

### 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.

**Results:** During the 40-month study period, we identified 1,370 children with influenza including 966 (71%) inpatients. Among 1,368 patients with birth information, 490 (36%) were <24 months and 1,048 (77%) were <60 months of age. Influenza A ( $n=811$ ) peaked during December-January and March-May and influenza B ( $n=407$ ) showed a peak only during March-May. Among inpatients, the mean LOS was 4 days. Seventy-one percent ( $n=681$ ) had a LOS of 1–5 days and 25% ( $n=238$ ) had LOS of 6–10 days. Among the episodes of LOS > 10 days, there were twice as many episodes in children aged < 24 months ( $n=30$ ) compared with those in the rest of the age groups of patients combined ( $n=15$ ). The mean total direct cost associated with laboratory-confirmed influenza was \$1,205. Stratified by LOS groups, the mean total direct cost for LOS of 1–5 days was \$874 compared to that of \$1415 for LOS of 6–10 days. The mean direct costs for treatment, testing, and admission were \$319, \$383, and \$441, respectively.

**Conclusions:** More than three-quarters of pediatric influenza occurred within the first five years of life. The economic burden of this disease in Korea suggests the need to consider inclusion of infants and toddler in annual routine influenza immunization program.

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#### ***Chlamydia pn.* and *Mycoplasma pn.* Infections in Exacerbation of Asthma in Children**

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In addition to viral infections exacerbation of asthma in children may be resulted from atypical bacterial infections. The aim of our study was to investigate the relationship between *Chlamydia pn.* and *Mycoplasma pn.* infections and asthma exacerbation. The study included 67 children aged 6–18 years with asymptomatic asthma ( $n=24$ ) and asthma exacerbation ( $n=43$ ). The presence of infections was analysed by PCR with specific primers of DNA sequence for both pathogens in the induced sputum. *Chlamydia pn.* infections were found in 14 cases out of 67 and *Mycoplasma pn.* in 4 cases out of 67 children. It is interested, that children with severe asthma according to the GINA 2002 criteria exhibited *Chlamydia pn.* infection in 66%, and *Mycoplasma pn.* in 33% of cases. It can be concluded that atypical bacterial infection may play significant role in severe asthma exacerbation in children.

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#### **Incidence and Etiology of Umbilical Infections in Newborns in Low-income Communities of Karachi, Pakistan**

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**Background:** Bacteria colonizing or infecting the umbilical stump of newborn can readily access the systemic circulation and predispose the infant to sepsis.

**Objective:** To determine the incidence and etiology of umbilical infections (omphalitis) in 3 low-income communities of Karachi over a 3 year period.

**Methods:** Prospective study of omphalitis in young infants (0–59 days) in communities with established newborn surveillance systems (birth cohort 6904) was conducted. Almost 80% of babies in these areas are delivered at home by unskilled birth attendants. Babies were referred to Primary Health centers by trained community health workers if pre-defined signs were present. Infections were categorized as mild (redness around umbilical stump of <2 cm, with no pus) moderate (redness around umbilical stump of 2 cm around umbilical stump with or without purulent discharge).

**Results:** A total of 1501 babies were diagnosed with omphalitis. The incidence of omphalitis was 217/1000 live births. Among babies with omphalitis 24% had mild infection, 68% moderate, and 7.8% severe infections. Common pathogens isolated from 675 cases with purulent discharge sent for culture were *Staphylococcus aureus* (304), *Beta hemolytic streptococci* 179 (Group A: 104, group B: 61, others: 14), and *Pseudomonas spp* (52); 20.8% were polymicrobial. Of 1501 babies, 141 had concurrent signs of sepsis (9.3%). Overall, 6.2% of *S. aureus* isolated were methicillin-resistant. **Conclusions:** Umbilical infections are common problems in home-delivered babies and may predispose newborns to sepsis. Most are due to *S. aureus* and beta-hemolytic streptococci. Prevention, early diagnosis and guidelines for appropriate therapy in community settings are imperative.

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#### **Surveillance of Causes of Sepsis in Young African Infants**

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**Background and Aims:** Bacterial infections are a leading cause of morbidity and mortality in young infants in developing countries. At Chris Hani Baragwanath Hospital (CHBH), Soweto, intrapartum antibiotics are administered to women whose infants are considered to be at high risk for sepsis. Surveillance of significant pathogens in septic young infants may assist in improving management programs of pregnant women and their infants.