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Review:

Influenza Project in Myanmar

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The epidemiological study of influenza in Southeast Asia is limited. We surveyed influenza in Myanmar from 2007 to 2013. Nasopharyngeal swabs were collected from patients in the two cities of Yangon and Nay Pyi Taw. Samples were screened using rapid influenza diagnostic kits and identified by virus isolation. Isolates were characterized by cycling-probe-based real-time PCR, drug susceptibility assay, and sequencing. Samples collected numbered 5,173, from which 1,686 influenza viruses were isolated during the seven-year study period. Of these, 187 strains were of seasonal influenza A(H1N1), 274 of influenza A(H1N1)pdm09, 791 of influenza A(H3N2), and 434 of influenza B. Interestingly, two zanamivir and amantadine-resistant strains each were detected in 2007 and 2008. These rare dual-resistant strains had a Q136K mutation in the NA protein and S31N substitution in the M2 protein. Our collaboration raised the influenza surveillance laboratory capacity in Myanmar and led Yangon's National Health Laboratory – one of the nation's leading research institutes – to being designated a National Influenza Center by the World Health Organization.

Keywords: influenza virus, Myanmar, surveillance, epidemiology

1. Collaborative Study Setup

Myanmar is an important transmission route of infectious diseases in Southeast Asia. Until recently, however, information from Myanmar on influenza and other infectious diseases was very limited for political reasons. Niigata University researchers started collaborating with Myanmar in 2000 to provide medicines for tuberculosis and other bacterial infections to a medical doctor who had obtained her Ph.D. at Niigata University during the 1990s through a Ministry of Education, Culture, Sports, Science and Technology, Japan, scholarship. Medical support continued for years and we built a strong relationship before starting an influenza project in Myanmar. Outbreaks of SARS and avian influenza prompted this doctor to start voluntarily collecting influenza samples in Yangon, Myanmar, in 2003 to use in screening for emerging infectious diseases. Influenza virus infections were first screened only using rapid test kits [1], but we started isolating influenza viruses at Niigata University in 2004. Our collaboration was officially recognized through signing of a memorandum of understanding (MOU) between Niigata University and the Ministry of Health of the Union of Myanmar in 2005.

Since then, we have collected influenza samples continuously at medical facilities in Yangon and stored samples at the National Health Laboratory (NHL). The NHL, one of Myanmar's leading research institutions, has staff members skilled in isolating the polio virus under the expanded programme on immunisation (EPI) of the World Health Organization (WHO). The NHL facility was not updated for over a decade, due to economic sanctions. At the request of the Minister of Health under the MOU, we started supporting the NHL in Yangon. We upgraded the influenza laboratory to enable influenza isolation and PCR detection. The laboratory was designated in February 2008 as the WHO National Influenza Center (**Fig. 1**). To promote staff capacity, we have trained 5 Myanmar researchers from the NHL at Niigata University. They have learned how to detect influenza viruses by virus isolation and PCR and have played a central role at their home laboratory after returning to Myanmar.

Sample collection and training of the staff started at Nay Pyi Taw, Myanmar's capital, in 2008, through cooperation with the Department of Medical Research of Central Myanmar (**Fig. 1**). The doctor playing a key role in this effort was also a Ministry of Education, Culture, Sports, Science and Technology, Japan, scholarship Ph.D. student in the 1990s and obtained her Ph.D. at Kumamoto University. Since 2008, our project has established two regular study sites, even though it is very rare in Myanmar for foreign researchers to conduct medical projects at regular study sites. We visited the Mandalay Health Laboratory in 2009 to check laboratory capacity and are currently planning to renovate the laboratories.

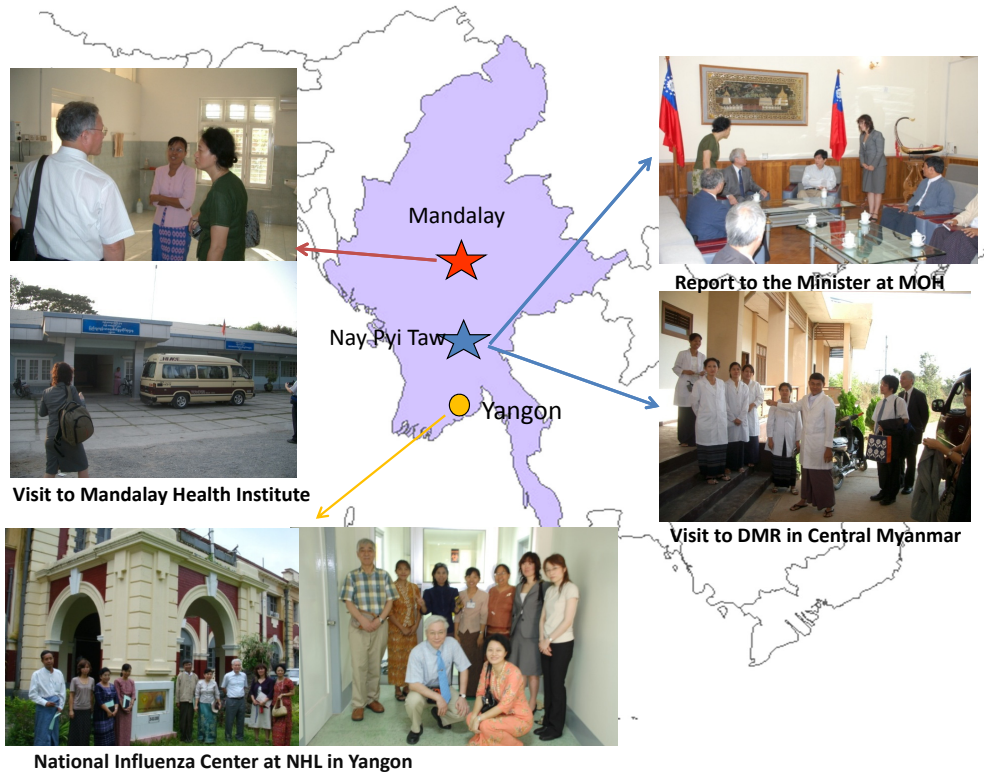

Fig. 1. Influenza research centers and project activities in Myanmar.

2. Influenza Epidemiology in Myanmar

During seven years of research from 2007 to 2013, 5,173 patients with influenza-like symptoms were screened using rapid influenza test kits (Quick-Navi Flu, Denka Seiken Co., Ltd.) at medical facilities in Yangon and Nay Pyi Taw (**Table 1**). Influenza A was found to be positive in 2,206 patients and influenza B in 890. Nasopharyngeal samples were collected from patients whom the kits had indicated positive. Samples were stored at Sanpya Hospital, NHL, in Yangon and at the Department of Medical Research (DMR) in Nay Pyi Taw. Samples were regularly shipped to Niigata University for virus isolation and characterization. The project was approved by ethics committees at each Myanmar study site.

Nasopharyngeal samples were inoculated onto Madin-Darby canine kidney (MDCK) cells and viral cultures monitored 3-7 days for cytopathic effects. Viral RNA was extracted using Extragen II kits. First-strand complementary DNA synthesis was performed using universal primers of influenza A and B [2]. Virus isolates were identified by subtyping real-time PCR assay, called a cycling probe, as reported elsewhere [3].

Of the 1,686 influenza viruses isolated during the study period, 187 were influenza seasonal A/H1N1, 274 A/H1N1pdm09, 791 A/H3N2, and 434 influenza B (**Table 1**). Influenza was detected yearly during the rainy season, i.e., from June to November with peaks in July and August (**Fig. 2**). The influenza circulation period is the same as reported previously for 2005-2007 [2]. Circulating types and subtypes changed yearly, as did predominant types and subtypes. Seasonal A/H1N1 was detected in 2008 and 2009, but replaced by influenza A/H1N1pdm09 in 2010. A/H1N1pdm09 was first detected in 2009 in North America, causing a global pandemic that year in most countries, including Japan [4]. In Myanmar, however, A/H1N1pdm09 started circulating locally one year later, in 2010. Pandemic strains arrived "late" presumably due to limited international movement to the country because of governmental policy. Influenza A/H3N2 was detected almost annually, except when A/H1N1pdm09 and B cocirculated in 2012. Notably, new influenza A/H3N2 variants tended to appear half a year earlier in Myanmar than in Japan. One example was indicated by the early appearance of community-acquired amantadine-resistant A/H3N2 in 2005 [2]. Drug-resistant influenza viruses generally emerge after antiinfluenza drugs such as M2 inhibitor – amantadine – or neuraminidase inhibitors – oseltamivir, peramivir, zanamivir, or laninamivir – have been administered. Amantadine-resistant A/H3N2 strains started circulating, in the community, however, without amantadine having been administered since 2003 [5]. These resistant community-acquired strains were first observed in China around 2000, then spread to Asia in subsequent years. Community-acquired drug-resistant viruses were detected in Japan in 2005-2006. We found the same resistant strains in Myanmar during July-September 2005 – six months ahead of their appearance in Japan. A WHO influenza research group reported that new

Table 1. Number of samples collected, rapid influenza kit positives, and influenza isolates during 2007 and 2013.

	2007	2008	2009	2010	2011	2012	2013	Total
Primary samples	1,250	1,417	533*	694*	444*	521*	314	5,173
Rapid test								
Flu A	124	327	529	474	376	170	206	2,206
Flu B	160	133	0	220	26	351	0	890
Total	284	460	529	694	402	521	206	3,096
Virus isolates								
A(H3N2)	63	134	350	17	165	0	62	791
A(H1N1)	0	66	121	0	0	0	0	187
A(H1N1)pdm09	0	0	16	232	0	25	1	274
B	129	102	0	114	10	79	0	434
Total	192	302	487	363	175	104	63	1,686

*No. of test kit positive samples received.

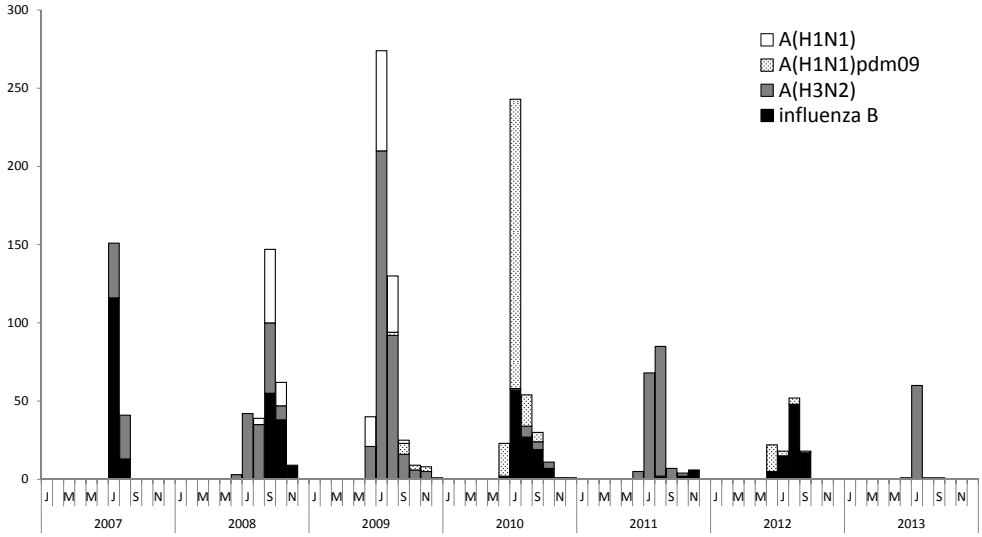

Fig. 2. Monthly distribution of Myanmar Influenza Isolates (2007-2013).

influenza A/H3N2 variants are likely to emerge from China and Southeast Asia before spreading elsewhere [6]. Myanmar thus becomes one of the best sites for observing newly emerging A/H3N2 influenza variants. Our study results indicate that circulating types and subtypes in Yangon and Nay Pyi Taw were similar in a season, suggesting frequent human movement between the two cities (data not shown).

We actively monitored antiviral resistance in Myanmar isolates. Neuraminidase inhibitor (NAI) susceptibility testing was performed using a fluorescent-based assay that measures IC_{50} values of influenza isolates for the 4 commercially available drugs, i.e., oseltamivir (Tamiflu®), zanamivir (Relenza®), peramivir (Rapiacta®), and laninamivir (Inavir®). Although influenza is not commonly treated antivirally in Myanmar, we detected community-acquired NAI-resistant influenza viruses. We found rare strains resistant to zanamivir and amantadine – one each in 2007 and 2008 [7]. Genetic sequencing revealed that resistant strains possessed a Q136K mutation in the NA protein. We then detected seasonal oseltamivir-resistant A/H1N1 strains possessing an H275Y mutation in the NA [8]. In 2009, virtually all seasonal H1N1 – 120 of 121 cases or 99.2% – were resistant to oseltamivir without the drug having been used. It was speculated that the oseltamivir resistant seasonal H1N1 strains were transmitted from other countries to Myanmar. This resistant virus was first observed in Europe in 2007-2008 and then rapidly spread to North America, Asia and the Southern Hemisphere by the middle of 2008 [9]. It is thus important to monitor influenza viruses in Myanmar to determine newly emerged strains and community-acquired drug-resistant strains.

3. Sustainability of Myanmar Influenza Project

During our decade-plus collaboration with Myanmar, we established two influenza study sites that analyzed thousands of influenza viruses. The project's driver has been the enthusiasm of Myanmar doctors and their efforts toward self-sustainability. The Niigata University group also visited Myanmar regularly – roughly twice a year – to discuss project progress and problem solving. We pro-

vided primers for our Myanmar counterparts, for example, to detect H1N1pdm09 in May 2009 soon after WHO declared the start of a global influenza pandemic caused by the strain [4]. Our assistance resulted in entry screening being quickly set up at major airports in Myanmar for international passengers with influenza symptoms.

As indicated earlier, our collaboration dates back to an exchange program for Ph.D. students through a Ministry of Education, Culture, Sports, Science and Technology, Japan, scholarship supported by the Japanese government. We built close relationships with Myanmar doctors studying in Japan. Development of human resources needs time, but in a long term, it is the most effective way to achieve success in overseas projects.

The political situation in Myanmar has changed rapidly after national elections at the end of 2010. Diplomatic mission teams, including those from Japan, visit Myanmar often to strengthen relationships. The sanctions were lifted in 2012 and the economy is now booming. Myanmar is opening up very quickly. In progress in international relations between Myanmar and Niigata University, we became an associate member of the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) in 2012. We also signed a letter of intent (minutes of understanding) with University of Medicine 2 in Yangon in 2013 – one of the top medical universities in the country. Under this agreement, two universities facilitate the exchange of students and researchers and encourage medical projects.

In conclusion, we have set up influenza projects in Myanmar, but the research infrastructure is still on its way to maturing in the development of human resources and facilities. We plan to continue our influenza project to update facilities, to train more researchers in modern techniques overseas, and to bring Myanmar up to the global research level. We plan to expand our study sites northward in Myanmar around Mandalay, the hub of inland commercial traffic. We also plan to monitor more influenza viruses coming from other countries and plan sharing information with other J-GRID projects in analyzing influenza viruses that migrate globally.

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