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Vesa P. Hytönen, Timo Vesikari

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## Prevalence of norovirus GII-4 antibodies in Finnish children

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Manuscripts

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3 1 **PREVALENCE OF NOROVIRUS GII-4 ANTIBODIES**  
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5 2 **IN FINNISH CHILDREN**  
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56 29  
57 30 The work was performed in Vaccine Research Center, University of Tampere Medical School.  
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59 31  
60 32 Running head: NoV antibody prevalence in Finnish children  
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3 34 **ABSTRACT**  
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8 36 Noroviruses (NoVs) are the second most common cause of viral gastroenteritis after rotavirus in  
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10 37 children. NoV genotype GII-4 has emerged as the major type not only in outbreaks of NoV  
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12 38 gastroenteritis but also endemic gastroenteritis among infants and young children worldwide. Using  
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14 39 baculovirus-insect cell system virus-like particles (VLPs) of NoV genotype GII-4 and an  
15  
16 40 uncommon genotype GII-12 were produced. These VLPs were used in ELISA for detection of  
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18 41 NoV-specific IgG and IgA antibodies in 492 serum specimens from Finnish children 0 to 14 years  
19  
20 42 of age collected between 2006 and 2008. NoV IgG antibody prevalence was 47.3% in the age group  
21  
22 43 7-23 months and increased up to 91.2% after the age of five. Avidity of NoV IgG antibodies was  
23  
24 44 low in the primary infections while high avidity antibodies were detected in the recurrent infections  
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26 45 of the older children. In GII-4 infections, the homologous antibody response to GII-4 VLPs was  
27  
28 46 stronger than to GII-12 VLPs but cross-reactivity between GII-4 and GII-12 was observed. Binding  
29  
30 47 of GII-4 VLPs to a putative carbohydrate antigen receptor H-type 3 could be blocked by sera from  
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32 48 children not infected with NoV during a waterborne outbreak of acute gastroenteritis (AGE).  
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34 49 Therefore, protection against NoV infection correlated with strong blocking activity.  
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59 **KEYWORDS**

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

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For Peer Review

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3 84 **INTRODUCTION**  
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8 86 Noroviruses (NoVs) are common causative agents of acute non-bacterial gastroenteritis in children  
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10 87 and adults worldwide [Green. 2007]. After rotavirus, NoVs are the major cause of paediatric  
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12 88 gastroenteritis, often requiring hospitalization [Pang et al. 1999, Moreno-Espinosa et al. 2004].  
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15 89 NoVs belong to a genetically diverse family of *Caliciviridae* and there are currently at least 25 NoV  
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17 90 genotypes infectious to humans, of which 8 belong to genogroup GI and 17 to genogroup GII  
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20 91 [Zheng et al. 2006]. The prevalence of genotypes fluctuates annually [Gallimore et al. 2007] but  
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22 92 during the last decade variants of the GII-4 genotype have become the dominant genotypes in NoV  
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24 93 epidemics worldwide [Siebenga et al. 2009].  
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29 95 The inability to grow NoVs in *in vitro* cell cultures [Duizer et al. 2004] has impeded the  
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31 96 development of serotype-specific antibody assays. Thus, NoV capsid antigens used in serological  
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34 97 diagnostic assays have to be produced by recombinant protein production techniques including  
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36 98 baculovirus-insect cell expression system [Jiang et al. 1992]. NoV capsid genes are cloned into the  
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39 99 baculovirus genome and expressed in insect cells to yield capsid proteins which self-assemble to  
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41 100 form VLPs, mimicking the structural, antigenic and immunogenic properties of the native virus  
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43 101 [Jiang et al. 1992].  
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48 103 NoV seroprevalence studies using NoV VLPs have been conducted in several countries worldwide  
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50 104 [Gray et al. 1993, Lew et al. 1994, Nakata et al. 1998, Jing et al. 2000, Talal et al. 2000, Kobayashi  
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53 105 et al. 2009]. The prevalence of NoV specific antibodies has been observed to follow an age-related  
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55 106 pattern [Gray et al. 1993, Jing et al. 2000, Talal et al. 2000]; children acquire antibodies against  
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58 107 NoV at an early age and almost 100% of adults have NoV-specific antibodies [Jiang et al. 2000].  
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3 109 To distinguish primary from recurrent infections, the avidity of the immunoglobulin G (IgG)  
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5 110 antibodies may be determined [Gutierrez and Maroto. 1996, Kanno and Kazuyama. 2002]. The  
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8 111 significance of antibody level or avidity as a correlate for protection from NoV infection is not  
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10 112 clear but some studies have shown that high preexisting IgG levels may be protective in children  
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13 113 but not in adults [Ryder et al. 1985, Gray et al. 1994, Lew et al. 1994]. In the absence of a cell  
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15 114 culture model, a surrogate NoV neutralization antibody assay is used to demonstrate the ability of  
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17 115 antibodies to block the binding of NoV VLPs to ABH histo-blood group antigens (HBGAs) [Rockx  
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20 116 et al. 2005, Cannon et al. 2009]. HBGAs likely play an important role in virus entry to the gut  
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22 117 mucosal cells being the docking site or receptor for NoVs [Harrington et al. 2002]. High blocking  
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25 118 ability of the antibodies has been suggested to correlate with protection against infection  
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27 119 [Harrington et al. 2002, Lindesmith et al. 2003, LoBue et al. 2006].  
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32 121 The purpose of this study was to determine the seroprevalence of NoV GII-4 IgG and IgA  
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34 122 antibodies in Finnish children, and to study if the magnitude and the avidity of the antibodies  
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36 123 correlate with susceptibility to infection. In addition, cross-reactivity of NoV antibodies between a  
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39 124 common (GII-4) and a rare (GII-12) genotype was investigated.  
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3 134 **MATERIALS AND METHODS**  
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8 136 **Study material**  
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13 138 A total of 492 acute stage sera were collected from 0 to 14 year-old children hospitalized for AGE  
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15 139 in Tampere University Hospital and Kuopio University Hospital from August 2006 to August 2008  
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17 140 [S. Räsänen et al., manuscript in preparation]. The study protocol and consent forms had been  
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20 141 approved by the ethics committee in the Pirkanmaa hospital district in 2006 and the patients or the  
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22 142 legal guardians volunteered for the study after having given an informed consent. NoV infection  
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25 143 was detected in the stool samples of 97 children by reverse transcription (RT)-PCR which is  
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27 144 described in details elsewhere [S. Räsänen et al., manuscript in preparation]. Paired serum samples  
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29 145 were obtained from 14 of the children with NoV infection. Acute sera were collected within five  
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32 146 days and convalescent sera 2-6 weeks after onset of the disease. In addition, acute sera from six  
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34 147 adults were collected as a control. The sera were kept at -20° C before testing.  
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36 148  
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39 149 **Antigens**  
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43 151 Recombinant capsid proteins in the format of VLPs of the two NoV genotypes, GII-4 [004/95M-  
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45 152 14/1995/AU] and GII-12 [HU/NLV/Wortley/90/UK] were used as coating antigens in enzyme-  
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48 153 linked immunosorbent assays (ELISA). The VLPs were produced using baculovirus-insect cell  
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51 154 expression system as described previously [Jiang et al. 1992]. VLP production and purification are  
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53 155 described in details elsewhere [T. Koho et al., manuscript in preparation]. The morphology and the  
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55 156 antigenicity of the VLPs produced were verified with electron microscopy and western blot (data  
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58 157 not shown).  
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60**Serum antibody ELISA and avidity assay**

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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 (SIGMAFAST 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<sub>2</sub>SO<sub>4</sub>). Absorbance (optical density, OD) at a wavelength of 490 nm was measured in a microplate reader (Victor<sup>2</sup> 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

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

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

### 194 Carbohydrate blocking assay

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

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3 208 Background signal from the blank wells (wells lacking HBGA) was subtracted from all of the OD  
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10 211 **Statistical analyses**  
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15 213 Pearson's chi-square test was performed to examine the differences in antibody prevalence between  
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17 214 age groups. Student's *t*-test was used to assess the intergroup differences in antibody magnitude and  
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20 215 avidity. All hypothesis testing was two-tailed. Pearson's correlation coefficient was used to  
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22 216 determine the correlation between GII-4 and GII-12 specific antibody levels. Mann-Whitney U-test  
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25 217 was used to compare the intergroup differences in the blocking ability of the sera. Statistical  
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27 218 significance was defined as  $P < 0.05$ .  
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## 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  $61.5 \pm 5.1\%$ ) were detected in infants  $\leq 6$  months of age. The mean avidity index was significantly lower in children aged 7-12 months ( $40.4 \pm 3.9\%$ ) than in children aged 0-6 months ( $P < 0.05$ ). The highest of all mean avidity indexes ( $85.6 \pm 4.5\%$ ) were detected in older children, who had most probably

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3 258 encountered several NoV infections during their lifetime. The lowest frequency of the high avidity  
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6 259 antibodies was detected in the primary infections whereas the highest frequency was observed in the  
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8 260 recurrent infections ( $P<0.001$ ). All the adult control sera ( $n=6$ ) contained high avidity antibodies  
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10 261 (data not shown).

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### 15 263 **Cross reactivity of NoV antibodies**

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20 265 To determine the immunological cross-reactivity of the sera the seroprevalence was assayed against  
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22 266 GII-12 which so far has not been reported in Finland [Maunula and Von Bonsdorff. 2005, S.  
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25 267 Räsänen et al., manuscript in preparation]. Of the GII-4 positive sera 70.8% were also IgG positive  
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27 268 against GII-12 VLPs. A subset of GII-4 IgA positive samples (96 out of 197) was tested and found  
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29 269 that 47.6% of these were IgA cross-reactive for GII-12. When the GII-12 antibody responses were  
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32 270 compared to the responses obtained with GII-4 VLPs a positive correlation was detected overall  
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34 271 ( $r=0.64$ ) although the mean ODs were generally lower for GII-12-specific responses (data not  
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36 272 shown). Sera of the adult individuals were all positive for GII-12 (data not shown). Cross-reactivity  
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39 273 between the two genotypes was further confirmed using paired sera of three patients infected with  
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41 274 GII-4 NoV (Fig. 3). All the paired sera showed IgG seroconversion not only to GII-4 (Fig. 3A) but  
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44 275 also to GII-12 antigen, although with lower magnitude of the response (Fig. 3B).

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### 48 277 **Correlation of NoV infections to antibody prevalence and levels**

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53 279 Of the 492 children encountered in hospital because of AGE, 97 (19.7%) were diagnosed with NoV  
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55 280 infection by RT-PCR [S. Räsänen et al., manuscript in preparation]. The incidence of NoV  
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58 281 gastroenteritis was highest in children aged 7-23 months (53 out of 97 cases; 54.6%). Of these cases  
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60 282 60.4% were primary infections as judged by the absence of NoV antibodies at the acute stage while

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3 283 39.6% had pre-existing antibodies to NoV. To determine the possible protective effect of the pre-  
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6 284 existing NoV-specific antibodies against a subsequent NoV infection the OD values in the different  
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8 285 age groups obtained in ELISA IgG assay were compared. The lowest pre-existing antibody level  
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10 286 (mean OD  $0.66 \pm 0.06$ ) as well as antibody prevalence (47.3%, Fig. 1) were detected in the age  
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13 287 group 7-23 months (Fig. 4). In the age group 2-4 years mean OD level increased ( $1.11 \pm 0.07$ ) and  
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15 288 by the age of 14 years children had high levels of preexisting antibodies to NoV (mean OD  $1.62 \pm$   
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17 289  $0.10$ ) in the majority of individuals (91.2%, Fig. 1) and the least infections (14.7%) (Fig. 4).  
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20 290 Therefore, a trend to a negative association between the incidence of infection and the level of pre-  
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22 291 existing antibodies was observed.  
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### 26 293 **Antibody blocking**

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32 295 Acute sera obtained from children infected during a waterborne outbreak of AGE in the town of  
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34 296 Nokia, Finland [Räsänen et al. 2010] with NoV GII-4 or other agent causing acute gastroenteritis  
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36 297 were tested for blocking of the GII-4 VLPs binding to H-type-3 HBGA. Higher blocking activity  
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39 298 was seen in the sera of children not infected with NoV than in the sera of children with NoV  
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41 299 infection ( $P < 0.05$ ) (Fig 5A). In addition, blocking of the VLPs binding to H-type-3 antigen was  
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43 300 performed with 1:100 dilutions of paired sera from eight children with NoV gastroenteritis (Fig.  
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46 301 5B). The blocking activity increased after a recent NoV infection. The acute sera could block on  
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48 302 average 24.8% of the maximum GII-4 binding to H-type 3 whereas 61.6% of the binding was  
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51 303 blocked by the convalescent sera ( $P < 0.05$ ).  
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3 308 **DISCUSSION**

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8 310 A study conducted 20 years ago to determine the Norwalk virus (GI-1) seroprevalence in young  
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10 311 Finnish children [Lew et al. 1994] showed that 49% of children 4 to 23 months of age had NoV  
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12 312 antibodies. At that time NoV GII-4 was not common. Nearly identical seroprevalence for GII-4 in  
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15 313 the similar age group was found in the present study. The data therefore confirm that Finnish  
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17 314 children acquire NoV infection at an early age and with increasing age the number of natural  
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20 315 encounters with the virus accumulates, as judged by the increasing seroprevalence and the  
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22 316 prevalence of high avidity antibodies. Prospective studies in Finland have shown that clinically  
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25 317 manifest NoV gastroenteritis is seen in 20-30% of Finnish children by the age of 2 years [Pang et al.  
26  
27 318 1999, Zeng et al. 2010]. As the seroprevalence increases even more rapidly, it is likely that many  
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29 319 NoV infections are subclinical. The prevalence of NoV antibodies in children has been reported in  
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32 320 several European countries, Asia, Africa and Latin America [Gray et al. 1993, Nakata et al. 1998,  
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34 321 Jing et al. 2000, Talal et al. 2000, Farkas et al. 2006, Kobayashi et al. 2009] and the acquisition of  
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36 322 NoV GII-4 specific antibodies, especially in the developed countries, is consistent with the results  
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39 323 of the present study [Pelosi et al. 1999, Nicollier-Jamot et al. 2003]. Infants under 6 months of age  
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41 324 typically have high antibody levels, which are explained by the presence of maternal antibodies  
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43 325 [Gray et al. 1993, Numata et al. 1994]. After 6 months of age, when the maternal antibodies decline,  
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46 326 the seroprevalence decreases and starts rising again after 12-24 months of age reaching 90-100% in  
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48 327 older children and adult population [Matsui and Greenberg. 2000].

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53 329 All the adult sera and the majority of children's sera tested in this study recognized both GII-4 and  
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55 330 GII-12 VLPs in ELISA, although the level of reactivity was higher against GII-4. The likely reason  
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57  
58 331 may be cross reactive epitopes rather than previous exposure to NoV GII-12 as GII-12 genotype has  
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60 332 not been reported in Finland [Maunula and Von Bonsdorff. 2005, Räsänen et al., manuscript in

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3 333 preparation]. In addition, these results imply the existence of highly conserved antibody epitopes  
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6 334 between the genotypes within a genogroup. We also showed that a seroconversion caused by GII-4  
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8 335 infection could be detected with a heterotypic antigen (GII-12) although the response to homotypic  
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10 336 VLPs was stronger. Other reports also found that seroconversion can be observed in convalescent  
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12 337 sera using VLPs from genetically distinct virus from the one causing the primary infection [Belliot  
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15 338 et al. 2001]. Despite the cross-reactivity, greater seroresponses are detected against homologous  
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17 339 strain, and strains belonging to the same genogroup react more strongly than the strains across the  
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19 340 genogroups [Treanor et al. 1993, Farkas et al. 2003, Lindesmith et al. 2010]. Because of the cross  
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21 341 reactive immunity and the presence of the pre-existing antibodies detected, ELISA method as the  
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23 342 one described in this study and routinely used by others [Rockx et al. 2005, Iritani et al. 2007] does  
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25 343 not give precise serotype-specific determination of the immune response.  
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31 345 The avidity and the level of NoV specific IgG antibodies are low after primary infections in young  
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33 346 children. The avidity increases with time and particularly as a result of repeated infections when  
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35 347 molecular evolution specifies the antigen binding domains of the antibodies to recognize the target  
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37 348 epitopes more precisely [Murphy et al. 2008]. Indeed, high avidity antibodies were detected in older  
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39 349 children and adults who are likely to have experienced NoV infections repeatedly throughout life.  
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41 350 The high avidity antibodies detected in infants less than 6 months of age presumably represent  
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43 351 transplacentally acquired maternal IgG antibodies [Gray et al. 1993, Numata et al. 1994]. The  
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45 352 results show that 20% of infants under 6 months of age already had NoV infection as judged by the  
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47 353 presence of NoV IgA antibodies. Such incidence of infection suggests that transplacentally acquired  
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49 354 IgG antibodies do not offer full protection.  
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55 356 The present study showed that children who were exposed to NoV in a waterborne outbreak of  
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57 357 AGE [Räsänen et al. 2010] but were not infected by NoV, had higher blocking activity of GII-4  
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3 358 VLPs binding to H type 3 HBGA compared to those who were infected. Interestingly, the acute sera  
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6 359 from not-infected children had similar blocking ability as the convalescent sera from children  
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8 360 recently suffering from a NoV infection. The increase in the blocking ability of the serum after the  
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10 361 NoV infection has been hypothesised to be associated with a short-term immunity to NoV infection  
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13 362 [Rockx et al. 2005]. The results shown in the current study also suggest that high blocking activity  
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15 363 of VLPs binding to HBGA in the acute serum may confer protection against reinfection. Blocking  
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17 364 activity of the antibodies may be a relevant surrogate marker of NoV protection when considering  
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20 365 different vaccine approaches.  
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25 367 In this study, the majority of sporadic NoV cases treated in hospital occurred in children 7-23  
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27 368 months of age. This age group also had the lowest pre-existing antibody levels. Therefore, a trend to  
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29 369 a negative correlation between the susceptibility to NoV infection and pre-existing IgG antibodies  
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32 370 was observed. A previous study conducted in Finland [Lew et al. 1994] suggested that children with  
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34 371 low NoV-specific IgG titers (<1:50) are more susceptible to NoV infection in the next 10 months  
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36 372 than children with higher titers (>1:50). In the current study it was shown that older children with  
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39 373 higher NoV specific antibody levels and avidity had also fewer infections than children less than 2  
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41 374 years old. It may be speculated that NoV infections before the age of two years induce protection  
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43 375 for later ages as the children develop protective immune response to NoV. This is in support of the  
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46 376 concept of NoV vaccination in young infants in order to decrease the burden NoV of infection as  
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48 377 well as the hospitalization cases due to NoV gastroenteritis.  
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3 383 ACKNOWLEDGEMENTS  
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6 384  
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14 388 samples and Marjo Salonen for organizing serum collection.  
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3 556 **FIGURE CAPTIONS**

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8 558 Fig. 1. Age-related prevalence of immunoglobulin (Ig) G and IgA serum antibodies to norovirus  
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10 559 GII-4 in Finnish children and adults.

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15 561 Fig. 2. Avidity indexes of immunoglobulin G antibodies to norovirus GII-4 in three age groups.  
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17 562 Bars represent the mean avidity index of the group. Boxed values indicate the frequency (%) of  
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20 563 high avidity antibodies in the age group. \* $P < 0.05$ , \*\* $P < 0.0001$  (Student's *t*-test).

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25 565 Fig. 3. Serum titration of three norovirus (NoV) GII-4 infected pediatric patients against NoV virus  
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27 566 like particles (VLPs). Sera were diluted 2-fold starting from 1:100 and tested in an enzyme-linked  
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29 567 immunosorbent assay using GII-4 VLPs (A) or GII-12 VLPs (B) as antigens. Acute sera titrations  
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32 568 are shown in dotted lines and convalescent sera titrations in full lines.

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36 570 Fig. 4. Age distribution of frequency of cases of norovirus (NoV) infections encountered in hospital  
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39 571 and the levels of NoV GII-4 specific immunoglobulin G antibodies by age group. The bars  
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41 572 represent the percentages of NoV infected children in each age group of 97 total infection cases.  
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43 573 The mean optical density (OD) values of each group sera (1:100 dilution) tested by enzyme-linked  
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46 574 immunosorbent assay are shown (◆) on the secondary y-axis.

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51 576 Fig. 5. Blocking of norovirus (NoV) GII-4 virus like particles (VLPs) binding to H-type-3 HBGA  
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53 577 by the acute sera of NoV GII-4 infected (n=9) or not infected (n=6) children (A), and by the paired  
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55 578 sera from children (n=8) infected with NoV GII-4 (B). Shown are mean optical density (OD) values  
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58 579 of the tested sera (1:100 dilutions). The mean OD of the binding control indicates the maximum  
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60 580 binding of the GII-4 VLPs to H-type-3. Error bars represent the standard error of the mean (SEM).

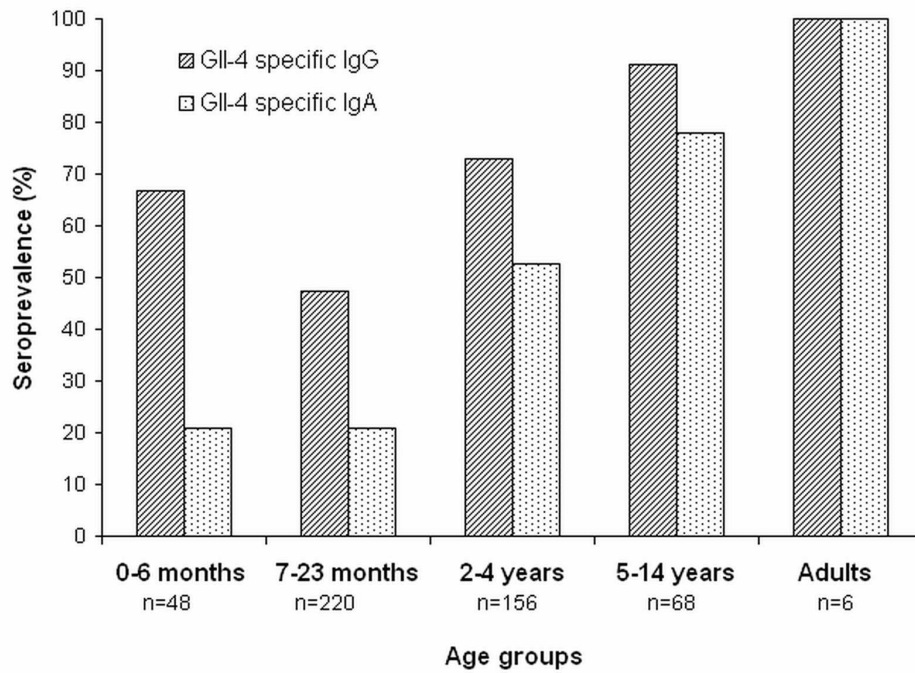


Fig. 1. Age-related prevalence of immunoglobulin (Ig) G and IgA serum antibodies to norovirus GII-4 in Finnish children and adults.  
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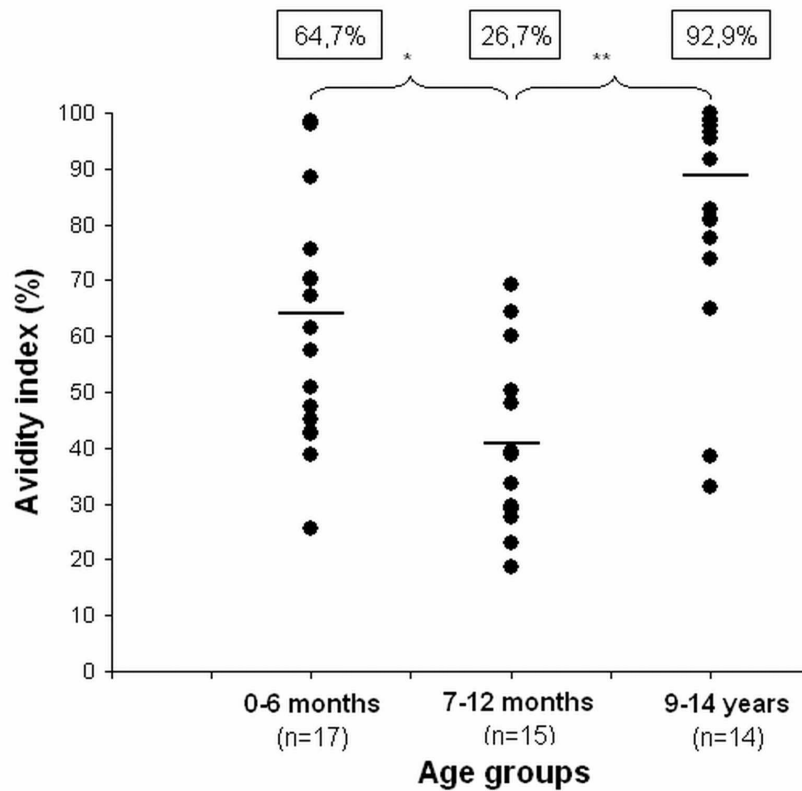


Fig. 2. Avidity indexes of immunoglobulin G antibodies to norovirus GII-4 in three age groups. Bars represent the mean avidity index of the group. Boxed values indicate the frequency (%) of high avidity antibodies in the age group. \* $P < 0.05$ , \*\* $P < 0.0001$  (Student's t-test).  
80x65mm (600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

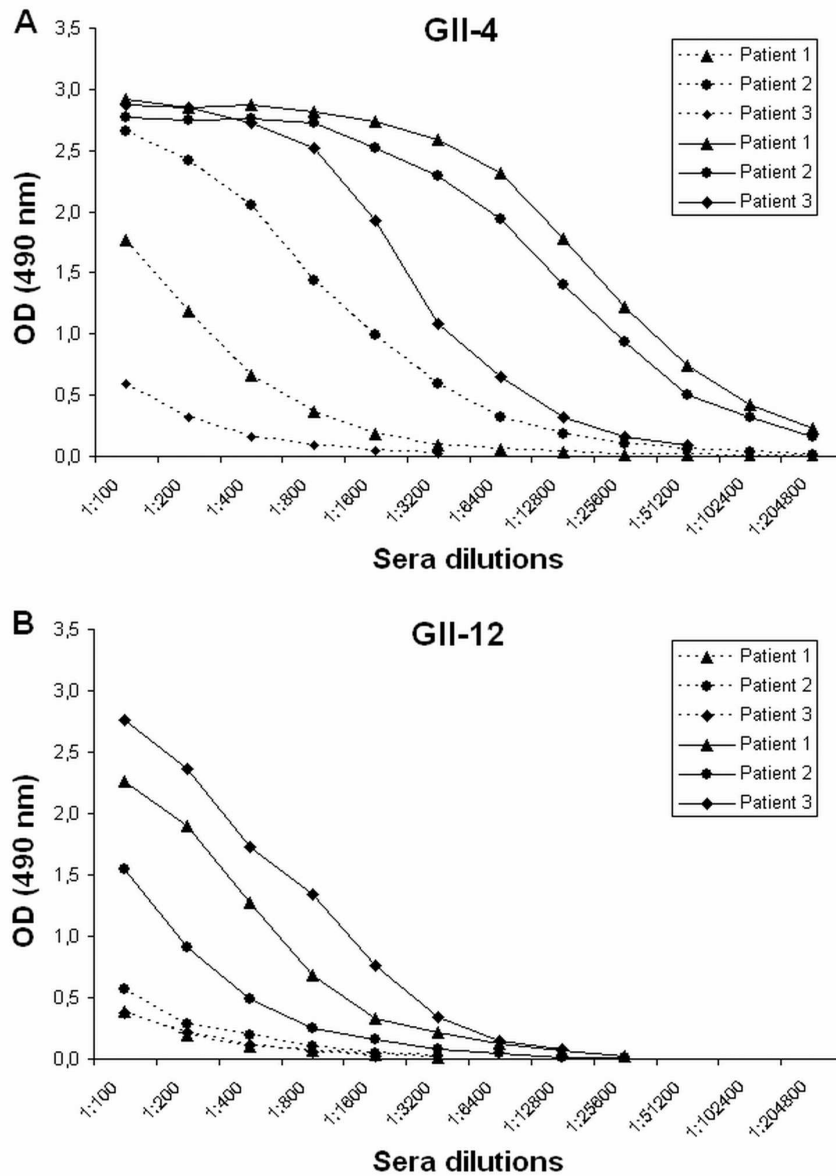


Fig. 3. Serum titration of three norovirus (NoV) GII-4 infected pediatric patients against NoV virus like particles (VLPs). Sera were diluted 2-fold starting from 1:100 and tested in an enzyme-linked immunosorbent assay using GII-4 VLPs (A) or GII-12 VLPs (B) as antigens. Acute sera titrations are shown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

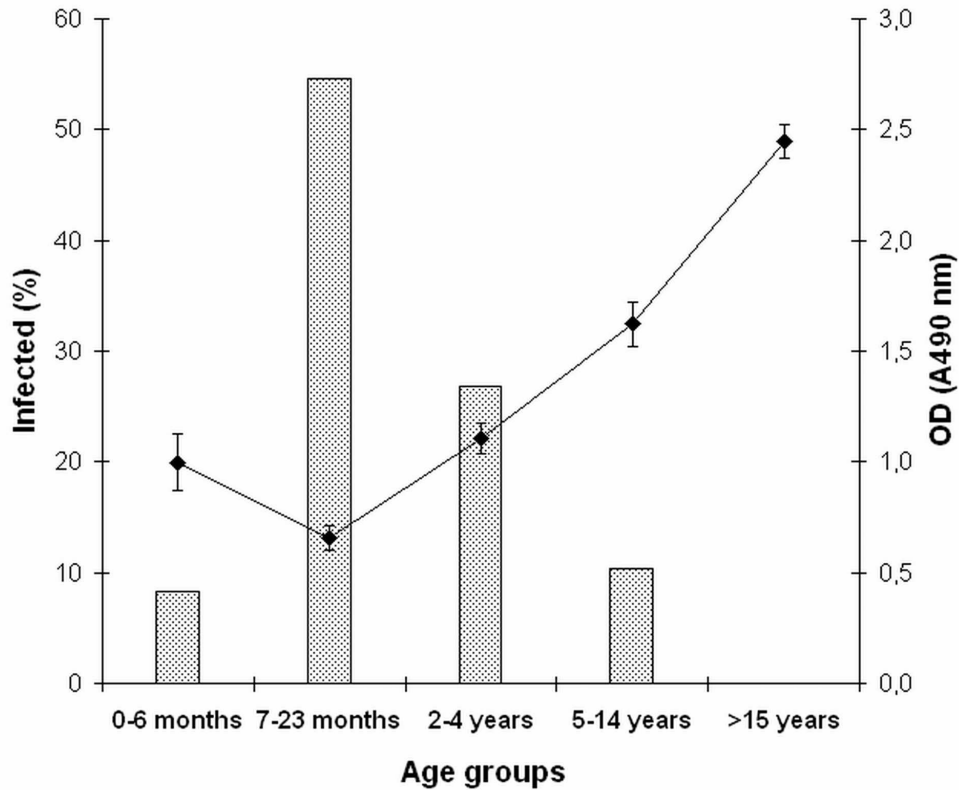


Fig. 4. Age distribution of frequency of cases of norovirus (NoV) infections encountered in hospital and the levels of NoV GII-4 specific immunoglobulin G antibodies by age group. The bars represent the percentages of NoV infected children in each age group of 97 total infection cases. The mean optical 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)

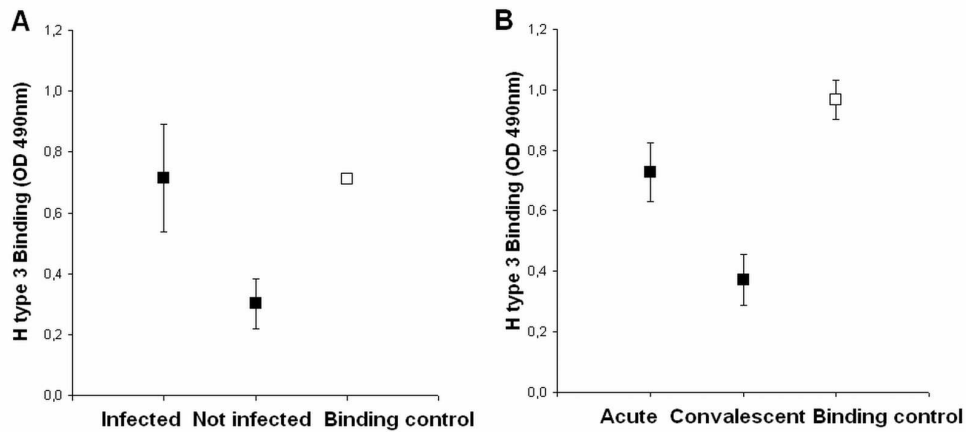


Fig. 5. Blocking of norovirus (NoV) GII-4 virus like particles (VLPs) binding to H-type-3 HBGA by the acute sera of NoV GII-4 infected (n=9) or not infected (n=6) children (A), and by the paired sera from children (n=8) infected with NoV GII-4 (B). Shown are mean optical density (OD) values of the tested 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)