HAL
open science

Prevalence of norovirus GII-4 antibodies in Finnish children

Kirsi Nurminen, Vesna Blazevic, Leena Huhti, Sirpa Räsänen, Tiia Koho, Vesa P. Hytönen, Timo Vesikari

To cite this version:

Kirsi Nurminen, Vesna Blazevic, Leena Huhti, Sirpa Räsänen, Tiia Koho, et al.. Prevalence of norovirus GII-4 antibodies in Finnish children. Journal of Medical Virology, 2011, 83 (3), pp.525. 10.1002/jmv.21990 . hal-00610734

HAL Id: hal-00610734

https://hal.science/hal-00610734v1

Submitted on 24 Jul 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Journal of Medical Virology

Prevalence of norovirus GII-4 antibodies in Finnish children

Journal:	*Journal of Medical Virology*
Manuscript ID:	JMV-10-2016.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	01-Sep-2010
Complete List of Authors:	Nurminen, Kirsi; University of Tampere Medical School, Vaccine Research Center Blazevic, Vesna; University of Tampere Medical School, Vaccine Research Center Huhti, Leena; University of Tampere Medical School, Vaccine Research Center Räsänen, Sirpa; University of Tampere Medical School, Vaccine Research Center; Department of Pediatrics, Tampere University Hospital Koho, Tiia; Institute of Medical Technology, University of Tampere and Tampere University Hospital Hytönen, Vesa; Institute of Medical Technology, University of Tampere and Tampere University Hospital Vesikari, Timo; University of Tampere Medical School, Vaccine Research Center; Department of Pediatrics, Tampere University Hospital
Keywords:	NoV, antibody responses, ELISA, avidity, blocking antibodies

SCHOLARONE™
Manuscripts

PREVALENCE OF NOROVIRUS GII-4 ANTIBODIES IN FINNISH CHILDREN

Nurminen K.[1], Blazevic V.[1], Huhti L.[1], Räsänen S.[1,3], Koho T.[2], Hytönen VP.[2], Vesikari T.[1,3],

[1] Vaccine Research Center, University of Tampere Medical School, Tampere, Finland

[2] Institute of Medical Technology, University of Tampere and Tampere University Hospital, Tampere, Finland

[3] Department of Pediatrics, Tampere University Hospital, Finland

Authors:

Kirsi Nurminen, M.Sc.

Vesna Blazevic, Ph.D.

Leena Huhti, M.Sc.

Sirpa Räsänen, M.D.

Tiia Koho, M.Sc.

Vesa P. Hytönen, Ph.D.

Timo Vesikari, M.D.

Corresponding author:

Kirsi Nurminen, M.Sc.

University of Tampere Medical School

Vaccine Research Center

Biokatu 10

FIN-33520 Tampere, Finland

E-mail: kirsi.nurminen@uta.fi

Tel: +358 40 190 1504

Fax: +358 3 3551 8450

The work was performed in Vaccine Research Center, University of Tampere Medical School.

Running head: NoV antibody prevalence in Finnish children

For Peer Review

ABSTRACT

Noroviruses (NoVs) are the second most common cause of viral gastroenteritis after rotavirus in children. NoV genotype GII-4 has emerged as the major type not only in outbreaks of NoV gastroenteritis but also endemic gastroenteritis among infants and young children worldwide. Using baculovirus-insect cell system virus-like particles (VLPs) of NoV genotype GII-4 and an uncommon genotype GII-12 were produced. These VLPs were used in ELISA for detection of NoV-specific IgG and IgA antibodies in 492 serum specimens from Finnish children 0 to 14 years of age collected between 2006 and 2008. NoV IgG antibody prevalence was 47.3% in the age group 7-23 months and increased up to 91.2% after the age of five. Avidity of NoV IgG antibodies was low in the primary infections while high avidity antibodies were detected in the recurrent infections of the older children. In GII-4 infections, the homologous antibody response to GII-4 VLPs was stronger than to GII-12 VLPs but cross-reactivity between GII-4 and GII-12 was observed. Binding of GII-4 VLPs to a putative carbohydrate antigen receptor H-type 3 could be blocked by sera from children not infected with NoV during a waterborne outbreak of acute gastroenteritis (AGE). Therefore, protection against NoV infection correlated with strong blocking activity.

For Peer Review

KEYWORDS

NoV, antibody responses, ELISA, antibody avidity, blocking antibodies

For Peer Review

INTRODUCTION

Noroviruses (NoVs) are common causative agents of acute non-bacterial gastroenteritis in children and adults worldwide [Green. 2007]. After rotavirus, NoVs are the major cause of paediatric gastroenteritis, often requiring hospitalization [Pang et al. 1999, Moreno-Espinosa et al. 2004]. NoVs belong to a genetically diverse family of *Caliciviridae* and there are currently at least 25 NoV genotypes infectious to humans, of which 8 belong to genogroup GI and 17 to genogroup GII [Zheng et al. 2006]. The prevalence of genotypes fluctuates annually [Gallimore et al. 2007] but during the last decade variants of the GII-4 genotype have become the dominant genotypes in NoV epidemics worldwide [Siebenga et al. 2009].

The inability to grow NoVs in *in vitro* cell cultures [Duizer et al. 2004] has impeded the development of serotype-specific antibody assays. Thus, NoV capsid antigens used in serological diagnostic assays have to be produced by recombinant protein production techniques including baculovirus-insect cell expression system [Jiang et al. 1992]. NoV capsid genes are cloned into the baculovirus genome and expressed in insect cells to yield capsid proteins which self-assemble to form VLPs, mimicking the structural, antigenic and immunogenic properties of the native virus [Jiang et al. 1992].

NoV seroprevalence studies using NoV VLPs have been conducted in several countries worldwide [Gray et al. 1993, Lew et al. 1994, Nakata et al. 1998, Jing et al. 2000, Talal et al. 2000, Kobayashi et al. 2009]. The prevalence of NoV specific antibodies has been observed to follow an age-related pattern [Gray et al. 1993, Jing et al. 2000, Talal et al. 2000]; children acquire antibodies against NoV at an early age and almost 100% of adults have NoV-specific antibodies [Jiang et al. 2000].

For Peer Review

To distinguish primary from recurrent infections, the avidity of the immunoglobulin G (IgG) antibodies may be determined [Gutierrez and Maroto. 1996, Kanno and Kazuyama. 2002]. The significance of antibody level or avidity as a correlate for protection from NoV infection is not clear but some studies have shown that high preexisting IgG levels may be protective in children but not in adults [Ryder et al. 1985, Gray et al. 1994, Lew et al. 1994]. In the absence of a cell culture model, a surrogate NoV neutralization antibody assay is used to demonstrate the ability of antibodies to block the binding of NoV VLPs to ABH histo-blood group antigens (HBGAs) [Rockx et al. 2005, Cannon et al. 2009]. HBGAs likely play an important role in virus entry to the gut mucosal cells being the docking site or receptor for NoVs [Harrington et al. 2002]. High blocking ability of the antibodies has been suggested to correlate with protection against infection [Harrington et al. 2002, Lindesmith et al. 2003, LoBue et al. 2006].

The purpose of this study was to determine the seroprevalence of NoV GII-4 IgG and IgA antibodies in Finnish children, and to study if the magnitude and the avidity of the antibodies correlate with susceptibility to infection. In addition, cross-reactivity of NoV antibodies between a common (GII-4) and a rare (GII-12) genotype was investigated.

For Peer Review

MATERIALS AND METHODS

Study material

A total of 492 acute stage sera were collected from 0 to 14 year-old children hospitalized for AGE in Tampere University Hopital and Kuopio University Hospital from August 2006 to August 2008 [S. Räsänen et al., manuscript in preparation]. The study protocol and consent forms had been approved by the ethics committee in the Pirkanmaa hospital district in 2006 and the patients or the legal guardians volunteered for the study after having given an informed consent. NoV infection was detected in the stool samples of 97 children by reverse transcription (RT)-PCR which is described in details elsewhere [S. Räsänen et al., manuscript in preparation]. Paired serum samples were obtained from 14 of the children with NoV infection. Acute sera were collected within five days and convalescent sera 2-6 weeks after onset of the disease. In addition, acute sera from six adults were collected as a control. The sera were kept at -20° C before testing.

Antigens

Recombinant capsid proteins in the format of VLPs of the two NoV genotypes, GII-4 [004/95M-14/1995/AU] and GII-12 [HU/NLV/Wortley/90/UK] were used as coating antigens in enzyme-linked immunosorbent assays (ELISA). The VLPs were produced using baculovirus-insect cell expression system as described previously [Jiang et al. 1992]. VLP production and purification are described in details elsewhere [T. Koho et al., manuscript in preparation]. The morphology and the antigenicity of the VLPs produced were verified with electron microscopy and western blot (data not shown).

For Peer Review

Serum antibody ELISA and avidity assay

Sera were tested for anti-GII-4 and anti-GII-12 immunoglobulin G (IgG) and immunoglobulin A (IgA) by ELISA as previously described [Iritani et al. 2007] with modifications. VLPs were used to coat (4°C overnight) 96-well microtiter plates (Nunc Immuno Maxisorp, Thermo Fisher Scientific Inc., Waltham, MA, USA) in 0.01 M carbonate-bicarbonate buffer (pH 9.6) at a concentration 0.5 µg/ml for IgG and 2 µg/ml for IgA detection (100 µl/well). The optimal antigen concentration for coating was determined in preliminary experiments by testing serial dilutions of the antigens with positive and negative sera and plotting saturation curves. After washing three times with 10 mM phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBS-T) the plates were blocked at room temperature (RT) for 1 h with PBS containing 5% skimmed milk (Sigma-Aldrich, St. Louis, MO, USA). The wells were then washed three times with PBS-T and incubated 1 h at 37°C with 100 µl of serum diluted 1:100 or two-fold dilution series in PBS-T containing 1% skimmed milk. All serum samples were tested in duplicate wells. After washing six times, horseradish peroxidase (HRP) conjugated anti-human IgG or IgA (Zymed, Invitrogen, Carlsbad, CA, USA) diluted 1:4000 in 1% milk in PBS-T was added to the wells. After incubation (1 h, 37°C) the plates were washed and o-phenylenediamine dihydrochloride (SIGMA*FAST* OPD, Sigma-Aldrich) substrate was added at a concentration of 0.4 mg/ml. The plates were incubated at RT in the dark for 30 minutes and the reaction was stopped with 2 M sulphuric acid (H_2SO_4). Absorbance (optical density, OD) at a wavelength of 490 nm was measured in a microplate reader (Victor^2 1420, Perkin Elmer, Waltham, MA, USA). One known positive and one known negative serum sample was added to all plates as controls. Background signal from the blank wells (wells without serum) was subtracted from all of the OD readings at a plate. A sample was considered positive if the net absorbance value was above the set cut-off value, calculated as follows: $\geq$ 2x mean OD readings from the negative control serum wells at the same dilution and at least 0.100 OD. Negative antigen control wells included Sf-9 mock

For Peer Review

baculovirus infected insect cell lysate and were routinely <0.100 OD. A seroconversion was considered as a ≥4-fold rise in the titer between acute and convalescent sera.

To determine the IgG avidity of NoV antibodies a urea elution was used to remove the low avidity antibodies [Kanno and Kazuyama. 2002]. After incubation of sera on VLP coated plates the sera were aspirated from the plate and 8 M urea in PBS-T was added. After 5 minutes of incubation the treatment was repeated. Plates were washed 4 times prior to the addition of HRP-conjugated anti-human IgG as described above. The avidity index was calculated as [OD with urea/OD without urea] x 100% and the index value >50% was considered as high avidity.

Carbohydrate blocking assay

The assay for blocking the binding of HBGAs to NoV VLPs with human serum was performed as described previously [Harrington et al. 2002]. Briefly, microtiter plates were coated with GII-4 VLPs in PBS (pH 7.2) at a concentration of 2 µg/ml and incubated 4 h at RT. After washing, the plates were incubated with 5% milk in PBS overnight at 4˚C for blocking. Sera diluted 1:100 were added to the wells and the plates were incubated 1 h at 37°C and then aspirated from the plate. 100 µl of biotinylated H-type-3 or Lewis B (for negative control) (Lectinity Holdings, Inc., Moscow, Russia) were added at a concentration of 40 µg/ml in 1% milk in PBS-T. After 4 h at 37°C the wells were washed and a 1:2000 dilution of streptavidin-conjugated HRP (Thermo Fisher Scientific Inc.) was added and incubated 1 h at 37°C. The development of the colour reaction and the measurement of absorbance at a wavelength 490 nm were conducted as described above. OD reading from the wells incubated without serum was considered as a maximum signal for the binding of H-type 3 to GII-4 VLP. No binding of VLPs to the negative control HBGA (Lewis B) was detected.

For Peer Review

Background signal from the blank wells (wells lacking HBGA) was subtracted from all of the OD values of tested samples.

Statistical analyses

Pearson's chi-square test was performed to examine the differences in antibody prevalence between age groups. Student's *t*-test was used to assess the intergroup differences in antibody magnitude and avidity. All hypothesis testing was two-tailed. Pearson's correlation coefficient was used to determine the correlation between GII-4 and GII-12 specific antibody levels. Mann-Whitney U-test was used to compare the intergroup differences in the blocking ability of the sera. Statistical significance was defined as $P<0.05$.

For Peer Review

RESULTS

Overall and age-related serum IgG and IgA antibodies against GII-4

Of the 492 serum specimens 63.4% and 38.9% sera were positive for GII-4 specific IgG and IgA, respectively. The age-related seroprevalence is shown in Figure 1. The prevalence of IgG antibodies to GII-4 was 66.7% in the age group 0-6 months and 47.3% in the age group 7-23 months. After the age of two years the prevalence increased to 73.1% and continued to increase up to 91.2% in children 5-14 years of age. The NoV IgG antibody prevalence in the age group 7-23 months was significantly lower compared to the other age groups ($P<0.001$ to 0.05). The corresponding seroprevalence for GII-4 specific IgA antibodies was low (20.8%) in infants 0-6 months of age and rose with increasing age. Overall the prevalence for IgG was significantly higher in each age group compared to IgA seroprevalence ($P<0.001$ to 0.05). Samples positive for both IgG and IgA were detected in 16.8% of 0-6 months old infants, 20.5% of 7-23 months old, 51.3% of 2-4 years and 79.4% of 5-14 years old children (data not shown). IgG and IgA GII-4 specific antibodies were detected in 100% of the adult sera (Fig. 1).

Antibody avidity

The avidity indexes of NoV IgG antibodies in the three age groups representing newborns (0-6 months of age), presumable primary/recent infections (7-12 months of age) and recurrent infections (9-14 years of age) are shown on Figure 2. High avidity IgG antibodies (mean avidity index 61.5 ± 5.1%) were detected in infants ≤6 months of age. The mean avidity index was significantly lower in children aged 7-12 months (40.4 ± 3.9%) than in children aged 0-6 months ($P<0.05$). The highest of all mean avidity indexes (85.6 ± 4.5%) were detected in older children, who had most probably

For Peer Review

encountered several NoV infections during their lifetime. The lowest frequency of the high avidity antibodies was detected in the primary infections whereas the highest frequency was observed in the recurrent infections ($P<0.001$). All the adult control sera (n=6) contained high avidity antibodies (data not shown).

Cross reactivity of NoV antibodies

To determine the immunological cross-reactivity of the sera the seroprevalence was assayed against GII-12 which so far has not been reported in Finland [Maunula and Von Bonsdorff. 2005, S. Räsänen et al., manuscript in preparation]. Of the GII-4 positive sera 70.8% were also IgG positive against GII-12 VLPs. A subset of GII-4 IgA positive samples (96 out of 197) was tested and found that 47.6% of these were IgA cross-reactive for GII-12. When the GII-12 antibody responses were compared to the responses obtained with GII-4 VLPs a positive correlation was detected overall (r=0.64) although the mean ODs were generally lower for GII-12-specific responses (data not shown). Sera of the adult individuals were all positive for GII-12 (data not shown). Cross-reactivity between the two genotypes was further confirmed using paired sera of three patients infected with GII-4 NoV (Fig. 3). All the paired sera showed IgG seroconversion not only to GII-4 (Fig. 3A) but also to GII-12 antigen, although with lower magnitude of the response (Fig. 3B).

Correlation of NoV infections to antibody prevalence and levels

Of the 492 children encountered in hospital because of AGE, 97 (19.7%) were diagnosed with NoV infection by RT-PCR [S. Räsänen et al., manuscript in preparation]. The incidence of NoV gastroenteritis was highest in children aged 7-23 months (53 out of 97 cases; 54.6%). Of these cases 60.4% were primary infections as judged by the absence of NoV antibodies at the acute stage while

For Peer Review

39.6% had pre-existing antibodies to NoV. To determine the possible protective effect of the pre-existing NoV-specific antibodies against a subsequent NoV infection the OD values in the different age groups obtained in ELISA IgG assay were compared. The lowest pre-existing antibody level (mean OD 0.66 ± 0.06) as well as antibody prevalence (47.3%, Fig. 1) were detected in the age group 7-23 months (Fig. 4). In the age group 2-4 years mean OD level increased (1.11 ± 0.07) and by the age of 14 years children had high levels of preexisting antibodies to NoV (mean OD 1.62 ± 0.10) in the majority of individuals (91.2%, Fig. 1) and the least infections (14.7%) (Fig. 4). Therefore, a trend to a negative association between the incidence of infection and the level of pre-existing antibodies was observed.

Antibody blocking

Acute sera obtained from children infected during a waterborne outbreak of AGE in the town of Nokia, Finland [Räsänen et al. 2010] with NoV GII-4 or other agent causing acute gastroenteritis were tested for blocking of the GII-4 VLPs binding to H-type-3 HBGA. Higher blocking activity was seen in the sera of children not infected with NoV than in the sera of children with NoV infection ($P<0.05$) (Fig 5A). In addition, blocking of the VLPs binding to H-type-3 antigen was performed with 1:100 dilutions of paired sera from eight children with NoV gastroenteritis (Fig. 5B). The blocking activity increased after a recent NoV infection. The acute sera could block on average 24.8% of the maximum GII-4 binding to H-type 3 whereas 61.6% of the binding was blocked by the convalescent sera ($P<0.05$).

For Peer Review

DISCUSSION

A study conducted 20 years ago to determine the Norwalk virus (GI-1) seroprevalence in young Finnish children [Lew et al. 1994] showed that 49% of children 4 to 23 months of age had NoV antibodies. At that time NoV GII-4 was not common. Nearly identical seroprevalence for GII-4 in the similar age group was found in the present study. The data therefore confirm that Finnish children acquire NoV infection at an early age and with increasing age the number of natural encounters with the virus accumulates, as judged by the increasing seroprevalence and the prevalence of high avidity antibodies. Prospective studies in Finland have shown that clinically manifest NoV gastroenteritis is seen in 20-30% of Finnish children by the age of 2 years [Pang et al. 1999, Zeng et al. 2010]. As the seroprevalence increases even more rapidly, it is likely that many NoV infections are subclinical. The prevalence of NoV antibodies in children has been reported in several European countries, Asia, Africa and Latin America [Gray et al. 1993, Nakata et al. 1998, Jing et al. 2000, Talal et al. 2000, Farkas et al. 2006, Kobayashi et al. 2009] and the acquisition of NoV GII-4 specific antibodies, especially in the developed countries, is consistent with the results of the present study [Pelosi et al. 1999, Nicollier-Jamot et al. 2003]. Infants under 6 months of age typically have high antibody levels, which are explained by the presence of maternal antibodies [Gray et al. 1993, Numata et al. 1994]. After 6 months of age, when the maternal antibodies decline, the seroprevalence decreases and starts rising again after 12-24 months of age reaching 90-100% in older children and adult population [Matsui and Greenberg. 2000].

All the adult sera and the majority of children's sera tested in this study recognized both GII-4 and GII-12 VLPs in ELISA, although the level of reactivity was higher against GII-4. The likely reason may be cross reactive epitopes rather than previous exposure to NoV GII-12 as GII-12 genotype has not been reported in Finland [Maunula and Von Bonsdorff. 2005, Räsänen et al., manuscript in

For Peer Review

preparation]. In addition, these results imply the existence of highly conserved antibody epitopes between the genotypes within a genogroup. We also showed that a seroconversion caused by GII-4 infection could be detected with a heterotypic antigen (GII-12) although the response to homotypic VLPs was stronger. Other reports also found that seroconversion can be observed in convalescent sera using VLPs from genetically distinct virus from the one causing the primary infection [Belliot et al. 2001]. Despite the cross-reactivity, greater seroresponses are detected against homologous strain, and strains belonging to the same genogroup react more strongly than the strains across the genogroups [Treanor et al. 1993, Farkas et al. 2003, Lindesmith et al. 2010]. Because of the cross reactive immunity and the presence of the pre-existing antibodies detected, ELISA method as the one described in this study and routinely used by others [Rockx et al. 2005, Iritani et al. 2007] does not give precise serotype-specific determination of the immune response.

The avidity and the level of NoV specific IgG antibodies are low after primary infections in young children. The avidity increases with time and particulary as a result of repeated infections when molecular evolution specifies the antigen binding domains of the antibodies to recognize the target epitopes more precisely [Murphy et al. 2008]. Indeed, high avidity antibodies were detected in older children and adults who are likely to have experienced NoV infections repeatedly throughout life. The high avidity antibodies detected in infants less than 6 months of age presumably represent transplancentally acquired maternal IgG antibodies [Gray et al. 1993, Numata et al. 1994]. The results show that 20% of infants under 6 months of age already had NoV infection as judged by the presence of NoV IgA antibodies. Such incidence of infection suggests that transplacentally acquired IgG antibodies do not offer full protection.

The present study showed that children who were exposed to NoV in a waterborne outbreak of AGE [Räsänen et al. 2010] but were not infected by NoV, had higher blocking activity of GII-4

For Peer Review

VLPs binding to H type 3 HBGA compared to those who were infected. Interestingly, the acute sera from not-infected children had similar blocking ability as the convalescent sera from children recently suffering from a NoV infection. The increase in the blocking ability of the serum after the NoV infection has been hypothesised to be associated with a short-term immunity to NoV infection [Rockx et al. 2005]. The results shown in the current study also suggest that high blocking activity of VLPs binding to HBGA in the acute serum may confer protection against reinfection. Blocking activity of the antibodies may be a relevant surrogate marker of NoV protection when considering different vaccine approaches.

In this study, the majority of sporadic NoV cases treated in hospital occurred in children 7-23 months of age. This age group also had the lowest pre-existing antibody levels. Therefore, a trend to a negative correlation between the susceptibility to NoV infection and pre-existing IgG antibodies was observed. A previous study conducted in Finland [Lew et al. 1994] suggested that children with low NoV-specific IgG titers (<1:50) are more susceptible to NoV infection in the next 10 months than children with higher titers (>1:50). In the current study it was shown that older children with higher NoV specific antibody levels and avidity had also fewer infections than children less than 2 years old. It may be speculated that NoV infections before the age of two years induce protection for later ages as the children develop protective immune response to NoV. This is in support of the concept of NoV vaccination in young infants in order to decrease the burden NoV of infection as well as the hospitalization cases due to NoV gastroenteritis.

For Peer Review

ACKNOWLEDGEMENTS

We thank Eeva Jokela for her technical assistance during the study. We are grateful for Marjo Salminen and Suvi Lappalainen for coordinating the epidemiological study. We also thank the personnel of Tampere University Hospital and Kuopio University Hospital for collecting the serum samples and Marjo Salonen for organizing serum collection.

For Peer Review

REFERENCES

Belliot G, Noel JS, Li JF, Seto Y, Humphrey CD, Ando T, Glass RI, Monroe SS. 2001. Characterization of capsid genes, expressed in the baculovirus system, of three new genetically distinct strains of "Norwalk-like viruses". J Clin Microbiol 39:4288-4295.

Cannon JL, Lindesmith LC, Donaldson EF, Saxe L, Baric RS, Vinje J. 2009. Herd immunity to GII.4 noroviruses is supported by outbreak patient sera. J Virol 83:5363-5374.

Duizer E, Schwab KJ, Neill FH, Atmar RL, Koopmans MP, Estes MK. 2004. Laboratory efforts to cultivate noroviruses. J Gen Virol 85:79-87.

Farkas T, Deng X, Ruiz-Palacios G, Morrow A, Jiang X. 2006. Development of an enzyme immunoassay for detection of sapovirus-specific antibodies and its application in a study of seroprevalence in children. J Clin Microbiol 44:3674-3679.

Farkas T, Thornton SA, Wilton N, Zhong W, Altaye M, Jiang X. 2003. Homologous versus heterologous immune responses to Norwalk-like viruses among crew members after acute gastroenteritis outbreaks on 2 US Navy vessels. J Infect Dis 187:187-193.

Gallimore CI, Iturriza-Gomara M, Xerry J, Adigwe J, Gray JJ. 2007. Inter-seasonal diversity of norovirus genotypes: emergence and selection of virus variants. Arch Virol 152:1295-1303.

Gray JJ, Cunliffe C, Ball J, Graham DY, Desselberger U, Estes MK. 1994. Detection of immunoglobulin M (IgM), IgA, and IgG Norwalk virus-specific antibodies by indirect enzyme-

For Peer Review

linked immunosorbent assay with baculovirus-expressed Norwalk virus capsid antigen in adult volunteers challenged with Norwalk virus. J Clin Microbiol 32:3059-3063.

Gray JJ, Jiang X, Morgan-Capner P, Desselberger U, Estes MK. 1993. Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. J Clin Microbiol 31:1022-1025.

Green KY. 2007. Caliciviridae: the noroviruses. In: Fields BN, editor. Fields virology, 5. ed. ed. Lippincott Williams & Wilkins: Philadelphia (Pa.). p 949-980.

Gutierrez J, Maroto C. 1996. Are IgG antibody avidity assays useful in the diagnosis of infectious diseases? A review. Microbios 87:113-121.

Harrington PR, Lindesmith L, Yount B, Moe CL, Baric RS. 2002. Binding of Norwalk virus-like particles to ABH histo-blood group antigens is blocked by antisera from infected human volunteers or experimentally vaccinated mice. J Virol 76:12335-12343.

Iritani N, Seto T, Hattori H, Natori K, Takeda N, Kubo H, Yamano T, Ayata M, Ogura H, Seto Y. 2007. Humoral immune responses against norovirus infections of children. J Med Virol 79:1187-1193.

Jiang X, Wang M, Graham DY, Estes MK. 1992. Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein. J Virol 66:6527-6532.

For Peer Review

Jiang X, Wilton N, Zhong WM, Farkas T, Huang PW, Barrett E, Guerrero M, Ruiz-Palacios G, Green KY, Green J, Hale AD, Estes MK, Pickering LK, Matson DO. 2000. Diagnosis of human caliciviruses by use of enzyme immunoassays. J Infect Dis 181 Suppl 2:S349-59.

Jing Y, Qian Y, Huo Y, Wang LP, Jiang X. 2000. Seroprevalence against Norwalk-like human caliciviruses in beijing, China. J Med Virol 60:97-101.

Kanno A, Kazuyama Y. 2002. Immunoglobulin G antibody avidity assay for serodiagnosis of hepatitis C virus infection. J Med Virol 68:229-233.

Kobayashi S, Fujiwara N, Takeda N, Minagawa H. 2009. Seroepidemiological study of norovirus infection in Aichi Prefecture, Japan. Microbiol Immunol 53:356-359.

Lew JF, Valdesuso J, Vesikari T, Kapikian AZ, Jiang X, Estes MK, Green KY. 1994. Detection of Norwalk virus or Norwalk-like virus infections in Finnish infants and young children. J Infect Dis 169:1364-1367.

Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, Stewart P, LePendu J, Baric R. 2003. Human susceptibility and resistance to Norwalk virus infection. Nat Med 9:548-553.

Lindesmith LC, Donaldson E, Leon J, Moe CL, Frelinger JA, Johnston RE, Weber DJ, Baric RS. 2010. Heterotypic humoral and cellular immune responses following Norwalk virus infection. J Virol 84:1800-1815.

For Peer Review

LoBue AD, Lindesmith L, Yount B, Harrington PR, Thompson JM, Johnston RE, Moe CL, Baric RS. 2006. Multivalent norovirus vaccines induce strong mucosal and systemic blocking antibodies against multiple strains. Vaccine 24:5220-5234.

Matsui SM, Greenberg HB. 2000. Immunity to calicivirus infection. J Infect Dis 181 Suppl 2:S331-5.

Maunula L, Von Bonsdorff CH. 2005. Norovirus genotypes causing gastroenteritis outbreaks in Finland 1998-2002. J Clin Virol 34:186-194.

Moreno-Espinosa S, Farkas T, Jiang X. 2004. Human caliciviruses and pediatric gastroenteritis. Semin Pediatr Infect Dis 15:237-245.

Murphy K, Travers P, Walport M. 2008. The adaptive immune response. In: Masson S, Lawrence E, Lucas G, Goatly B, editors. Janeway's immunobiology, 7th ed. ed. Garland Science: New York, US; Abingdon, UK. p. 323-490.

Nakata S, Honma S, Numata K, Kogawa K, Ukae S, Adachi N, Jiang X, Estes MK, Gatheru Z, Tukei PM, Chiba S. 1998. Prevalence of human calicivirus infections in Kenya as determined by enzyme immunoassays for three genogroups of the virus. J Clin Microbiol 36:3160-3163.

Nicollier-Jamot B, Pico V, Pothier P, Kohli E. 2003. Molecular cloning, expression, self-assembly, antigenicity, and seroepidemiology of a genogroup II norovirus isolated in France. J Clin Microbiol 41:3901-3904.

For Peer Review

Numata K, Nakata S, Jiang X, Estes MK, Chiba S. 1994. Epidemiological study of Norwalk virus infections in Japan and Southeast Asia by enzyme-linked immunosorbent assays with Norwalk virus capsid protein produced by the baculovirus expression system. J Clin Microbiol 32:121-126.

Pang XL, Joensuu J, Vesikari T. 1999. Human calicivirus-associated sporadic gastroenteritis in Finnish children less than two years of age followed prospectively during a rotavirus vaccine trial. Pediatr Infect Dis J 18:420-426.

Pelosi E, Lambden PR, Caul EO, Liu B, Dingle K, Deng Y, Clarke IN. 1999. The seroepidemiology of genogroup 1 and genogroup 2 Norwalk-like viruses in Italy. J Med Virol 58:93-99.

Räsänen S, Lappalainen S, Kaikkonen S, Hamalainen M, Salminen M, Vesikari T. 2010. Mixed viral infections causing acute gastroenteritis in children in a waterborne outbreak. Epidemiol Infect 22:1-8.

Rockx B, Baric RS, de Grijs I, Duizer E, Koopmans MP. 2005. Characterization of the homo- and heterotypic immune responses after natural norovirus infection. J Med Virol 77:439-446.

Ryder RW, Singh N, Reeves WC, Kapikian AZ, Greenberg HB, Sack RB. 1985. Evidence of immunity induced by naturally acquired rotavirus and Norwalk virus infection on two remote Panamanian islands. J Infect Dis 151:99-105.

Siebenga JJ, Vennema H, Zheng DP, Vinje J, Lee BE, Pang XL, Ho EC, Lim W, Choudekar A, Broor S, Halperin T, Rasool NB, Hewitt J, Greening GE, Jin M, Duan ZJ, Lucero Y, O'Ryan M, Hoehne M, Schreier E, Ratcliff RM, White PA, Iritani N, Reuter G, Koopmans M. 2009. Norovirus

For Peer Review

illness is a global problem: emergence and spread of norovirus GII.4 variants, 2001-2007. J Infect Dis 200:802-812.

Talal AH, Moe CL, Lima AA, Weigle KA, Barrett L, Bangdiwala SI, Estes MK, Guerrant RL. 2000. Seroprevalence and seroincidence of Norwalk-like virus infection among Brazilian infants and children. J Med Virol 61:117-124.

Treanor JJ, Jiang X, Madore HP, Estes MK. 1993. Subclass-specific serum antibody responses to recombinant Norwalk virus capsid antigen (rNV) in adults infected with Norwalk, Snow Mountain, or Hawaii virus. J Clin Microbiol 31:1630-1634.

Zeng SQ, Halkosalo A, Salminen M, Szakal ED, Karvonen A, Vesikari T. 2010. Norovirus gastroenteritis in young children receiving human rotavirus vaccine. Scand J Infect Dis 42:540-544.

Zheng D, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. 2006. Norovirus classification and proposed strain nomenclature. Virology 346:312-323.

For Peer Review

FIGURE CAPTIONS

Fig. 1. Age-related prevalence of immunoglobulin (Ig) G and IgA serum antibodies to norovirus GII-4 in Finnish children and adults.

Fig. 2. Avidity indexes of immunoglobulin G antibodies to norovirus GII-4 in three age groups. Bars represent the mean avidity index of the group. Boxed values indicate the frequency (%) of high avidity antibodies in the age group. *P<0.05, **P<0.0001 (Student's t-test).

Fig. 3. Serum titration of three norovirus (NoV) GII-4 infected pediatric patients against NoV virus like particles (VLPs). Sera were diluted 2-fold starting from 1:100 and tested in an enzyme-linked immunosorbent assay using GII-4 VLPs (A) or GII-12 VLPs (B) as antigens. Acute sera titrations are shown in dotted lines and convalescent sera titrations in full lines.

Fig. 4. Age distribution of frequency of cases of norovirus (NoV) infections encountered in hospital and the levels of NoV GII-4 specific immunoglobulin G antibodies by age group. The bars represent the percentages of NoV infected children in each age group of 97 total infection cases. The mean optical density (OD) values of each group sera (1:100 dilution) tested by enzyme-linked immunosorbent assay are shown (◆) on the secondary y-axis.

Fig. 5. Blocking of norovirus (NoV) GII-4 virus like particles (VLPs) binding to H-type-3 HBGA by the acute sera of NoV GII-4 infected (n=9) or not infected (n=6) children (A), and by the paired sera from children (n=8) infected with NoV GII-4 (B). Shown are mean optical density (OD) values of the tested sera (1:100 dilutions). The mean OD of the binding control indicates the maximum binding of the GII-4 VLPs to H-type-3. Error bars represent the standard error of the mean (SEM).

For Peer Review

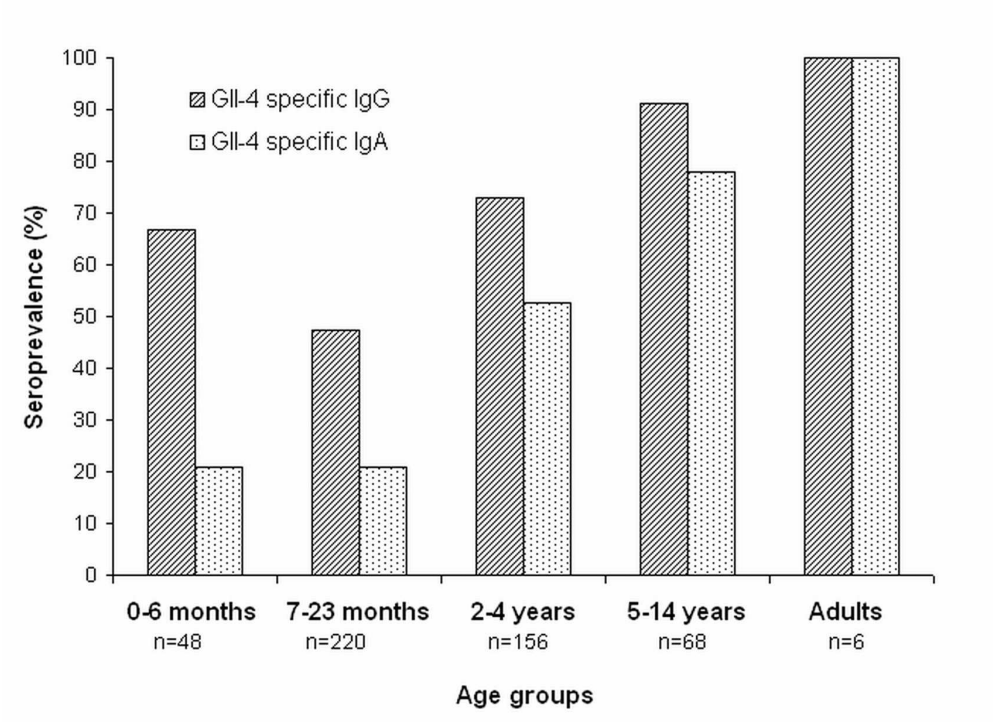

Fig. 1. Age-related prevalence of immunoglobulin (Ig) G and IgA serum antibodies to norovirus GII-4 in Finnish children and adults.
72x52mm (600 x 600 DPI)

Review

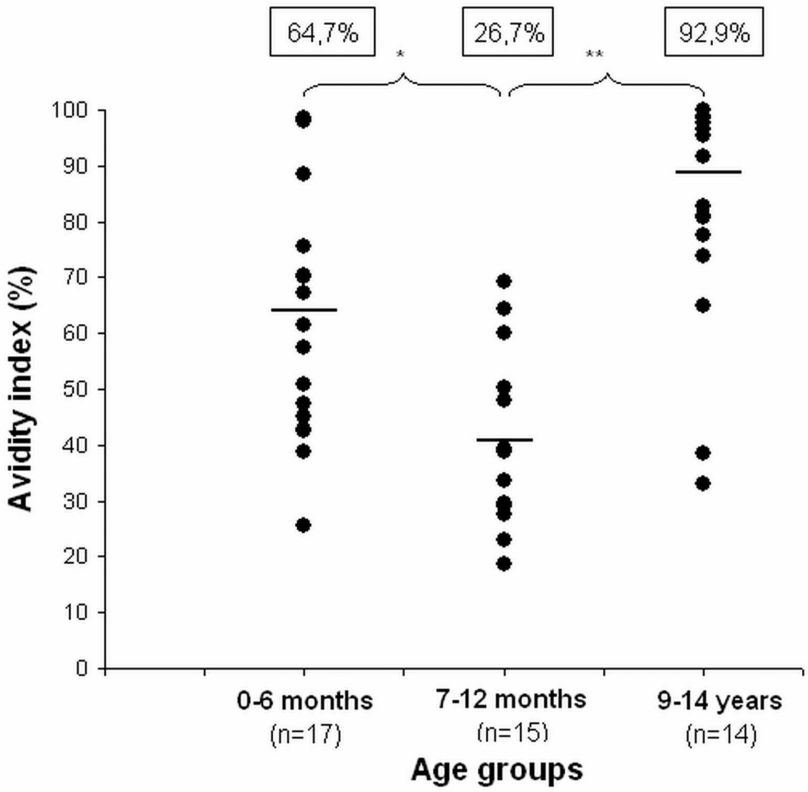

Fig. 2. Avidity indexes of immunoglobulin G antibodies to norovirus GII-4 in three age groups. Bars represent the mean avidity index of the group. Boxed values indicate the frequency (%) of high avidity antibodies in the age group. *P<0.05, **P<0.0001 (Student's t-test).
80x65mm (600 x 600 DPI)

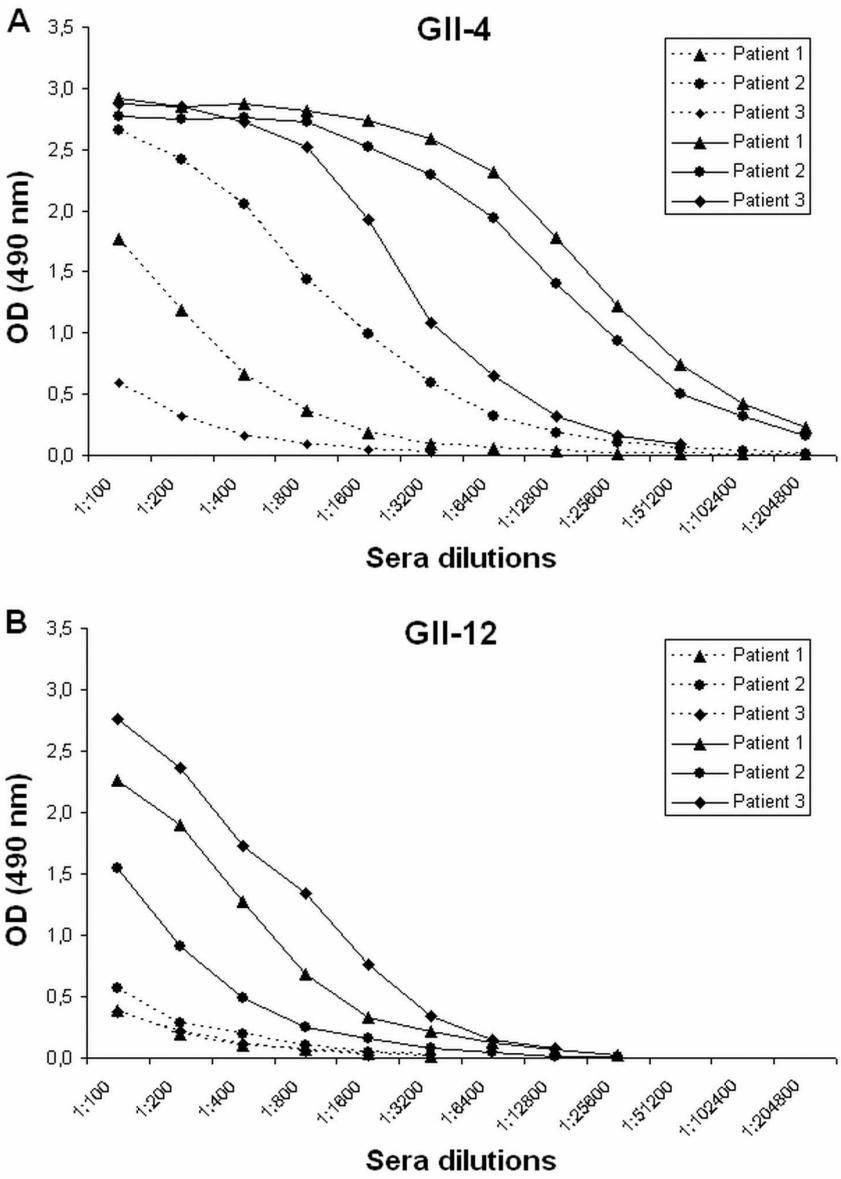

Fig. 3. Serum titration of three norovirus (NoV) GII-4 infected pediatric patients against NoV virus like particles (VLPs). Sera were diluted 2-fold starting from 1:100 and tested in an enzyme-linked immunosorbent assay using GII-4 VLPs (A) or GII-12 VLPs (B) as antigens. Acute sera titrations are shown in dotted lines and convalescent sera titrations in full lines.
132x174mm (600 x 600 DPI)

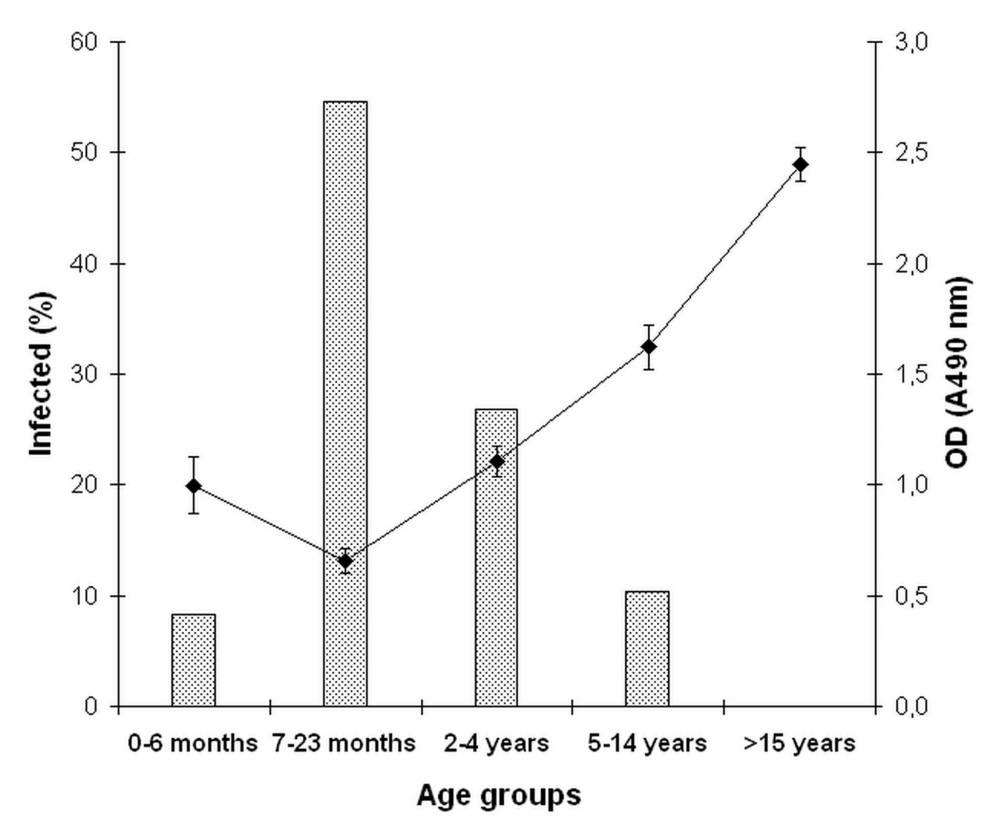

Fig. 4. Age distribution of frequency of cases of norovirus (NoV) infections encountered in hospital and the levels of NoV GII-4 specific immunoglobulin G antibodies by age group. The bars represent the percentages of NoV infected children in each age group of 97 total infection cases. The mean optical density (OD) values of each group sera (1:100 dilution) tested by enzyme-linked immunosorbent assay are shown (u) on the secondary y-axis.
82x68mm (600 x 600 DPI)

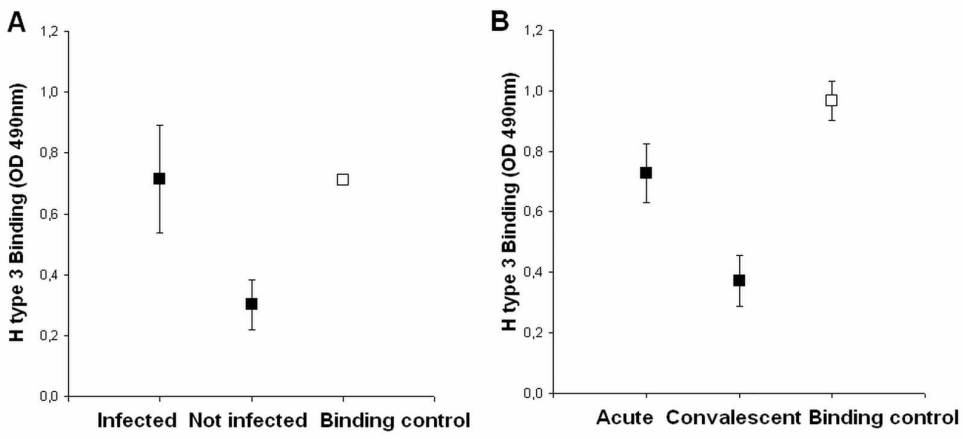

Fig. 5. Blocking of norovirus (NoV) GII-4 virus like particles (VLPs) binding to H-type-3 HBGA by the acute sera of NoV GII-4 infected (n=9) or not infected (n=6) children (A), and by the paired sera from children (n=8) infected with NoV GII-4 (B). Shown are mean optical density (OD) values of the tested sera (1:100 dilutions). The mean OD of the binding control indicates the maximum binding of the GII-4 VLPs to H-type-3. Error bars represent the standard error of the mean (SEM).
69x32mm (600 x 600 DPI)

er Review