Virological, Clinical Characteristics and Viral Shedding of Children with Norovirus Gastroenteritis

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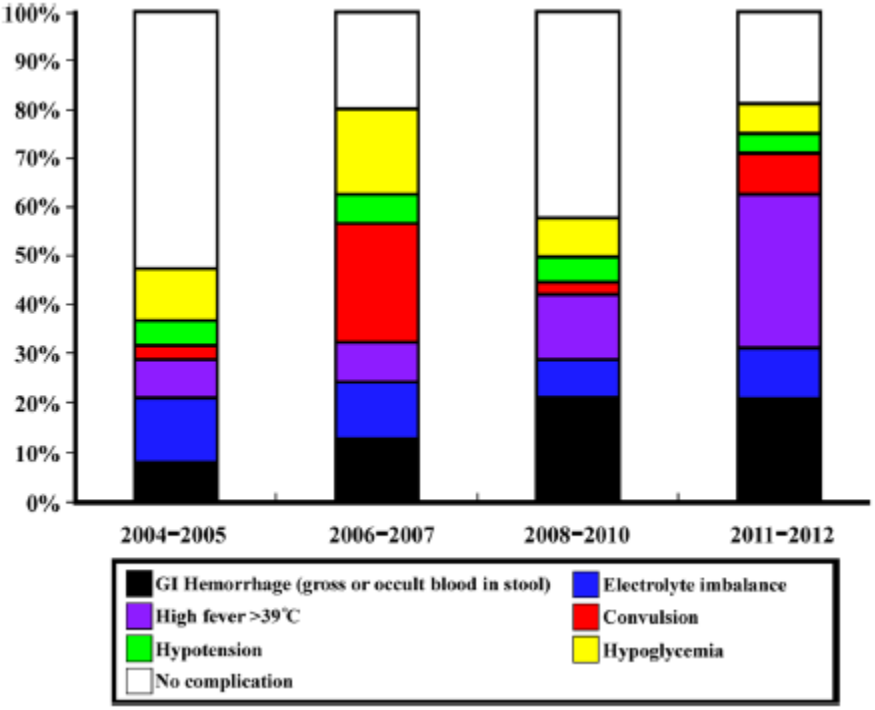
Background

- Norovirus (NoV) is an emerging enteric pathogen and being recognized as a global health burden as leading viral cause of outbreaks of gastroenteritis worldwide.
- The rapid transmission of NoV via person-to-person c ation o s C MC P uno- c Virol 2



Background

- Previous study indicated that NoV shedding is influenced by multiple factors which contain the age and viral copy numbers. *Kuribayashi K, Hosono Y, et al. Jpn J Infect Dis 2011; 64(2): 104-8.*
- Therefore, these patients should be managed carefully to prevent spread of the disease.

Background

- Real-time RT-PCR is an effective tool for the identification and monitoring of NoV transmission, which as a proxy measure of fecal viral load using cycle of threshold cycles (*CT* value) which may distinguish between asymptomatic viral shedding from clinically relevant disease. ***Gallimore CI, Cubitt D, du Plessis N J Clin Microbiol 2004; 42(5): 2271-4 .***

  but it cannot clearly to determine the correlated the viral load and viral shedding.
- Our study aimed to **standardize detection system for NoV infection** to investigate the clinical features, disease significances and NoV viral load with viral shedding.

Materials and Methods

- Fecal specimens from recruited children in **Chang Gung Children's Hospital** under diagnosis of norovirus gastroenteritis examined by RT-PCR and their clinical features of hospitalization were characterized.
- These patients did **not have underlying disease of immunity and chronic disease** and their clinical features of hospitalization were analyzed.

Materials and Methods

- Norovirus real time RT-PCR assay with viral copy numbers ((log)/g feces) calculation as viral load were performed.
- The primer sequence based on an adaption of a previously described, were modified through the use of VP2 gene to enhance amplification s
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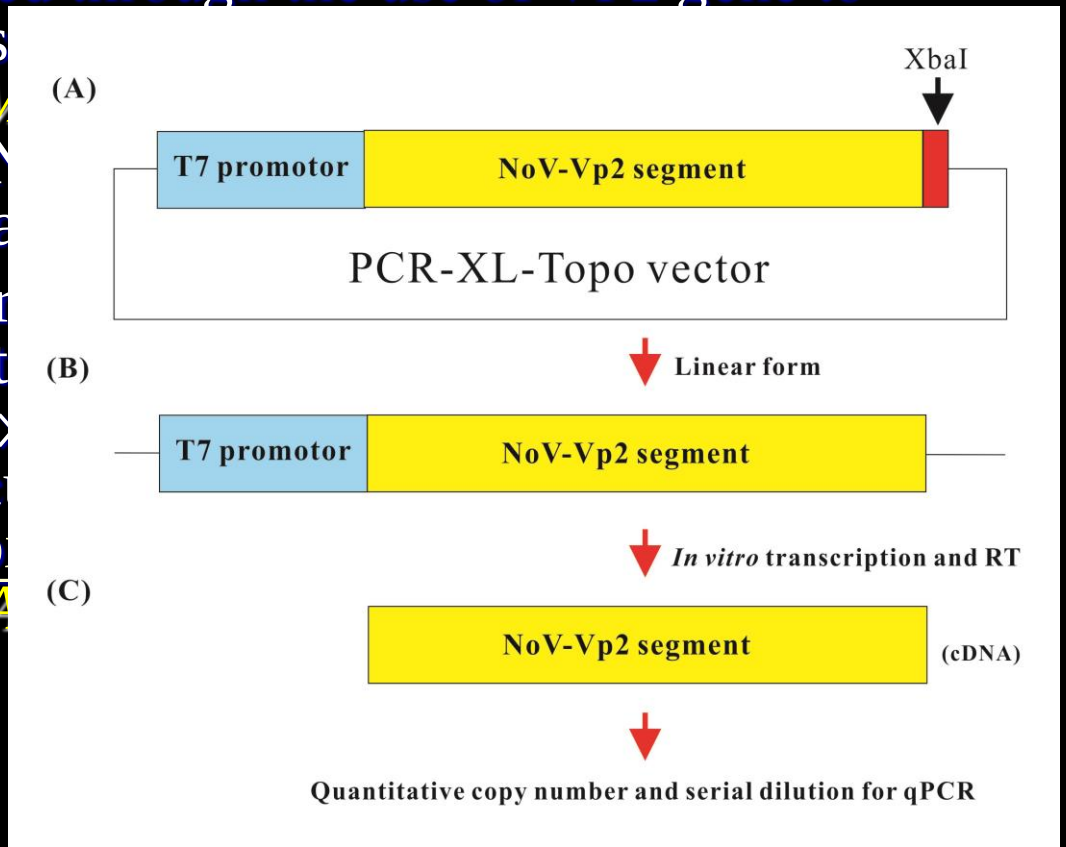

Materials and Methods

- The standard curve using 10-fold serial dilution of pVP2 plasmid. After determining the dilution range for each synthetic curve
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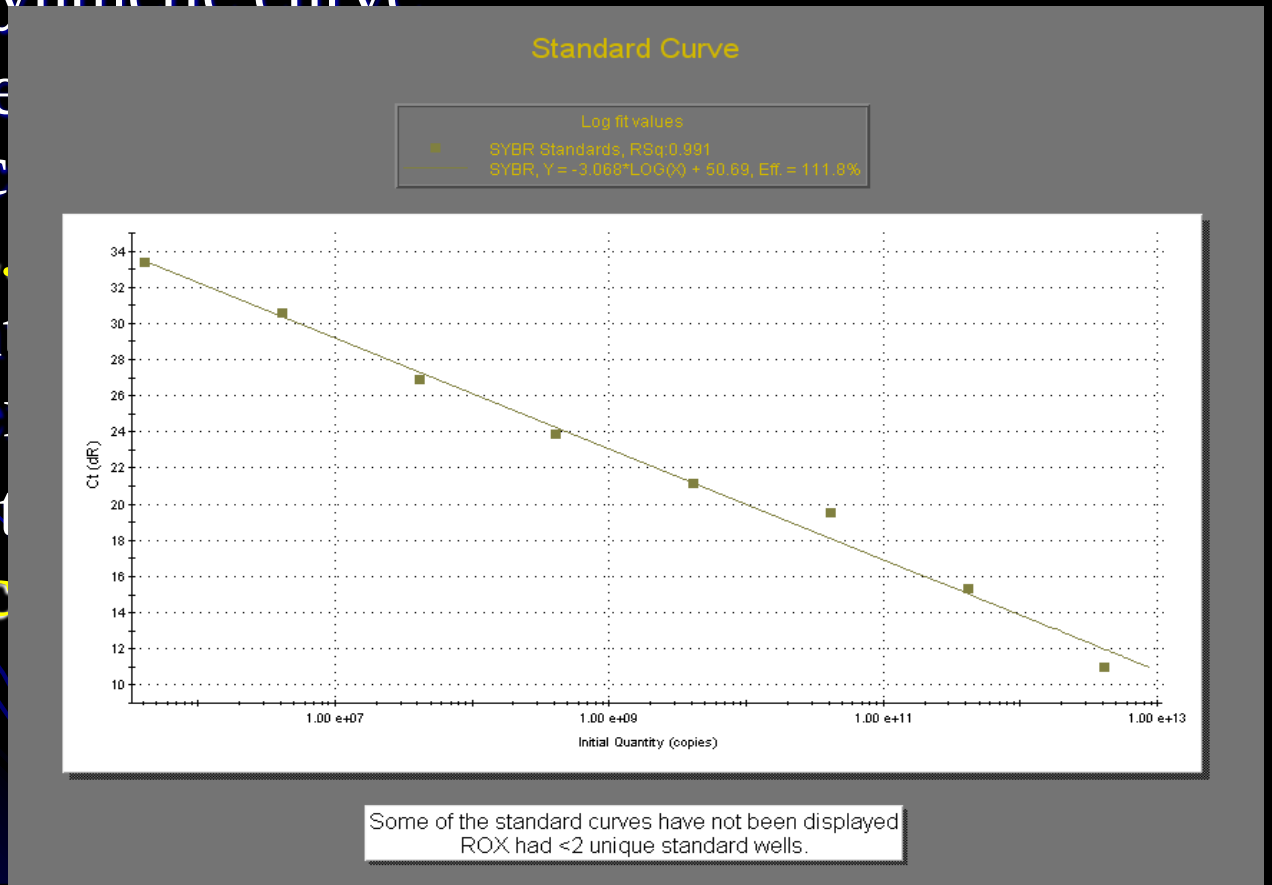


Materials and Methods

- The sequences of different PCR products from the same NoV positive specimen were used to reconstruct the near-full-length norovirus genome using the Vector NTi software package (Invitrogen) and further compared to reference sequences obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov) for NoV strain identification.

- Fisher exact test was used to determine differences between clinical features and based on infectious timing variances.
- Statistical significance was analyzed using a nonparametric Mann-Whitney U test for two independent samples series.

Results (1)

- A total of 44 fecal samples from NoV infected hospitalized children were collected for analysis viral load based on copy number quantification methods.
- The samples collection date varied from **day1 to day 19** from their disease onset. The results demonstrated 38 samples could be calculated for their viral load and of them, **the rising of viral load began with the third day from illness** (Figure 2).
- The viral load increasing varied from the 3rd day to the 8th day forming an unsmooth plateau feature without peaking. **After the 8 th day, the viral load declined and sheded at the 15 th day after illness onset averagely.**

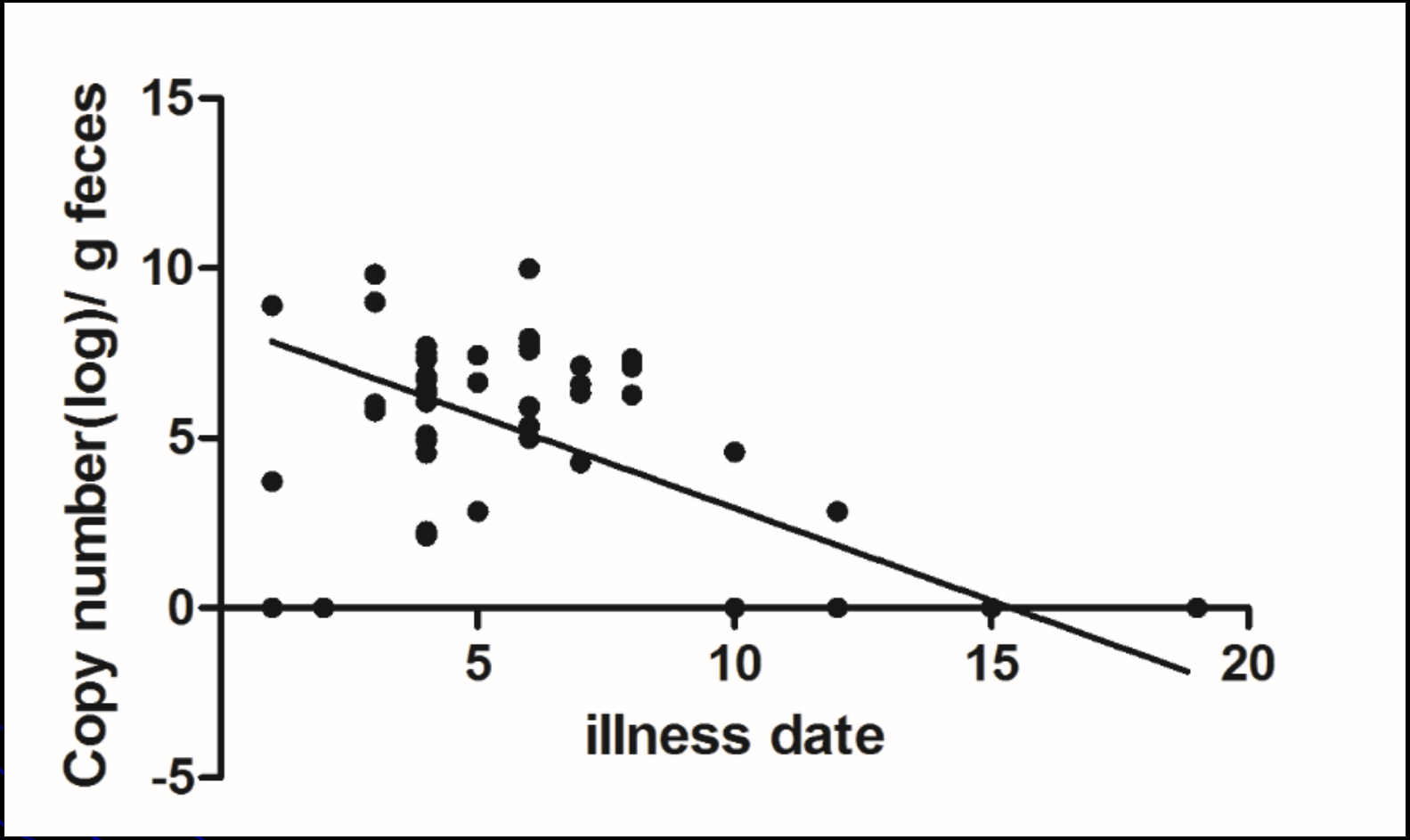


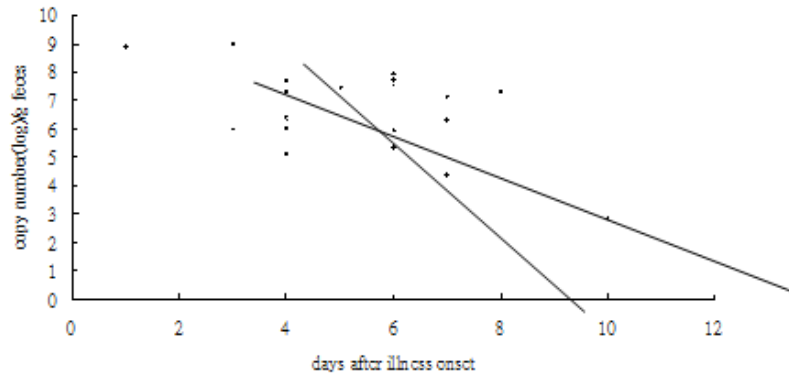
Fig. 37 fecal samples from NoV infected hospitalized children were collected for analysis viral load based on copy number quantification methods.

Results (2)

- Our results showed **56.8% (all) and 72%** (between 3-7 days illness) detection rate based on copy numbers cut-off value of > 10^5 and 43.2% (all) and **52%** (between 3-7 days illness) based on cut-off value > 10^6/g feces.
- In regards to correlate viral load with clinical manifestations, we found there is a longer shedding period in patients in 17 **febrile patients** (16.3 days after disease onset) than in 21 afebrile ones (12.7 days after disease onset) (P=0.03)
- A significantly longer shedding period of patients infected by **GII.4 Sydney strain** (17.6 days after illness onset) then by non- GII.4 Sydney(12.3 days after disease onset) strain norovirus (P <0.01).

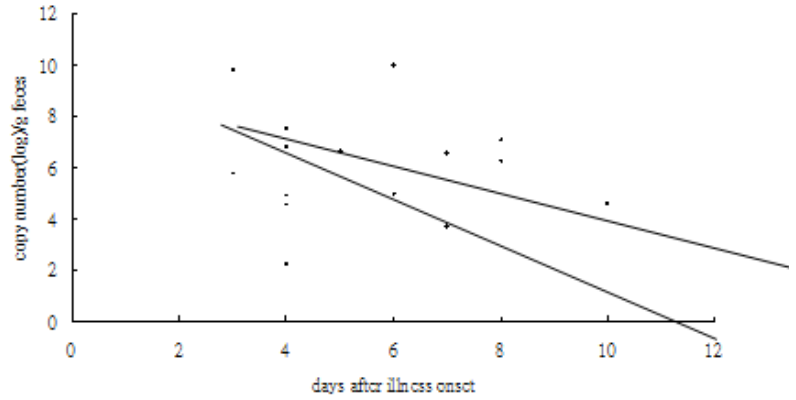
Correlate viral load with Fever

NO fever



9.6-14.2

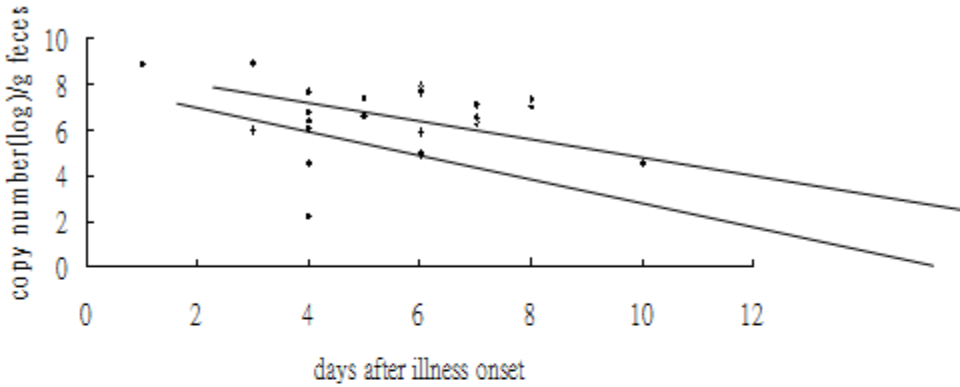
Fever



11.3-19.8

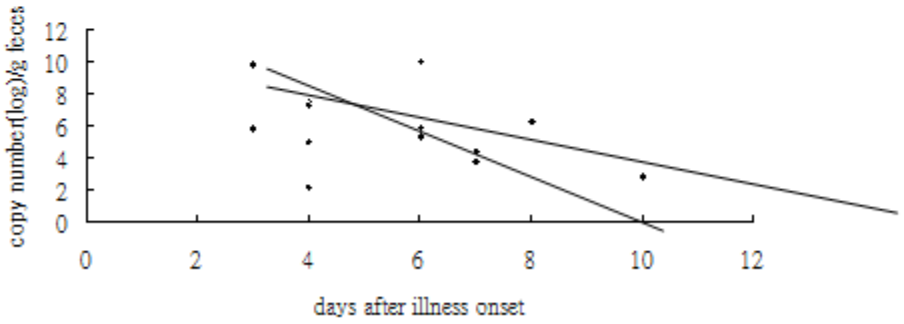
Correlate viral load with NoV Genotypes

GII.4 2012 Sydney



14.6-20.1

Non-GII.4 Sydney



9.8-13.4

Discussion

- In this study, the specific VP2 primer was designed to calculate the NoV copies number in patients' stool and this approach could understand the relationship with **clinical manifestations and viral shedding** in the hospitalized patient.
- Clinical correlation results showed in febrile patients, a longer shedding period with higher viral load was not ever reported. Virus infection **induced immune response and inflammation process** with febrile symptom may drive viral replication with a longer shedding time in acute infection stage. ***Partridge DG, Evans CM, Raza M, J Hosp Infect 2012; 81(1): 25-30.***

Discussion

- This indicated **norovirus detection** rate of 50-70% by ELISA methods with a lower threshold (> 10^5/g feces) while the reduction of detection ratio of 40-50% based on a higher threshold (> 10^6/g feces).
- In regards to viral shedding and NoV strains, we found a **longer shedding duration of GII.4 Sydney strain** that ever caused severe symptoms with gastrointestinal hemorrhage and high fever in Taiwan. ***Chen SY,1 Ye Feng,2 Hsun-Ching Chao Journal of Medical Microbiology, 2015 May;64(Pt 5):544-50.***
- In conclusion, the copy numbers based method viral load evaluation provide a more specific and precise way for assessment noroviruses detection, viral shedding, transmissibility, and even clinical correlation

Thank you for listening.

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