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Sharing experiences from a reference laboratory in the public health response for Ebola viral disease, MERS-CoV and H7N9 influenza virus investigations

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ABSTRACT

An efficient public health preparedness and response plan for infectious disease management is important in recent times when emerging and exotic diseases that hitherto were not common have surfaced in countries with potential to spread outside borders. Stewardship from a reference laboratory is important to take the lead for the laboratory network, to proactively set up disease surveillance, provide referral diagnostic services, on-going training and mentorship and to ensure coordination of an effective laboratory response. In Malaysia, the Institute for Medical Research has provided the stewardship for the Ministry of Health's laboratory network that comprises of hospital pathology, public health and university laboratories. In this paper we share our experiences in recent infectious disease outbreak investigations as a reference laboratory within the Ministry of Health infectious disease surveillance network.

The International Health Regulations was enforced in June 2007, emphasizing the obligation of State Parties to detect, report, and respond to public health emergencies [1]. An effective public health response requires a coordinated laboratory response in the Ministry of Health (MOH) network with credible laboratories capable of providing accurate and reliable results in a timely manner to enable effective public health measures to contain the spread of the outbreak. Most countries have limited expertise and experience for detecting exotic diseases that may be imported and depend on the World Health Organization (WHO) and network laboratories or the Centers for Disease Control, United States for technical expertise, kits or controls and laboratory confirmation. Although the information and resources exchange during any crisis is available through this network, this process takes time. Time is a critical factor in the public health response for preventing spread, surge and for efficient containment of an outbreak. Stewardship from a reference laboratory is important to take the lead for the laboratory network, to provide referral

diagnostic services, on-going training and mentorship and to ensure coordination of an effective laboratory response. Ebola virus, Middle East respiratory syndrome corona virus (MERS-CoV) and avian influenza A (H7N9) are examples of emerging and reemerging viruses that have gripped the attention of many nations worldwide. It is vital that countries should have the ability to quickly identify potential cases as part of their pandemic preparedness plan. In Malaysia, the Institute for Medical Research (IMR) is the reference laboratory for specialized diagnostics and infectious disease outbreak management. This paper highlights the stewardship role of IMR and its important contribution to the national public health response network.

MERS-CoV was identified in 2012 as the cause of severe acute respiratory syndrome cases in Saudi Arabia. It was found that all cases of MERS were linked to countries in or near the Arabian Peninsula, or had travel history to those countries. On April 16, 2014, MOH Malaysia reported the first case of laboratory-confirmed MERS-CoV infection in a 54 year-old man, who came back from performing pilgrimage in Saudi Arabia. The patient who developed pneumonia with respiratory distress, after returning to Malaysia on March 29, succumbed to his illness three days after admission at a local hospital. Combination of throat and nasal swabs sample taken from the patient was sent to a State Hospital laboratory that has the capability to

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perform real-time reverse-transcription (rRT-PCR) assay for MERS-CoV. The rRT-PCR screening and initial confirmatory assays were performed on April 14 at the Melaka Hospital and both assays were positive for MERS-CoV. Since this is the first case that has ever yielded a positive result in Malaysia, a sample was sent to the IMR, Kuala Lumpur which acts as the National MERS-CoV Confirmation Laboratory, for further investigations. IMR was able to conclusively confirm by DNA sequencing the genes that code for the *nucleocapsid* and RNA-dependent RNA polymerase proteins of the MERS-CoV. Based on partial sequencing of these genes, it was revealed that the strain was closely related to strains from Jeddah. However, following full *nucleocapsid* gene sequencing the Malaysian case actually was 99.9% identical to the sequence derived from the Qatari and Saudi Arabian camels and clustered together with human-derived MERS-CoV sequences isolated from human cases in 2014, among them were those reported in Greece and USA [2]. H7N9 infections in humans were first reported in China in March 2013. In 2014, IMR also successfully confirmed Malaysia's first influenza AH7N9 case outside Greater China (including Hong Kong and Taiwan) on February 12 in a Chinese tourist. The patient was a 67-year-old woman who was previously treated by a general practitioner in China on January 30, four days before travelling to Malaysia with symptoms of fever, cough, fatigue and joint pain. She arrived in Kuala Lumpur on February 4, and travelled to Sabah the following day. On February 7, she was admitted to the intensive care unit at Tuaran District Hospital for acute respiratory distress. She was intubated and upon family request was transferred to a private hospital in Kota Kinabalu. On February 9, the first respiratory sample was tested positive for H7 by IMR and on February 11, H7N9 by DNA sequencing was further confirmed. Sequencing of the *hemagglutinin* and *neuraminidase* genes revealed that this strain was closely related to strains from Guangzhou and Guangdong [3]. Mutations associated with drug resistance were not found in this patient. Patient subsequently recovered and was discharged on March 13, and she returned to China on March 16.

Ebola virus disease (EVD), which first appeared in 1976 in Central Africa, was reported for the first time in West African countries in March 2014. On August 8, WHO declared the outbreak of a Public Health Emergency of International Concern. In August 2014, IMR was given the task by the MOH to be in charge of laboratory diagnosis of EVD for Malaysia [4]. In preparation for a possible surge, IMR started active surveillance for any importation of EVD suspect cases and trained the National Public Health Laboratory staff in management of samples from suspected EVD cases and in laboratory diagnosis of EVD. Also, as part of preparedness in dealing with the possibility of EVD outbreak in Malaysia, IMR organised training for 9 hospital laboratories in working in the biosafety level2 with the biosafety level3 (BSL3) practices. The first sample for EVD investigation arrived in IMR on September 3. The sample was inactivated in BSL3 laboratory following that the Ebola virus rRT-PCR test was performed and sample was found to be negative for Ebola virus. Since then, 11 more cases have been found negative by both IMR and National Public Health Laboratory.

The timely laboratory detection for the MOH has ensured that the above infectious disease outbreaks were effectively contained to a single case. The quick response and ability to

accurately identify the virus and also to confirm a negative case, was due to the initiatives of the reference laboratory in the Virology Unit, IMR which had as early as 2011 proactively set up laboratory tests for exotic disease surveillance and investigation. As part of the preparedness planning, the unit had taken steps to upgrade their molecular testing technologies with various rRT-PCR platforms for different exotic viruses to ensure high throughput and rapid turn-around time. The laboratory has the capability to detect rabies, Ebola, Marburg, Rift Valley fever, St Louis encephalitis, West Nile, Crimean Congo haemorrhagic fever, Lassa, Yellow fever, Hanta viruses and also Influenza A from H1 to H15. The primers and probes database for these viruses and other emerging viruses are regularly updated and a collection of synthetic positive controls for use in rRT-PCR were also prepared. Staff are regularly trained with blinded and coded controls to increase their competency. The Unit has also put up an on-call list for Malaysian healthcare practitioners to contact IMR for any urgent laboratory investigations for such cases.

As part of the national preparedness plan and in an effort to cope with the surge of laboratory cases during an active outbreak, influenza type A, influenza type B and MERS-CoV tests were decentralised to all hospitals and public health laboratories with PCR facilities. For Ebola, testing was decentralised to laboratories with BSL3 facilities only. As an early preparation for decentralization of the tests to these laboratories, hands-on training and workshops were conducted to build the capacity of their laboratory staff on the diagnosis of these viruses. In order to ensure the quality of tests performed, the IMR has initiated a quality assurance programme known as the National External Quality Assessment Scheme for Molecular Detection of Influenza Viruses and MERS-CoV; and the programme is carried out twice a year. The objective of the programme is to assess test performance of the participating laboratories in detecting Influenza A & B Viruses and MERS-CoV using molecular technique.

The WHO Western Pacific Region Office together with the South East Asia Office developed an Asia Pacific Strategy for Emerging Diseases (APSED) – a strategic framework for countries to build the capacity required under the International Health Regulations 2005 [5]. The MOH, Malaysia embraced the vision of APSED and drafted Malaysia's own strategic document and action plan [6]. Rapid laboratory confirmation of the aetiological agent is important to implement public health measures to limit community spread by permitting the timely isolation of cases and contact tracing. A stewardship role for a reference laboratory is important to make the APSED plan workable.

Conflict of interest statement

We declare that we have no conflict of interest.

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