

Phenotypic and Genotypic Characterization of Two Mouse Adapted Enterovirus 71 Strains that Showed Differences in Murine CNS Infection

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Background: Generally, most enterovirus 71 (EV71) strains isolated from human clinical cases are unable to cause paralysis in suckling mice (> 6 days old) by oral or parenteral routes of inoculation. After 5 serial passages, an EV71 strain was able to infect 2-week-old mice and seems to largely reproduce the pathology of human EV71 encephalomyelitis. Two EV71 variants (P5A1 and P5D1) purified from the original adapted strain were further studied for differences in phenotype and genotype as the P5A1 variant showed attenuated virulence while the P5D1 variant retained virulence in 2-week-old mice.

Design and Methods: We analyzed viral phenotypic markers (plaque morphology and temperature sensitivity) of the viral strains. The plaque diameter was determined on virus-infected Vero cell monolayers after 7 days of incubation at 36 °C under a 0.5% agar overlay. The temperature sensitivity of viruses was evaluated by determining the viral titers in Vero cells at 36 °C and 39 °C. To map out genotypic differences, if any, whole viral genomes were sequenced and compared. Briefly, viral genomic RNA was extracted from the supernatant of infected Vero cells using viral RNA kit and RT-PCR was performed by using Titan One-Tube RT-PCR system. Direct sequencing was carried out on the full-length genomic sequence, using DNA fragments amplified by RT-PCR. The sequence of the 5' and 3' end of viral genome was determined using 5' and 3' RACE methods.

Results: Both EV71 strains P5D1 and P5A1 exhibited similar plaques diameter. However, EV71-P5A1 was temperature sensitive (ts) at 39 °C. Nucleotide sequence comparison revealed seven nucleotide changes, including 1 each on 5'NTR, VP2, VP3, VP4, 2A, 3A and 3'NTR regions, resulting in only 1 amino acid change at position 2A-906 (serine \diamond phenylalanine).

Conclusion: It is possible that these phenotypic/genotypic differences contribute to the differences in virulence of these variants. To confirm the molecular determinants of neuro- and myotropism, and/or the ts phenotype in greater detail, cDNA-derived infectious clones of our mouse adapted EV71 strains could be useful.

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Incidence of Respiratory Illness and Associated Expenditures of Households Among Children Under Five in Urban Dhaka, Bangladesh

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Background: Respiratory infection is a primary cause of childhood mortality and morbidity in Bangladesh. The

episode among children under age 5 years in the catchment population of two pediatric hospitals of urban Dhaka, Bangladesh.

Methods: The study team identified 70 hospitalized children with a diagnosis of pneumonia from two large pediatric hospitals of Dhaka, who lived within 60 minutes travel distance. For each child, field workers enrolled 100 neighbors who had children under-5 years. Interviewers collected data from primary caregiver of children on specific illness symptoms and health care expenditures. Illness episodes with either cough or difficulty breathing as the primary symptom or fever as the primary symptom with either cough or difficulty breathing present were classified as respiratory illness.

Results: The study team collected disease information of 7992 children under-5 years from 6970 households. The incidence of respiratory illness treated by practitioners as outpatient case was 2599 episodes per 1000 child-year-observed and hospitalization for respiratory illness was 27 episodes per 1000 child-year-observed. Outpatient episodes averaged 9 days, and cost an average US\$3.7. Hospitalized episodes averaged 20 days with average direct expenditure of US\$98. For each 1000 children under-5 years, this urban population had an estimated out-of-pocket expenditure of US\$9616 for outpatient episodes and US\$2667 for hospitalized episodes per year.

Conclusion: The out-of-pocket expenditure per hospitalized episode was about twice the monthly income of the poorest 16% of households (US\$58 or less), which might lead these families to further impoverishment. Vaccines that prevent childhood pneumonia, including HiB and pneumococcal vaccine, would benefit these communities, especially the poorest group. Alternative health financing strategies might be of use to protect this vulnerable group.

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Disease Morbidity and Cost Analysis Associated with Laboratory-Confirmed Influenza Among Children <15 Years in South Korea, 2004–2007

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Background: We analyzed medical chart records of inpatients and outpatients as well as costs incurred during hospitalizations to describe the impact of influenza among Korean children < 15 years of age.

Methods: From March 2004 – June 2007, we identified 1,370 laboratory-confirmed influenza episodes at five tertiary care hospitals in South Korea. Demographic, epidemiological and medical information were analyzed to describe the distribution of patients by age, season, virus type, and length of stay. We analyzed hospitalization costs associated with testing, treatment and admission for a subset of 926 patients from three hospitals.