

newly introduced RV vaccines, RotaTeq® which contains G1, G2, G3, G4, and P1A viruses (Merck) and Rotarix™ which contains a G1P1A[8] virus (GlaxoSmithKline).

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#### 16.017

##### Study on the Circulation of HEV71 on Selected Communities in Sarawak

Y. Podin\*, D. Perera, K. Aslan Joe, M.J. Cardoso

*Institute of Health and Community Medicine, UNIMAS, Kota Samarahan, Sarawak, Malaysia*

**Background:** Human enterovirus 71 (HEV71) is a common aetiology of hand-foot-and-mouth-disease (HFMD) where it occasionally causes CNS illnesses. In 1997, Sarawak experienced a HEV71-associated HFMD outbreak with 34 deaths reported. Following the outbreak, a prospective HFMD surveillance study showed that HEV71-associated HFMD outbreaks occurred in Sarawak every three years, each caused predominantly by different genogroups of HEV71; genogroup-B3 in 1997, genogroup-B4 in 2000, and genogroup-B5 in 2003. This study attempts to investigate the circulation of HEV71 in between the three-year cycle outbreaks before an expected outbreak in 2006 in Sarawak.

**Methods:** Three communities, Villages-A, B and C, were selected based on their locations with reference to the Rajang river, the epicenter of the 1997 outbreak. Stool samples were collected through 2 visits; the first collection in mid-2005 and the second in February 2006. Filter-sterilized samples were inoculated into rhabdomyosarcoma and QB1-293A cells. Virus cultures were observed for cytopathic effect (CPE) daily for 14-days. Upon blind-passage, cultures without CPE were considered negative. Reverse-transcription-polymerase-chain-reaction (RT-PCR) on positive cultures was done using primers for partial VP1 previously described by Oberste *et al.* RT-PCR positive products were subjected to DNA sequencing and molecular analysis.

**Results:** During the first collection, 106 samples were collected from Village-A, 428 from Village-B and 183 from Village-C. The second collection yielded 88 samples from Village-A, 410 from Village-B and 177 from Village-C. While, no virus was isolated from the first collection of Village-A, 4% of samples from Village-B and 6% of samples from Village-C yielded enteroviruses respectively. From the second collection, 8% of samples from Village-A, 6.8% from Village-B and 4.5% from Village-C, yielded enteroviruses. The viruses isolated were identified mostly as echovirus-6 (E6), E7, E19, coxsackievirus-A (CA) 20, CA21, CA24 and coxsackievirus-B (CB) 4.

**Conclusion:** HEV71 was not circulating in sampled villages but enteroviruses species B and C were.

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#### 16.018

##### HCV Genotypes in Haemodialysis Units: A Preliminary Study

H.H. Aini<sup>1,\*</sup>, M.I.A. Mustafa<sup>1</sup>, M.R. Seman<sup>2</sup>, B.A. Nasuruddin<sup>1</sup>

<sup>1</sup> *Kulliyah of Medicine, International Islamic University, Kuantan, Pahang, Malaysia*

<sup>2</sup> *Nephrology Unit, Tengku Ampuan Afzan Hospital, Kuantan, Pahang, Malaysia*

**Introduction:** Serological verification among haemodialysis patients in Kuantan, Pahang, an East Coast town in Peninsular Malaysia is routinely updated for every 3 months by Ministry of Health. However, the determination of the HCV genotypes for each infected patient has not yet been attempted. This study aims at the determination of HCV genotypes among infected haemodialysis patients.

**Methodology:** 22 seropositive patients out of 208 (10.5%) from 4 haemodialysis units (HDUs) were enrolled in this study during the period from March to August 2007. Detection of HCV RNA from serum was done by using RT-PCR technique targeting 212 bp of the 5'UTR region. Meanwhile, the base sequences of the above regions were deduced using the same primers as for the RT-PCR. The nucleotide sequences data were analyzed with Bioedit software and classification of the genotypes was done using neighbor-joining method together with known sequences obtained from NCBI homepage.

**Result:** Out of 21 samples (male=57%), 2 are negatives. The 19 positive HCV-RNA samples were subjected for sequencing analysis. The analysis showed that 63.2% (12/19) were of genotype 3, 26.3% (5/19) of genotype 1 followed by 5.3% (1/19) of genotype 4 and 1 isolate (5.3%) is undetermined. Furthermore, the genotype distribution showed discrete clustering among units. Sequence comparisons revealed that 4 strains from one HDU have a G insertion (-208) which is not detected in other strains including ones that were retrieved from NCBI sequence database.

**Conclusion:** Genotype 3 is the most prevalent genotype found among haemodialysis patients in Kuantan which is in concordance with previously published HCV genotype prevalence in Malaysia. Furthermore, genotype clustering suggests a nosocomial and blood transfusion infection which requires further subtyping test. Meanwhile, nucleotide sequence analysis suggested that HCV adaptation to certain environment may lead to mutations in the well conserved region (5'UTR).

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#### 16.019

##### Molecular Characterization of Rotavirus A Associated with Outbreaks of Acute Gastroenteritis in Sarawak in 2001 and 2007

M.S. Sum\*, D. Perera, N. Ramji, F. Elie, M.J. Cardoso

*Institute of Health and Community Medicine, Universiti Malaysia Sarawak, Kota Samarahan, Sarawak, Malaysia*

**Background:** An unusual increase in the burden of gastroenteritis cases was noted in Serian, in the south of Sarawak in 2001. A similar occurrence was also observed in

Miri, in the north of Sarawak in 2007. Stool samples collected from these two isolated outbreaks were tested for rotavirus, which is a primary etiological agent of gastroenteritis. This study aimed to characterize the G (VP7) and P (VP4) genotypes of group A human rotavirus circulating in these two outbreaks. The G and P types of the rotavirus-positive faecal specimens were determined by reverse-transcription PCR and sequencing.

**Methods:** Faecal specimens were resuspended in PBS and were subjected to nucleic acid extraction by using the Chemagic Viral Extraction Kit (Chemagen, Germany). Rotavirus was detected by RT-PCR using published primers specific for VP4 and VP7. Positive samples were subjected to DNA sequencing and partial sequences of the G and P gene were analysed using Lasergene (DNASTAR, Madison WI, USA) software and phylogenetic tree analysis using a neighbour-joining method.

**Results:** A total of 272 samples were tested for rotavirus and of these 75 (72%) and 68 (40%) in Serian and Miri respectively, were positive for group A human rotavirus. A total of 105 sequences were obtained, and five different G-P combinations were identified. In both outbreaks, G1[P8] were the most common strains found which constituted of 87.6% of all the rotavirus typed. Other genotypes include the emergent strains G9[P8] (7.6%), G2[P4] (3.8%) and a rare G3[P9] (1%). Out of the 5 combinations only G1[P8] and G9[P8] strains were identified in the 2001 outbreak in Serian while G1[P8], G2[P4] and G3[P9] were found circulating in the 2007 outbreak in Miri.

**Conclusion:** Given variations observed in circulating rotavirus strains, continued monitoring of genotypes is essential to identify emerging strains, track common strains and evaluate impact of rotavirus vaccines.

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16.020

#### Phylogenetic Analysis of the VP1 Gene of Human Echovirus 30 Isolates from Sarawak Reveals An Emerging Genotype

D. Perera<sup>1,\*</sup>, M.H. Ooi<sup>1</sup>, Y. Podin<sup>1</sup>, S. Shah Jahan<sup>2</sup>, R.S.D. Sedi<sup>2</sup>, M.J. Cardosa<sup>1</sup>

<sup>1</sup>Institute of Health, University Malaysia Sarawak, Malaysia

<sup>2</sup>Institute of Health & Community Medicine, Universiti Malaysia Sarawak, Malaysia

**Background:** Human enteroviruses are the leading cause of aseptic meningitis (ASM) worldwide. Human echovirus 30 (E30), together with E9 and E11, are the enteroviruses most frequently implicated in community outbreaks of ASM. In Sarawak, in 2001 and 2004, we have isolated E30 from a number of patients during a prospective study of central nervous system infection.

**Methods:** The complete nucleotide sequences of the VP1 gene for 23 E30 isolates from Sarawak were determined by RT-PCR and sequencing using published and in-house designed primer sets. The relationship between the 23 Sarawakian E30 VP1 sequences and an additional 470 E30 VP1 sequences obtained from the GenBank database were investigated by phylogenetic analysis using a maximum likelihood approach.

**Results:** The phylogenetic analysis of all 493 E30 VP1 sequences revealed 3 separate genotype groups which included two previously published genotypes (I and II) and a previously undescribed genotype that we have designated as genotype III. The Sarawak strains belonged to two separate genotypes (II and III). Strains isolated in 2001 belonged to genotype II whereas strains isolated in 2004 were from genotypes II and III. A global comparison of E30 VP1 sequences revealed that multiple lineages (within or in separate genotype groups) of E30 strains may circulate at any one time. This is in contrast to previously published reports that a single virus lineage appear to circulate at a time and that temporal displacement of the lineage occurs with time. Our results also showed that E30 strains can be divided geographically into 'continental' and 'cosmopolitan' types.

**Conclusions:** Increased capacity for virus diagnostics has improved our appreciation for the disease burden of E30. This has led to the description of an emerging genotype (III) and a better understanding of the molecular epidemiology of E30.

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16.021

#### Surveillance of Acute Hepatitis B in Taiwan

C.C. Liu<sup>1</sup>, W.J. Su<sup>1</sup>, T.M. Huang<sup>1</sup>, Y.H. Ni<sup>2</sup>, C.H. Chiu<sup>1</sup>, D.P. Liu<sup>1</sup>, B.H. Wu<sup>1</sup>, H.C. Lin<sup>1,\*</sup>

<sup>1</sup>Centers for Disease Control, Taiwan, Taipei, Taiwan

<sup>2</sup>Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan

**Backgrounds and Purpose:** In Taiwan, hepatitis B virus (HBV) infection is the leading cause of acute viral hepatitis. An effective universal HBV vaccination program was launched since 1984. We assessed the impact of vaccination on acute hepatitis B in terms of incidence and risk factors.

**Methods:** Cases definition of acute hepatitis B meets the clinical and laboratory criteria. HBsAg existed less than 6 months and IgM-anti-HBc positive are necessary. From 1994 to 2007, reported cases to Taiwan CDC via the National Notifiable Diseases Surveillance System were enrolled. The incidence of birth cohorts who were born between 1974 and 1993 (10 years after or before vaccine introduction) was further analyzed. Risk factors were recorded.

**Results:** Totally, 4255 patients (gender: 1326F/2929M; birth years: 1902–2007) were diagnosed as acute hepatitis B, representing 52% of all acute viral hepatitis patients in 14 years. The median age of onset was 31 years (range, 0–94 years), and the overall incidence was 1.43/100,000 (range, 1.01–1.88/100,000). For people's birth years between 1974 and 1983 ( $n=1388$ ), the incidence of acute hepatitis B was 34.59/100,000 (range, 9.65–44.46/100,000). It decreased to 4.06/100,000 (range, 0.62–12.13/100,000) for those birth years between 1984 and 1993 ( $n=131$ ) and their HBV vaccine coverage was 86.19% (range, 75.70–92.05%). Possible risk factors were investigated for cases reported during 2006 and 2007. The occurrence of acute hepatitis B may be related to people who had recent surgery (22.9%), lived with family members of HBV carriers (12.5%), or had HCV infection (7.6%). Sharing razors or toothbrushes (6.9%), acupuncture