Molecular typing of human rhinovirus associated with acute respiratory tract infections in hospitalized children in Sarawak, Borneo, from 2012 to 2015

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**Purpose**

An estimated 120 to 156 million cases of acute lower respiratory tract infections occur globally with mortality rate of 1.4 millions per year.\textsuperscript{1,2} Human rhinovirus (HRV) was first isolated in 1956 and since then, it has been known to associate with numerous respiratory illnesses. HRV is a highly ubiquitous enterovirus that transmits through contact surfaces or aerosol and mainly infects the tracheobronchial tree. Several studies have shown that HRV is strongly associated with at least half of the acute exacerbations of asthma and chronic obstructive pulmonary disease cases in adults and likely to be substantially higher among children.\textsuperscript{3,4} It infects upper and lower respiratory tracts which leads to diseases including rhinosinusitis, otitis media, and even pneumonia and bronchiolitis. Due to its biology and antigenic diversity, no vaccine or antiviral therapies are currently available for HRV. The virus includes three species (A, B, and C) with over 160 serotypes. This study aims to characterize the circulating HRV types among hospitalized pediatrics in Sarawak to better understand their epidemiology.
Methods and Materials

1. Sample collection

Respiratory samples were collected from children hospitalized with ARTI symptoms between August 2012 to October 2015 from three major public hospitals in Sarawak.

<table>
<thead>
<tr>
<th>Location:</th>
<th>3 Sarawak hospitals</th>
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<tr>
<td>Inclusion criteria:</td>
<td>Symptoms indicative of acute lower respiratory tract infection</td>
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</tbody>
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Fig. 1: Hospital locations, period of study and criteria for ARTI sampling.

References: Institute of Health and Community Medicine, Universiti Malaysia Sarawak (UNIMAS) - Kota Samarahan/MY

2. Cell culture and RNA extraction

Positive samples that were positively identified via a modified multiplex PCR assay were cultured in human rhabdomyosarcoma cell line. Positive isolates were then extracted with High Pure Viral Nucleic Acid kit (Roche).

3. Partial VP4/VP2 and complete VP1 RT-PCR

A published nested PCR assay was modified to target and sequence the partial VP4/VP2-coding region of HRV. An overlapping PCR was designed in-house with degenerate primers targeting VP1. The amplicons were sequenced to confirm the virus identity.
**Fig. 2:** Schematic diagram of the HRV-A genome and regions amplified for molecular typing.

**References:** Institute of Health and Community Medicine, Universiti Malaysia Sarawak (UNIMAS) - Kota Samarahan/MY

4. Sanger sequencing and phylogenetic analysis

Contigs were formed from raw sequences using SeqMan Pro and edited on BioEdit ver.7.2.5. The sequences were then aligned using MAFFT ver.7 and subsequently used for reconstructing phylogenetic trees and to examine the genetic relatedness among Sarawak strains with others using MEGA ver.7.8
**Location:**
3 Sarawak hospitals

**Period of study:**
Aug 2012 – Oct 2015

**Inclusion criteria:**
Symptoms indicative of acute lower respiratory tract infection

**Fig. 1:** Hospital locations, period of study and criteria for ARTI sampling.

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**Fig. 2:** Schematic diagram of the HRV-A genome and regions amplified for molecular typing.

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Results

A total of 191 individuals were previously tested positive for HRV in multiplex PCR. Samples from these clinical episodes were grown in RD cell lines and yielded 50 isolates positive for HRV-A and HRV-B only. RT-PCR targeting both partial VP4/VP2 and VP1 was performed and positive samples were sequenced. Additional sequences were obtained by direct sequencing on clinical materials including those positive for HRV-C. A total of 180 HRV partial VP4/VP2 sequences were successfully obtained which include sequences from 91 HRV-A, 5 HRV-B, and 84 HRV-C. About 74% of these cases were associated with pneumonia followed by 13% of cases with bronchial asthma. Only Sarawak strains representative of the identified HRV types were shown in Figure 3. Additionally, 118 VP1 sequences were obtained for type confirmation on most of these isolated strains (See Figure 3).

Phylogenetic analysis of these VP4/VP2 sequences revealed a diverse range of molecular types in each HRV species (44 HRV-A, 4 HRV-B types, 41 HRV-C types) as seen in Figure 4. These include two Sarawak isolates (A110, C58) which were genetically divergent from any currently known molecular type and thus, were identified as provisionally new types.
Fig. 3: Neighbor-joining trees of partial VP4/VP2 and complete VP1 sequences from Sarawak samples representing each prototype.

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**Fig. 4:** Distribution of HRV types by month from August 2012 to October 2015. Molecular types of each species are indicated below the axis. Red fonts indicate provisionally assigned new types.

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Conclusion

1. 44 HRV-A, 4 HRV-B, and 41 HRV-C types were identified through VP4/VP2 sequencing and verified by full VP1 sequences.
2. All detected strains are highly divergent from prototypes assigned for each type.
3. HRV-A and HRV-C were equally prevalent among hospitalized children.
4. Two provisionally new types were identified and proposed as HRV-A110 and HRV-C58.

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