electric potential. Together with computational predictions, we aim to extend the applicability of this technique," says Hajime Ito. "Our goal is to complement or at least partly replace existing photoredox approaches and provide an environmentally friendly and cost-efficient alternative to be used in industrial organic synthesis." ●

ORIGINAL ARTICLE
Kubota K., Pang Y., Miura A., Ito H. Redox Reactions of Small Organic Molecules Using Ball Milling and Piezoelectric Materials. *Science*, December 19, 2019.

FUNDING
The Japan Society for the Promotion of Science (JSPS)'s KAKENHI (JP18H03907, JP17H06370, JP19K15547), and the Japan Science and Technology Agency (JST)'s CREST grant (JPMJCR19R1).

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hajito@eng.hokudai.ac.jp

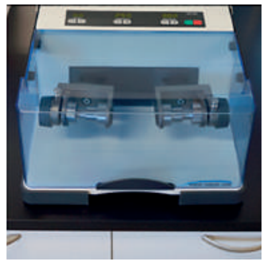
1) Ball was added to milling jar

2) Chemicals were added

3) Jar was closed

4) Jar was placed in Retch MM400

5) Grinding started



In ball milling, the chemicals and a steel ball are placed into steel jars which are then shaken vigorously.

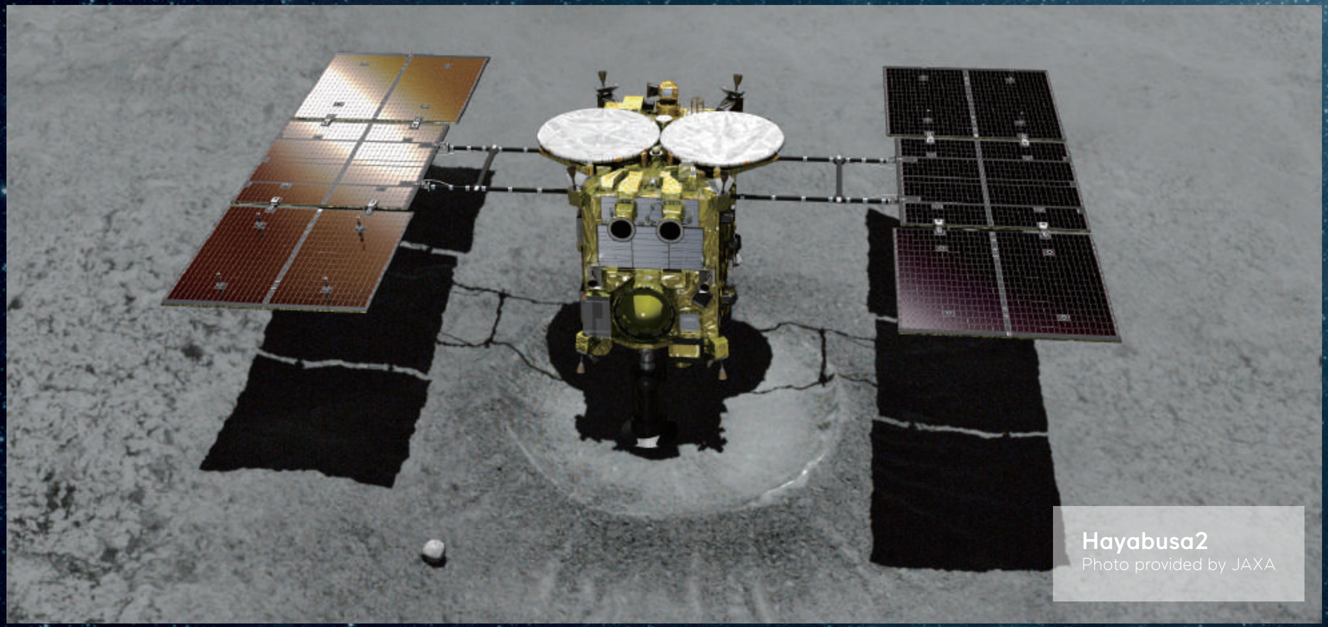
METEORITES

Message-bearers from 4.6 billion years ago

Hayabusa, a robotic spacecraft developed by the Japan Aerospace Exploration Agency (JAXA), returned to Earth in June 2010 after taking a sample from a small near-Earth asteroid named 25143 Itokawa. Professor Hisayoshi Yurimoto at the Faculty of Science, a member of the project is now analyzing the sample to find clues for elucidating how the solar system was born 4.6 billion years ago. He is also preparing to analyze a sample from another asteroid, Ryugu, which Hayabusa2 brought back to Earth in December 2020.

Yurimoto is a born scientist. He has had a keen interest in science, especially beautiful minerals, ever since he was young. "I was so elated when I found quartz in a nearby mikan tangerine field," Yurimoto said with a grin. Yurimoto researched minerals and obtained a doctor's degree in the study of peridots, a green transparent variety of olivine often used as semi-precious gems. Olivine is found in lava and meteorites.

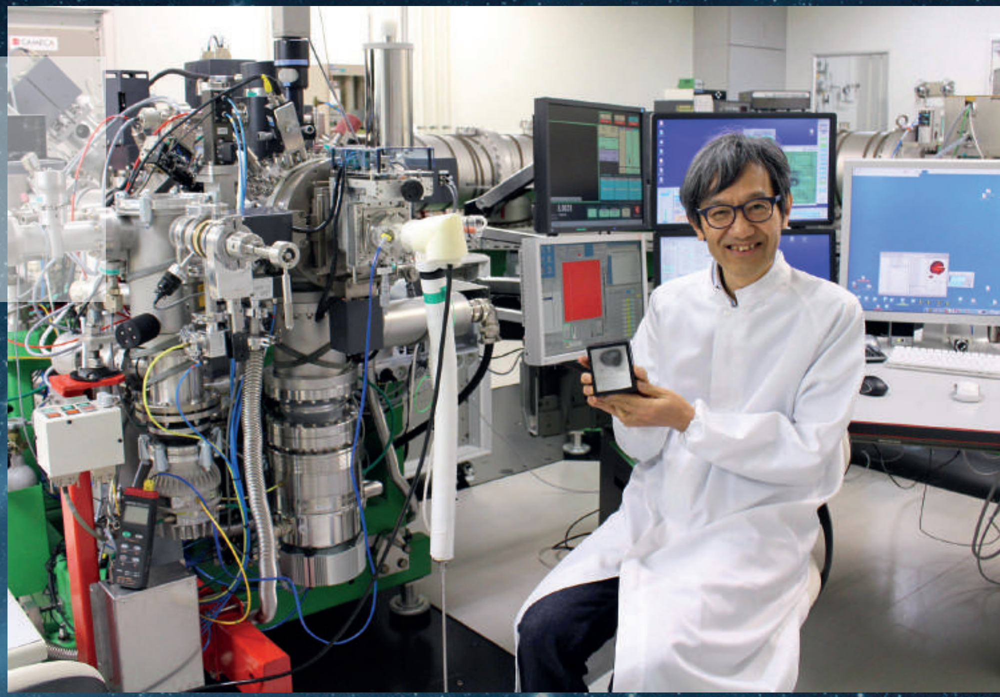
A turning point came more than two decades ago when Yurimoto was around 30 years old. During a meeting with a meteorite specialist, Yurimoto learned that meteorites contain minerals that are not found on Earth. More specifically, some minerals have isotopic ratios different from that of substances on Earth. Isotopes — two or more forms of the same element that contain equal numbers of protons but different numbers of neutrons in their nuclei, thus differing in their mass — exist in a certain ratio in elements on our planet.



Hayabusa2
Photo provided by JAXA

"I was astounded to discover a meteorite's isotopic ratio was different from the projected figure even though minerals in the meteorite were formed based on the same established laws of physics and chemistry," Yurimoto said, explaining why he started focusing his research on meteorites to unravel the origin and evolution of the solar system. In those days, however, meteorites were hard to come by in Japan. Yurimoto proceeded with his research by borrowing samples from the aforementioned specialist. He also started developing an isotope microscope, a one-of-a-kind device weighing 10 tons, to unlock the mysteries enveloping the isotopic ratio in meteorites.

A meteorite from one of Yurimoto's collections.



Dr. Yurimoto holding a meteorite in front of the isotope microscope.

The huge microscope, which was completed after 20 years of research and development, enabled researchers to distinguish isotopes of the same element in meteorites.

Yurimoto's group used this isotope microscope to analyze the sample from Itokawa. His group examined the isotopic ratio of oxygen, the most abundant element in meteorites, which has three kinds of isotopes. The results showed the isotopic ratio in the sample was the same as that of ordinary chondrites, which account for 80 percent of meteorites that fall to Earth, demonstrating that meteorites are asteroid fragments and contain vital information from the time when the solar system was born. This finding was reported by the media and fueled discussions around the globe.

It is possible to deduce the pressure, temperature and time required to form meteorites by examining meteorites with the isotope microscope. "We are trying to figure out how the mysterious conditions in meteorites were created, based on isotopic microscopic analyses," Yurimoto said, referring to the different isotopic ratios. "There are various theories about this. Some researchers suggest it is a result of lightening in space, and others point to the effects of collisions between celestial bodies. Nobody knows for certain. It is difficult to think about a phenomenon nobody has seen, but I'd definitely like to unlock that mystery."

Use of the microscope is not limited to space science. As it is suitable for examining molecular movements, the microscope is used for research in fields including medicine, biology, agricultural science and engineering. "It is interesting to talk to many researchers in different fields who would like to use the microscope," Yurimoto said. "Doing so sometimes has led to joint research with my team."

Yurimoto's curiosity about the unknown has not dimmed since he was looking for pieces of quartz as a boy. He is determined to decipher information hidden in meteorites and the original materials that created them, which Hayabusa collected from Itokawa. Driven by his unlimited curiosity that culminated in the development of a groundbreaking device, Yurimoto's ultimate goal is to take a glimpse into a world nobody has seen before. ●

Background photo Adobe Stock © Tryfonov

Center for Human-Nature, Artificial Intelligence, and Neuroscience (CHAIN) ESTABLISHED

(Left) The Center Director Shigeru Taguchi speaking at the inaugural symposium.

Hokkaido University launched the Center for Human-Nature, Artificial Intelligence, and Neuroscience, or CHAIN, in July 2019. It will conduct interdisciplinary research and education at the intersection of humanities, artificial intelligence, and neuroscience. The inaugural symposium was held at the university's Sapporo Campus on July 23rd to unravel its vision and ambitious plans for research and graduate-level education.

Professor Shigeru Taguchi, the Director of CHAIN, says, "Recent developments in neuroscience and artificial intelligence have made it possible for scientists to tackle problems that have been traditionally explored in humanities, such as consciousness, emotion, and self," explaining the ever-increasing demand for the integration of humanities and science. "We would like to open up new directions in understanding 'what human beings are'." The center aims, in particular, to integrate highly philosophical ideas with mathematical and empirical scientific methods and findings. Graduate-level education at CHAIN has also started in April 2020 for Master's and Ph.D. students in the university. ●

Hisayoshi Yurimoto bestowed the LEONARD MEDAL

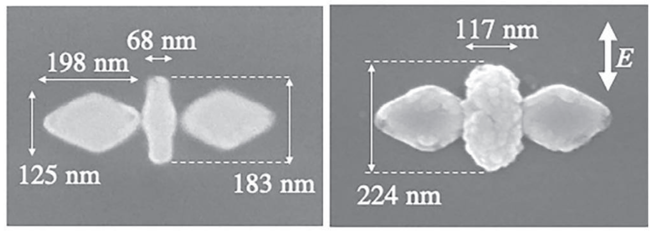
Professor Hisayoshi Yurimoto of the Faculty of Science received the 2019 Leonard Medal from the Meteoritical Society for his outstanding contributions to meteoritics, or the science of meteors, meteorites and meteoroids.

Established in 1933, the Meteoritical Society is an international organization dedicated to the research and education of planetary science, meteorites, and other extraterrestrial objects. The society annually presents the Leonard Medal, named after Frederick C. Leonard, the first president of the society, to one scientist.

Professor Yurimoto studies the origin and evolution of the solar system via chemical and isotopical analyses. Recently, he has been analyzing a meteorite sample named 25143 Itokawa from a small near-Earth asteroid taken by the Hayabusa spacecraft by the Japan Aerospace Exploration Agency (JAXA). ●

A gold butterfly can make its own semiconductor skin

A nanoscale gold butterfly provides a more precise route for growing/synthesizing nanosized semiconductors that can be used in nano-lasers and other applications.



Scanning electron microscope images of the nano-butterfly structure before (left) and after (right) laser irradiation. Semiconductor zinc oxide has crystalized on the surface of the gold nanorod. (Fujiwara H., et al, *Nano Letters*. December 23, 2019.)

Hokkaido University researchers have devised a unique approach for making nanosized semiconductors on a metal surface. The details of the method were reported in the journal *Nano Letters* and could further research into the fabrication of nanosized light and energy emitters.

The approach, developed by Hokkaido University's Research Institute for Electronic Science and Hokkai-Gakuen University, involves generating localized heat on a gold nanoparticle within a butterfly-shaped nanostructure. The heat causes hydrothermal synthesis in which semiconducting zinc oxide crystallizes on the gold nanoparticle.

Scientists have been investigating ways to carefully place nano-sized semiconductors on metallic particles to utilize them in nano-lasing and nano-lithography, for example. But current methods lack precision or are too costly.

The approach developed by the Japanese team overcomes these issues.

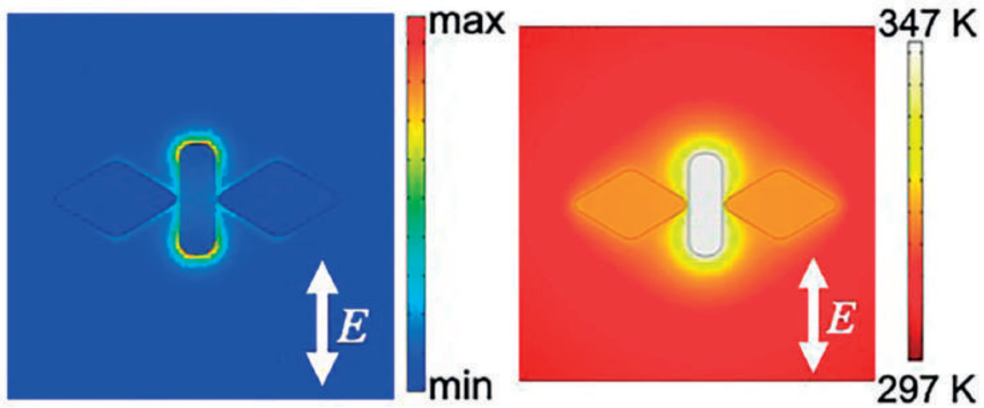
The team first conducted simulations to determine the optimal conditions for precisely controlling the generation of heat in nanostructures. They utilized a phenomenon called surface plasmon resonance, a process which partly converts light to heat in metallic materials.

According to the simulations, a butterfly-shaped nanostructure consisting of two rhombus gold particles placed on either side of a gold nanorod would lead to optimal conditions. In this system, the nanorod, or the body of the butterfly, works as a nanoheater using a specific polarized light. After rotating the light polarization 90 degrees, the rhombus particles, or the wings of the butterfly, should work as an antenna to gather light at subwavelength spots in the butterfly's semiconductor skin.

To test this theory, they fabricated the gold butterfly and placed it in water inside a glass chamber. A solution made from equal parts zinc nitrate hexahydrate and hexamethylene tetramine was added to the chamber, which was then sealed and placed on a microscopic stage. When the laser light was shone on the system inside the chamber, the nanorod heated up and semiconducting zinc oxide particles crystallized along its surface as they expected.

This demonstrated that the butterfly-shaped gold nano-antenna can precisely control where plasmon-assisted hydrothermal synthesis occurs, therefore enabling the localized formation of nano-sized semiconductors.

"Further research is expected to lead to the development of powerful nano-sized light sources, highly efficient photoelectric conversion devices, and photocatalysts," says Hokkaido University's Keiji Sasaki of the research team. "It could also lead to applications in semiconductor electronics and optical quantum information processing." ●



Calculated localized field (left) and temperature distribution (right) of the nano-butterfly structure. (Fujiwara H., et al, *Nano Letters*. December 23, 2019.)

ORIGINAL ARTICLE

Fujiwara H., et al, Localized ZnO Growth on a Gold Nanoantenna by Plasmon-Assisted Hydrothermal Synthesis, *Nano Letters*. December 23, 2019.

FUNDING

The Japan Society for the Promotion of Science (JSPS)'s KAKENHI (JP16H06506, JP17K05016, JP18H03882, JP18H05205, JP19H04529), Dynamic Alliance for Open Innovation Bridging Human, Environment and Materials, and the Nippon Sheet Glass Foundation for Materials Science and Engineering.

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There is no escaping from climate change, even in the deep sea

Even though the deeper layers of the ocean are warming at a slower pace than the surface, animals living in the deep ocean are more exposed to climate warming and will face increasing challenges to maintain their preferred thermal habitats in the future.

Reporting in the journal *Nature Climate Change*, an international team of scientists, led by the University of Queensland in Australia and involving Hokkaido University, analyzed contemporary and future global patterns of the velocity of climate change across the depths of the ocean. Their metric describes the temporal rate and direction of temperature changes, as a proxy for potential shifts of marine biota in response to climate warming.

Despite rapid surface warming, the team found that global mean climate velocities in the deepest layers of the ocean (>1,000 m) have been 2 to nearly 4-fold faster than at surface over the second half of the 20th century. The authors point to the greater thermal homogeneity of the deep ocean environment as responsible for these larger velocities. Moreover, while climate velocities are projected to slow down under scenarios contemplating strong mitigation of greenhouse gas emissions (RCP2.6), they will continue to accelerate in the deep ocean.

"Our results suggest that deep sea biodiversity is likely to be at greater risk because they are adapted to much more stable thermal environments," says Jorge García Molinos, a climate ecologist at Hokkaido University's Arctic Research Center, who contributed to the study. "The acceleration of climate velocity for the deep ocean is consistent through all tested greenhouse gas concentration scenarios. This provides strong motivation to consider the future impacts of ocean warming to deep ocean biodiversity, which remains worryingly understudied."

Climate velocities in the mesopelagic layer of the ocean (200-1000 m) are projected to be between 4 to 11 times higher than current velocities at the surface by the end of this century. Marine life in the mesopelagic layer includes great abundance of small fish that are food for larger animals, including tuna and squid. This could present additional challenges for commercial fisheries if predators and their prey further down the water column do not follow similar range shifts.

The authors also compared resulting spatial patterns of contemporary climate velocity with those of marine biodiversity for over 20,000 marine species to show potential areas of risk, where high biodiversity and velocity overlap. They found that, while risk areas for surface and intermediate layers dominate in tropical and subtropical latitudes, those of the deepest layers are widespread across all latitudes except for polar regions.

The scientists caution that while uncertainty of the results increases with depth, life in the deep ocean is also limited by many factors other than temperature, such as pressure, light or oxygen concentrations. "Without knowing if and how well deep ocean species can adapt to these changes, we recommend to follow a precautionary approach that limits the negative effects from other human activities such as deep-sea mining and fishing, as well as planning for climate-smart networks of large Marine Protected Areas for the deeper ocean," says García Molinos. ●

ORIGINAL ARTICLE

Isaac Brito-Morales et al., Climate velocity reveals increasing exposure of deep-ocean biodiversity to future warming. *Nature Climate Change*. May 25, 2020.

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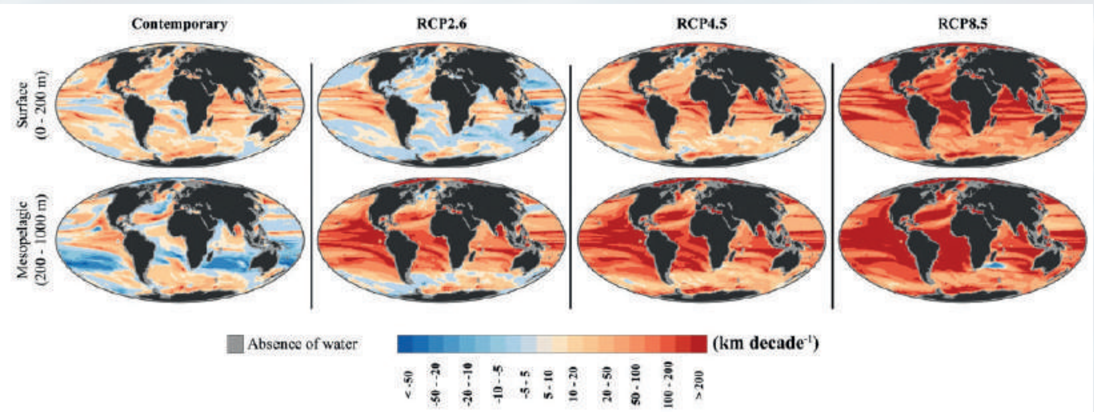


Climate velocity (km decade⁻¹) for contemporary (1955-2005) and projected future sea temperatures (2050-2100) at sea surface and the mesopelagic layer under three IPCC greenhouse gas emission scenarios (RCP2.6, RCP4.5 and RCP8.5). (Isaac Brito-Morales et al., *Nature Climate Change*. May 25, 2020)

Photo by YUCAR FotoGrafik on Unsplash

Deployment of the Philippines' first Micro-satellite "DIWATA-1" from the Kibo/ISS.
Photo: ©JAXA/NASA

MYANMAR-JAPAN COLLABORATION ON MICRO-SATELLITE DEVELOPMENT STARTED

Hokkaido University, Tohoku University, and Myanmar Aerospace Engineering University (MAEU) have started their first satellite development program from September 1st, 2019 funded by the government of the Republic of the Union of Myanmar. Over the course of five years, the program will develop and launch two 50kg-class Micro-satellites while carrying out capacity building for satellite development for students from Myanmar. It will contribute to agriculture, forestry, fisheries, and the mitigation of large-scale natural disasters.

Since 2015, Professor Yukihiro Takahashi of Hokkaido University and his collaborators at Tohoku University have accepted graduate students from the Republic of the Philippines and collaborated in the development, launch, and operation of Micro-satellites DIWATA-1 and -2 (PHL-Microsat program). This success urged the establishment of space agency PhilSA in the Philippines last year.

Similar to the Philippines program, this collaboration with Myanmar aims to develop and launch Micro-satellites for earth observation and to operate advanced observation instruments such as a multi-spectral camera installed on the satellite. Hokkaido University, Tohoku University, and MAEU will cooperate closely to promote research, development, and operation of the satellites. They aim to release the first satellite from the International Space Station in early 2021. ●

Bio-inspired hydrogel can rapidly switch to rigid plastic

A new material that stiffens 1,800-fold when exposed to heat could protect motorcyclists and racecar drivers during accidents.

Researchers have developed a hydrogel that does the opposite of what polymer-based materials, like plastic bottles, normally do: their material hardens when heated and softens when cooled. Their findings, published in the journal *Advanced Materials*, could lead to the fabrication of protective clothing items for traffic and sports-related accidents.

Takayuki Nonoyama and Jian Ping Gong of Hokkaido University and their colleagues were inspired by how proteins remain stable inside organisms that survive within extreme-heat environments, like hot springs and deep sea thermal vents. Normally, heat "denatures" proteins, altering their structure and breaking their bonds. But the proteins within thermophiles remain stable with heat thanks to enhanced electrostatic interactions such as ionic bonds.

The team developed an inexpensive, non-toxic polyacrylic gel based on this concept. A gel composed of polyelectrolyte poly (acrylic acid) (PAAc) was immersed in a calcium acetate aqueous solution. PAAc on its own acts like any other polymer-based material and softens when heated. But when calcium acetate is added, PAAc's side residues interact with the calcium acetate molecules, in a way similar to what happens inside thermophile proteins, causing PAAc to act very differently.

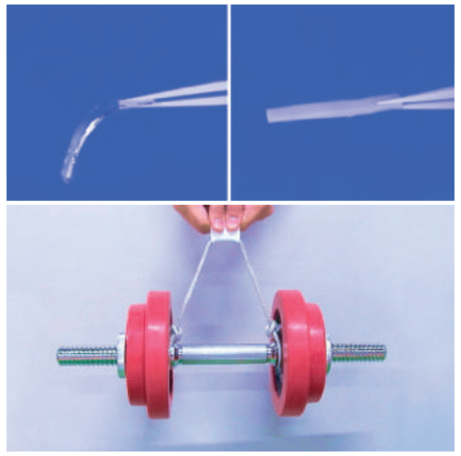
The team found that their originally uniform gel separates into a polymer dense "phase" and a polymer sparse one as the temperature rises. When it reaches to a critical temperature, in this case around 60°C, the dense phase undergoes less dehydration which strengthens ionic bonds and hydrophobic interactions between polymer molecules. This causes the material to rapidly transform from a soft, transparent hydrogel to a rigid, opaque plastic.

The heated material was 1,800 times stiffer, 80 times stronger, and 20 times tougher than the original hydrogel. The soft-to-rigid switching was completely reversible by alternatively heating and cooling the material. Moreover, the scientists could fine-tune the switching temperature by adjusting the concentration of the ingredients.

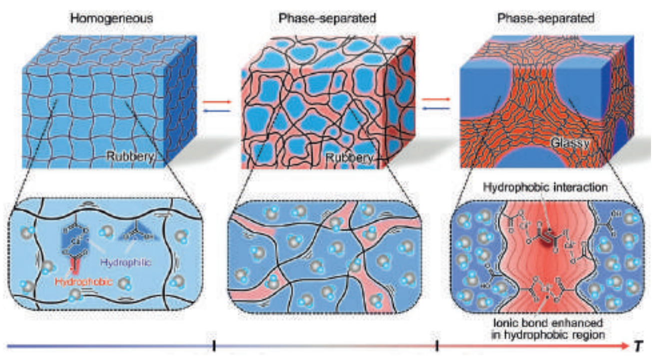
They then demonstrated a possible application of the material by combining it with a woven glass fabric. This new fabric was soft at room temperature, but when it was pulled against an asphalt surface for five seconds at a speed of 80 km/hour, the heat generated by the friction hardened the material with only minor abrasions forming on the contact surface.

Takayuki Nonoyama says "Clothing made from similar fabric could be used to protect people during traffic or sports-related accidents, for example. Our material could also be used as a heat-absorbent window coating to keep indoor environments cooler."

"This polymer gel can be easily made from versatile, inexpensive and non-toxic raw materials that are commonly found in daily life. Specifically, the polyacrylic acids are used in disposable diapers and calcium acetates are used in food additives," Jian Ping Gong added. "Our study contributes to basic research on new temperature-responsive polymers, and to applied research on temperature-responsive smart materials." ●



The gel is soft and transparent at 25°C (top left) and cannot support a 10 kg weight but it quickly becomes rigid and opaque when heated to 60°C (top right), becoming strong enough to support the weight (bottom). (Nonoyama T. et al., *Advanced Materials*, November 18, 2019)



Molecular structures and the mechanisms behind instant thermal hardening of the hydrogel. (Nonoyama T. et al., *Advanced Materials*, November 18, 2019)

ORIGINAL ARTICLE

Nonoyama T. et al., Instant Thermal Switching from Soft Hydrogel to Rigid Plastics Inspired by Thermophile Proteins. *Advanced Materials*, November 18, 2019.

FUNDING

The Japan Society for the Promotion of Science (JSPS)'s KAKENHI grant (JP17H06144, 17K19146, 17H06376).

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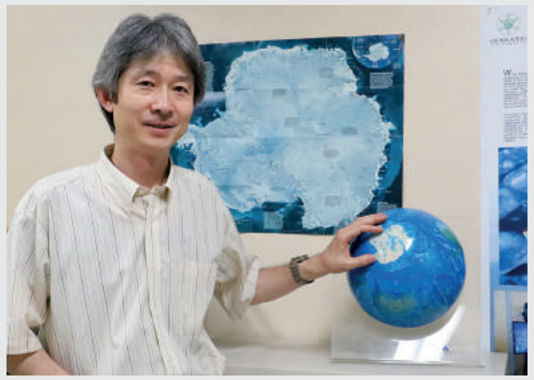
Antarctic Expedition ship *Shirase* during the 58th Japanese Antarctic Research Expedition in 2017.
Photo by Kazuya Ono, Institute of Low Temperature Science.

SHIGERU AOKI LEADS EXPEDITION TO ANTARCTICA

For the first time, a Hokkaido University researcher led the Japanese Antarctic Research Expedition. Associate Professor Shigeru Aoki of the Institute of Low Temperature Science was selected by the government to lead the 61st Japanese Antarctic Research Expedition, which left Japan for the Antarctic in November 2019 and continued until March 2020.

Aoki is an experienced scientist in the field of polar research, having participated in the 39th, 43rd, and 56th Japanese Antarctic Research Expeditions as well as a number of expeditions led by Australia. His role as the leader of the 61st expedition included managing the crew members and on-site research activities.

On board the observation ship Shirase, one of the main missions of the 61st expedition was to observe and uncover the relationship between ice and the ocean in the Antarctic regions, particularly in relation to climate change. ●

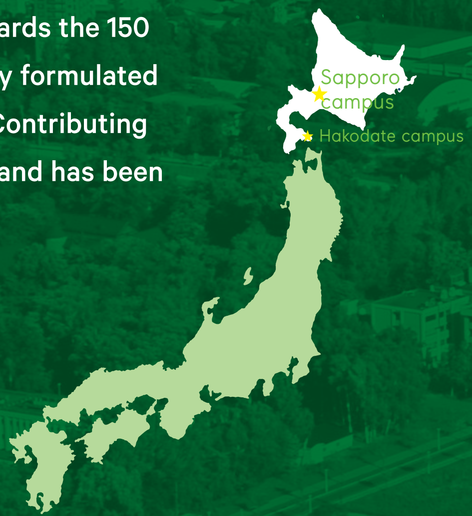


Associate Professor Shigeru Aoki at the Institute of Low Temperature Science.

Hokkaido University *at a glance*

A Long History

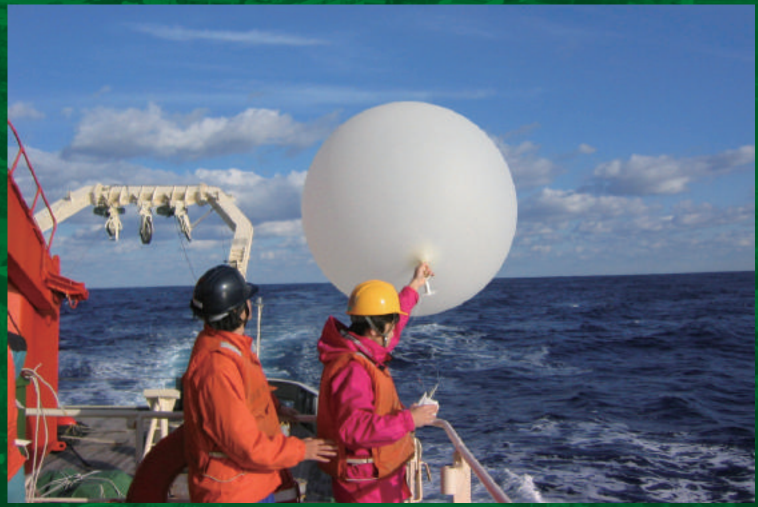
Founded in 1876 as Sapporo Agricultural College, Hokkaido University is one of the oldest, largest, and most prestigious universities in Japan. Boasting one of the largest campuses in Japan, the university houses cutting-edge research facilities, a university hospital, and a number of field research centers including one of the world's largest research forests. Towards the 150 anniversary of its founding, the university formulated an action strategy under the slogan of "Contributing towards the resolution of global issues," and has been implementing a number of reform plans.



Contributing towards the **resolution** of **global** issues

Hokkaido Universal Campus Initiative (HUCI)

Aiming to further internationalize the university and foster more world leaders who can contribute to the resolution of global issues, the university launched the Hokkaido Universal Campus Initiative (HUCI) in 2014 as part of Top Global University Projects by the Japanese government. Under the initiative, the university has implemented a number of programs to develop global leaders and promote international collaborations.



Research

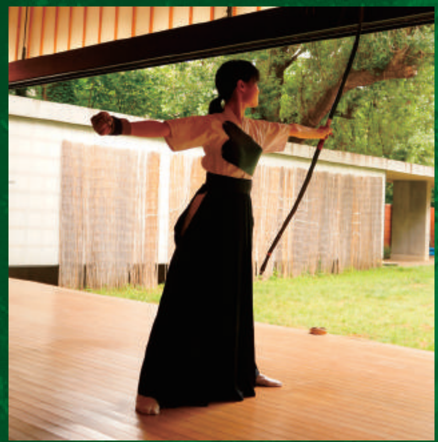
Since its establishment as an agricultural college, Hokkaido University has expanded its research strength to encompass a variety of fields in the sciences and humanities. It has produced experts in the areas such as low temperature science, life science, veterinary science, and fisheries science.

In 2014, to further strengthen international collaboration and conduct top-level research in strategic areas including quantum medical science and engineering, the university established the Global Institution for Collaborative Research and Education (GI-CoRE). In 2018, the university launched the Institute for Chemical Reaction Design and Discovery (ICReDD) as part of the World Premier Institutional Research Initiative (WPI) by the Japanese government.

International programs

In addition to the regular 12 undergraduate and 21 graduate schools, Hokkaido University runs a number of degree programs taught in English for international students such as Modern Japanese Studies Program (MJSP) and Integrated Science Program (ISP) for undergraduate students. At the graduate level, courses in engineering, veterinary medicine, agricultural science, science, and environmental science among others, are offered in English.

During the summer, the Hokkaido Summer Institute (HSI) offers more than 100 short programs in English covering a wide range of disciplines from material science to archaeology which are run by top-level researchers from the university and around the world.



Today

Data as of April 2020

660 km²
total area
campuses & facilities

12
undergraduate schools

21
graduate schools

1
Nobel prize
Akira Suzuki, Nobel Prize in Chemistry (2010)

8
overseas offices

227,860
alumni

18,105
students

12%
international students

RANKING

1st in Japan
76th in the world
THE World University Impact Rankings 2020

29th in Asia
132nd in the world
QS World University Rankings 2020

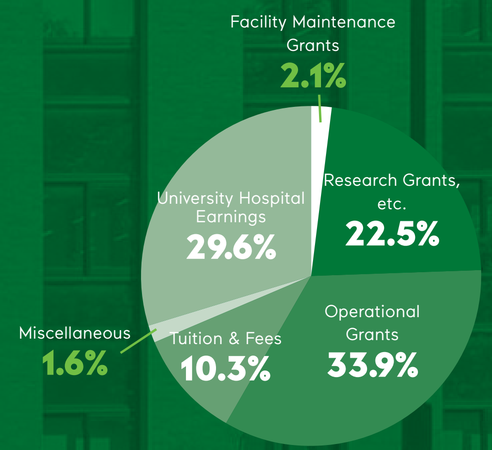
111th
in the world
Nature Index Top Academic Institutions 2020

3,917
faculty & staff

25
research centers and institutions

REVENUE

¥103,728m
revenue in 2020



Be ambitious.

Inazo Nitobe

HOKKAIDO UNIVERSITY

TOP GLOBAL UNIVERSITY JAPAN

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