

Impact Objectives

- Use the RAPINA system to improve the standard of laboratory-based rabies surveillance in the Philippines and beyond
- Further utilise the RAPINA system to understand VNA levels in HIV-positive patients so as to tailor their treatment accordingly
- Ultimately put an end to rabies once and for all even in rabies endemic countries



Leading the charge against rabies

Below Professor Akira Nishizono introduces RAPINA, an innovative new system his team has developed that will benefit all rabies endemic countries



Could you begin with an introduction to yourself?

I graduated from Oita

Medical University in Japan in 1985, and subsequently worked as an internal medicine physician and then began engaging in the virology research field. After obtaining my PhD, I began researching rabies and I am now Professor in the Department of Microbiology at Oita University. My main theme is comprehensive research on rabies; including virology, epidemiology, diagnosis, prevention and therapeutics.

What interests you most about this field?

Over the last 50 years, Japan has been a rabies-free country. However, the disease remains endemic in most other countries. It is one of the typical neglected tropical infectious diseases (NTDs). Unfortunately, there are few rabies researchers in this medical field in Japan. Therefore, I feel I must fulfil my mission to engage in research of this historic and important zoonotic disease.

Rabies is still incurable and leads to death. Until now, there have been no effective ways to treat the disease once symptoms appear. I want to develop therapeutic treatments against symptomatic rabies. That is my key motivation for continuing rabies research.

Can you explain more about your study into the effectiveness of the improved rapid neutralising antibody detection system (RAPINA)?

We demonstrated a novel, fast and easy test – RAPINA – as an alternative to virus-neutralising antibodies (VNA) determination assays. The test is based on the principle of immunochromatography combined with virus neutralisation (binding), both using the inactivated rabies virus as antigen and using two types of anti-G monoclonal antibody. The RAPINA test has good positive and negative predictive values corresponding to a VNA level of 0.5 IU/mL, as recommended by the World Health Organization (WHO) and the World Organization for Animal Health (OIE). The RAPINA test can be used on either a large or small number of samples, allowing VNA to be predicted at any time or for any purpose.

Does RAPINA have any additional uses?

In vaccine clinics RAPINA can also be utilised to evaluate the effect of a vaccine in immune-compromised hosts, including HIV-positive patients. The number of CD4+ T cells is a crucial factor for inducing a lasting immune response after the rabies vaccine. When changing the regimen or considering the booster vaccination schedule for HIV-positive patients, it may be important to know the precise level of VNA in these patients.

Can you explain the importance of your work in real-world terms?

Current rabies prevention and control strategies consist of different methods, including wild and domestic animal vaccination programmes, animal birth control, responsible pet ownership, rabies education and awareness, and cooperating with medical and veterinary approaches as the ‘One Health’ concept. Generally, the mass vaccination of dogs against RABV is a highly rational strategy for interrupting the natural transmission cycle of urban rabies from the veterinary side and proper post-exposure prophylaxis (PEP) even after being bitten by a rabid animal, and is one of the most effective tools for preventing rabies from the medical side. However, the above-mentioned effective strategies for rabies elimination have been hampered by several factors, including inferior priority of political decision-making and economic issues. If user-friendly tools were available in resource-limited countries or districts, rabies control programmes could easily be achieved in the future.

Can you discuss the work you have been engaged with in the Philippines?

We have launched an international collaborative project between Japan and the Philippines to improve laboratory-based rabies surveillance in the Philippines in support of rabies elimination efforts. Beyond this, our work will benefit all rabies endemic countries and districts, particularly those resource-limited areas in Asia and Africa. ►

Rabies elimination efforts

Collaborative work led by Oita University in Japan, is helping to move the world one step closer to the eradication of rabies

Despite Japan being free of rabies for 50 years now, the disease remains endemic in most countries. This is because these countries often do not have sufficient funds or the infrastructure required to employ the approved gold standard for the definitive diagnosis of the disease. The disease is extremely serious, infecting the brain and nerves and causing inflammation. What is more, once symptoms have appeared, the prognosis is nearly always death. More research surrounding the disease is required in order to successfully diagnose and ultimately combat it.

Research led by Chief Investigator Professor Akira Nishizono at Oita University in Japan, is seeking to ultimately combat the ongoing issue of endemic rabies using a new method – RAPINA. The novel approach has been specifically developed to quickly, easily and efficiently determine viral neutralising antibody (VNA) levels. This is crucial because rapidly and easily determining the VNA level against the rabies virus is key to evaluating the protective immunity of humans and dogs, and also for assisting in the strategy of eradication programmes.

Nishizono is Professor in the University's Department of Microbiology and specialises in comprehensive rabies research, encompassing virology, epidemiology, diagnosis, prevention and therapeutics. Using RAPINA, Nishizono and his team are working to improve laboratory-based rabies surveillance in the Philippines.

CRUCIAL SUPPORT

Nishizono's research is funded by: the Japan Agency for Medical Research

and Development; Japan International Cooperation Agency (JICA); Japan Society for the Promotion of Science; and US-Japan Cooperative Medical Sciences Programme. Nishizono explains more: 'The official request for 'Development of an improved laboratory surveillance system for rabies elimination in the Philippines' was submitted to the Japanese Government from the Government of the Philippines for possible JICA assistance under its Science and Technology Research Partnership for Sustainable Development (SATREPS). This promotes international joint-research studies supported by a grant from JICA and the Japan Agency for Medical Research and Development (AMED).'

The research underway is highly collaborative, with involvement from a number of key participants, including: Beatriz Quiambao of the Research Institute for Tropical Medicine, Clinical Research Division in the Philippines; and Mary Elizabeth Miranda of the Field Epidemiology Training Programme Alumni Foundation, Inc. in the Philippines. Nishizono explains why collaboration is so important to the research: 'Collaboration is key because there are no longer rabies cases in humans and animals in Japan, and what strongly promotes our project is intimate collaboration with researchers and health sector personnel in rabies endemic countries.'

A NOVEL ALTERNATIVE

RAPINA is an improved rapid neutralising antibody detection system that the team has developed with a view to providing an alternative to existing VNA determination

assays. The determination of the VNA response following immunisation against rabies is an indicator of how effective the vaccine has been. Existing systems have drawbacks in terms of their speed and cost, in addition to other factors. Nishizono explains more: 'To measure the level of VNA in human and animal serum, functional assays RFFIT or FAVN are internationally acceptable at the laboratory level,' he states. 'However, these assays are time-consuming (it requires about one and half days), expensive (requiring cell culture, fluorescent microscope, FITC-conjugated reagents), require the live rabies virus and can be performed only in restricted reference laboratories with biosafety level three.'

Indeed, the only test for the prediction of VNA is ELISA. Although this is cheaper, safer and easier to perform than the virus-neutralisation test, its reliability depends on the quality of the coating antigen and the specific secondary antibody against the species whose serum samples are to be tested. Furthermore, this test has a high-cost in many developing countries where VNA testing is needed. 'It does not provide a perfect alternative to the virus-neutralisation test,' Nishizono confirms.

PUTTING RAPINA TO THE TEST

Enter RAPINA, a novel, faster, easier and more cost-effective test that can be used as an alternative to VNA determination assays. It works by using immunochromatography combined with virus neutralisation, and uses the inactivated rabies virus as antigen and two types of anti-G monoclonal antibody. 'The advantages of the RAPINA test are low costs for laboratories in terms of both

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running cost (reagents, FITC-conjugates) and fixed costs (expensive equipment including fluorescent microscope, spaces including BSL-3, personnel),’ highlights Nishizono. ‘Secondly, the RAPINA test is less time consuming, taking approximately two hours. Thirdly, the RAPINA test can predict the VNA level in serum from humans or any kind of animal including dogs but not mice, because there is no need to use species-specific secondary antibody.’ What is more, the test can be used in laboratories where a large number of samples are screened.

In contrast, RFFIT/FAVN works by diluting serum samples and mixing them *in vitro* with the rabies virus before BHK-21 cells are added. Following a 24-hour period of incubation, cells are released by trypsin treatment, fixed and permeabilised with a p-formaldehyde solution, and stained with a rabies virus nucleocapsid protein-

specific antibody conjugate. Next, the percentage of virus infection inhibition caused by specific antibodies present in the serum are evaluated in a fluorescent microscope. Using a reference serum, a correlation curve between the IU/ml content and the percentage of infective inhibition is established and the VNA titers of serum samples are obtained by extrapolation.

ENCOURAGING FINDINGS

The team has reported the performance of RAPINA for evaluating the vaccination status of humans and dogs by conducting a multicentre comparison in Asia. The researchers discovered that, compared to the VNA level determined by standard neutralising tests, the positive and negative predicted values and concordance ratio of RAPINA were highly homologous and, importantly, reproducible among different laboratories.

In addition to concluding that RAPINA will be an effective tool for monitoring vaccination coverage in resource-limited countries, the researchers believe RAPINA has the potential to be adapted to determine neutralising antibody level in other infectious diseases. Looking ahead, the researchers intend to continue their focus and explore effective therapeutics against systematic rabies patients. ●



The RAPINA test is both faster and more cost effective than previous rabies test systems

Project Insights

FUNDING

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COLLABORATORS

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Akira Nishizono MD, PhD is Professor of Department of Microbiology, Oita University, Japan. His specialised area is the comprehensive research of rabies, including virology, epidemiology, diagnosis, prevention and therapeutics. His newly launched international collaborative project is to improve laboratory-based rabies surveillance in the Philippines in support of rabies elimination efforts.



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