Virus Antibody Survey in Different European Populations Indicates Risk Association Between Coxsackievirus B1 and Type 1 Diabetes

Running title: Group B Coxsackievirus Infections and Type 1 Diabetes

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ABSTRACT

Enteroviruses have been connected to type 1 diabetes in various studies. The current study evaluates the association between specific enterovirus subtypes and type 1 diabetes by measuring type-specific antibodies against the group B coxsackieviruses (CBV) which has been linked to diabetes in previous surveys. Altogether 249 children with newly diagnosed type 1 diabetes and 249 control children matched according to sampling time, gender, age and country were recruited in Finland, Sweden, England, France and Greece during the years 2001-2005 (mean age 9 years; 55 % boys). Antibodies against CBV1 were more frequent among diabetic children than in control children (OR=1.7, 95%CI=1.0-2.9) while other CBV types did not differ between the groups. CBV1-associated risk was not related to HLA genotype, age or gender. Finnish children had lower frequency of CBV antibodies than children in other countries. The results support previous studies suggesting an association between group B coxsackieviruses and type 1 diabetes, highlighting the possible role of CBV1 as a diabetogenic virus type.
INTRODUCTION

A connection between enterovirus (EV) infections and human type 1 diabetes has been documented in a variety of studies (1-3). Meta-analyses have indicated a clear risk effect in studies which have been based on direct detection of EVs in blood or tissues (Odds Ratio ranging between 5.5 and 17.4) (4). However, serological studies have shown inconsistent results (5). Accordingly, it is possible that an invasive infection, reflected by the presence of EV in blood or tissues, is needed for the development of β-cell damage rather than superficial i.e. mucosal infection.

On the other hand, many serological studies have been based on methods which cannot differentiate EV types from each other. In a scenario where only some of the more than 100 different EV types would induce type 1 diabetes, their effect could easily be missed if only pan-EV assays were used (antibodies against diabetogenic EV types can be masked by antibodies against all other EV types). For example, one can argue that if the etiology of poliomyelitis would have been evaluated in retrospective case-control studies it would have been impossible to identify the three EV types causing polio paralysis (poliovirus types 1-3) using such broadly reactive pan-EV antibody assays. This is due to the fact that the background frequency of EV infections was high when poliovirus antibody studies were conducted. This is a particularly relevant aspect if antibodies are measured a long time after the causative infection, e.g. at the time when clinical type 1 diabetes becomes clinically apparent.

The present study was designed to detect antibodies specifically against each of the six group B coxsackievirus serotypes (CBVs) in patients affected by type 1 diabetes and matched control subjects. We focused on the CBV group of EVs because previous studies have shown that human pancreatic islets strongly express the coxsackievirus and adenovirus receptor (CAR) (6). CAR is the major receptor for CBVs and it is not used by other EV types (7). Since the receptor binding explains partly the tropism of different EV serotypes to various organs (e.g. polioviruses to central
nervous system) the expression of CAR in the pancreatic islets fits with possible tropism of CBV group EVs to these cells. In fact, previous studies have documented the presence of EV RNA and EV proteins in the pancreatic islets of patients with type 1 diabetes (8-10), but the serotype of the involved EVs has not been identified. CBVs have also been linked to diabetes in mouse models, case-reports and case-control studies. In the early studies in the 1960s Gamble and Taylor reported neutralizing antibodies against CBV group EVs more frequently in patients affected by type 1 diabetes than in control subjects (11). Later studies have identified species B EV sequences in blood of type 1 diabetes patients and CBV4 has been isolated from the pancreas of a few patients with newly diagnosed type 1 diabetes (12, 13). In addition, we have recently observed that out of 41 different EV serotypes screened in prospectively observed children carrying HLA genes conferring susceptibility to type 1 diabetes, only CBV serotypes modulated the risk of β-cell autoimmunity and clinical type 1 diabetes (14).

In the current study the past exposure to different CBV serotypes was analyzed in children with type 1 diabetes and control subjects recruited in five European countries. The study was based on the measurement of neutralizing antibodies which are specific and sensitive indicators of past infection by a given EV serotype thus reflecting the infection history of the child (serological scar).

**RESEARCH DESIGN AND METHODS**

**Subjects**

The study population included 249 patients with newly diagnosed type 1 diabetes and 249 control subjects who were matched pair-wise according to the time of sampling, gender, age and country
They were recruited in Finland (103 case-control pairs), Sweden (58 pairs), England (13 pairs), France (43 pairs) and Greece (32 pairs) during the years 2001-2005 in the EU-funded “Viruses in Diabetes” (VirDiab) study. In Finland, the cases and controls were additionally matched for the HLA-conferred risk for type 1 diabetes and were recruited from the Diabetes Prediction and Prevention (DIPP) birth-cohort study which observes children who carry HLA risk genes (15). In other countries, control subjects were healthy schoolchildren or children coming for minor elective surgical operations to the local hospital. Children with malignant or autoimmune diseases, chronic infections (e.g. hepatitis etc.), patients from infectious disease wards and case child’s family members or classmates were not accepted as controls. However, children with sporadic and common acute infections were not excluded. The diagnosis of type 1 diabetes was based on WHO criteria. Serum samples were collected for virus antibody analyses shortly after the diagnosis of type 1 diabetes (mean 3 days; range 0-31 days). In the control group corresponding serum samples were collected on an average 68 days after the diagnosis of type 1 diabetes in the matching case child. Since neutralizing antibodies reflect past infections and their prevalence was not associated with the season of the year we accepted quite long time difference in older children (range from 512 days before and 514 days after the corresponding case sample). EDTA blood was collected at the same time for genetic studies. Samples were stored at -80 °C until analyzed.

**Virus antibodies**

Neutralizing antibodies were measured against all six CBV serotypes (ATCC prototype strains) using a plaque neutralization assay at the Department of Virology, University of Tampere, Finland. In addition, antibodies against CBV1 and CBV3 serotypes were measured using wild-type virus strains (PicoBank strains isolated in Finland). The serotype of all viruses was confirmed by sequencing the VP1 coding region of the viral genome (16). The serum was first mixed with 100
pfu of the virus and incubated for 1 h at 37°C followed by O/N incubation at RT. This mixture was then transferred on monolayer of green monkey kidney cells (GMK) on six-well plates in plaque assay medium containing The Minimal Essential Medium (MEM) supplemented with 1% fetal bovine serum, 40U/ml Penicillin-Streptomycin, 0.0023% Glucose, 1*L-GLutamine, 1,5mM MgCl2 and 1,5mM Carboxymethyl Cellulose (HEPES). The number of plaques was counted after 48h of incubation at 37°C. All test runs included both virus positive and virus negative control wells (17). Sera were tested using two dilutions (1/4 and 1/16) and sample was judged seropositive if either of these dilutions inhibited more than 80% of the plaques. Samples were also analyzed for higher titers of antibodies against ATCC CBV strains (titers 1/64, 1/256 and 1/1024).

**Diabetes-associated autoantibodies**

Islet cell antibodies (ICA) were detected by indirect immunofluorescence, while the three other autoantibodies insulin autoantibodies (IAA), glutamic acid decarboxylase autoantibodies (GADA), and insulinoma-associated protein 2 autoantibodies (IA-2A) were quantified with radiolabel binding assays as previously described (18). We used cut-off limits for positivity of 2.5 Juvenile Diabetes Foundation units for ICA, 3.48 relative units (RU) for IAA, 5.36 RU for GADA, and 0.43 RU for IA-2A representing the 99th percentile in more than 350 Finnish children. The disease sensitivity and specificity of the islet cell antibody assay were 100% and 98% in the fourth round of the International Workshops on Standardization of the ICA assay. The disease sensitivity of the IAA assay was 58% and the specificity 100% in the 2005 Diabetes Autoantibody Standardization Program Workshop. The same characteristics of the GADA assay were 82% and 96% and those of the IA-2A assay 72% and 100%, respectively.
HLA genotyping

Alleles in the HLA-DQB1, -DQA1 and DRB1 genes were defined using panels of sequence specific oligonucleotides and presence or lack of disease-associated HLA-DRB1*03-DQA1*05-DQB1*02 and HLA-DRB1*04-DQA1*03-DQB1*03:02 haplotypes documented in each subject (19). Altogether 189 patients and 171 controls were available for genetic analyses providing 148 HLA-typed case-control pairs.

Statistical analyses

Descriptive statistics are presented as proportions, frequencies, and means for demographic data and proportions and frequencies for genotypes and autoantibodies. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for factors associated with type 1 diabetes, using conditional logistic regression to account for the matched pair study design. $P<0.05$ (two-tailed) was considered statistically significant. Data were analyzed using STATA 12.1 (StataCorp LP; College Station, TX, USA).
RESULTS

Neutralizing antibodies against group B coxsackieviruses in different countries and age groups

The prevalence of antibodies against different CBV serotypes varied. CBV2, CBV3, CBV4 and CBV5 were the most common serotypes while CBV1 and CBV6 were less frequent (Fig 1). CBV antibody prevalence varied also considerably between countries. The most striking difference was that Finland had less CBV infections than other countries: All six CBV serotypes were most uncommon in Finland except CBV6 which was relatively rare in all countries (Fig. 1). The antibody prevalence increased by age in children younger than 8 years whereafter a plateau was observed (Fig. 2a). Altogether 28% of the children were seropositive for at least one CBV serotype before the age of 4 years compared to more than 80% of children older than 8 years ($P<0.001$). This age-association varied for different CBV serotypes. CBV1 was frequent already at a very young age but did not show as sharp increase at older age as CBV2-5 (Fig. 2b). The average antibody levels showed also clear variation between CBV types: Antibody responses against CBV3 and CBV4 were the strongest while the antibody responses against CBV1 and CBV6 were the weakest (Fig. 3).

Neutralizing antibodies against group B coxsackieviruses in patients with type 1 diabetes and control subjects

Neutralizing antibodies were first measured against all six CBV types using prototype ATCC CBV strains. Antibodies to CBV1 were the only ones whose prevalence differed between the case and control subjects, being more frequent in diabetic children (Table 2). This finding was confirmed using a wild-type CBV1 strain, which gave almost identical results showing the significantly higher
prevalence of CBV1 antibodies among cases (Table 2). The risk character of CBV1 was seen in both genders and in different age groups (data not shown). It was also observed when different antibody levels were used as a cut-off for seropositivity (titer 1/16: OR=1.7, 95% CI 0.9-3.4, \(P=0.133\); titer 1/64: OR=1.6, 95% CI 0.8-3.5, \(P=0.198\); titer 1/256: OR=2.0, 95% CI 0.7-5.9, \(P=0.206\)) and when the effect of CBV1 was adjusted for the effect of the HLA-DQ genotype (adjusted OR for ATCC-CBV1: 1.6; 95% CI 0.8-3.3; \(P=0.173\)), although these analyses did not reach statistical significance (only a subgroup of children were analyzed for different antibody levels and HLA alleles).

Since the prevalence of CBV antibodies was considerably lower in Finland than in other countries, we compared CBV antibodies between case and control subjects separately in Finland and in other countries. CBV1 showed a similar trend for increased frequency in case subjects both in Finland (OR=2.2; 95% CI 0.8-5.7) and in other countries (OR=1.5; 95% CI 0.8-3.0) although the difference was not statistically significant in these subgroups.

None of the other CBV serotypes differed between cases and controls. The overall cumulative number of antibodies to different CBV types did not differ between the case and control groups either (mean 1.8 vs. 1.7 different CBV types, respectively).
DISCUSSION

The results of the current study are in line with those from previous studies suggesting an association between group B coxsackieviruses and type 1 diabetes. In the current study CBV1 was the only CBV serotype showing this association. The significance of this finding is emphasized by the fact that the very same virus type has recently been observed to increase the risk of type 1 diabetes in the large prospective DIPP study (14). In that study EV infections were identified by measuring neutralizing antibodies against 41 different EV serotypes in children who were followed from birth and who developed multiple type 1 diabetes-associated autoantibodies as well as in control children. In that study the risk association of CBV1 was seen already before type 1 diabetes-associated autoantibodies appeared which suggests a potential role of CBV1 infections in the initiation of the β-cell damaging process (14).

The identification of CBV1 as a risk virus in these two independent studies supports the biological significance of this finding, since the detection of the same signal in both studies just by chance is unlikely, especially because the designs of these two studies were different in many ways. The current study is based on a cross-sectional study design including children who had recently been diagnosed with clinical type 1 diabetes while the DIPP study was based on a prospective birth cohort where samples had been taken during the prediabetic. In principle, the detection of neutralizing antibodies in the present study covered all past CBV infections, including those that did occur after the β-cell damaging process was initiated. In addition, antibodies were measured using ATCC reference strains while only wild-type strains were used in the DIPP study, and DIPP samples were also analyzed in another laboratory. Most importantly, the current study covered different countries and populations while the DIPP study was carried out only in Finland, and the children analyzed in these two studies were conceived during different time periods. However, in spite of these facts, it is still possible that the risk character of different CBVs may vary according
to the time and place, and we cannot exclude the possibility that other CBV types such as CBV4, could also be associated with type 1 diabetes in a certain time period or geographic region as suggested in previous studies (11, 12, 13).

One limitation of the current study is that cases and controls were not completely matched for type 1 diabetes-associated HLA genotypes. Previous studies have suggested that these genotypes may modulate antibody responses against EVs (20) and detection of EVs in patients with type 1 diabetes (21). The HLA genotype is also known to modulate the immune response and the course of many other virus infections (22, 23). However, such an HLA effect can hardly explain the elevated CBV1 antibodies in patients with type 1 diabetes since the risk effect of CBV1 remained when adjusted for the effect of HLA and it was seen also in Finland where cases and controls were matched for diabetes-associated HLA genotypes. In addition, the same risk effect of CBV1 was recently observed in the DIPP study where cases and controls were matched for type 1 diabetes-associated HLA genotypes (14). An additional limitation of the study is that the time of sample draw was not exactly the same in cases and their matched controls (the sample from controls was taken on an average 68 days after the diagnosis of T1D in the case child). However, considering the facts that neutralizing antibodies remain elevated for years after the infection and that the samples were taken randomly all year around and at a later time point in controls it is unlikely that this could explain the increased frequency of CBV1 antibodies in the case children.

The measurement of neutralizing antibodies allowed us to study the past exposure to individual CBV serotypes in an unbiased way. These antibodies persist for years or decades being a sensitive indicator of past infection. They are also highly specific for the EV serotype used in the assay suggesting that that elevated CBV1 antibodies in patients with type 1 diabetes are not elicited by unspecific immune reactivity. The specific nature of this observation is also supported by the facts that the difference in CBV1 antibodies between patients and controls was seen both at low and high
antibody levels and that the antibodies against all other CBV types did not differ between cases and controls. Neutralizing antibodies are also biologically active since they neutralize the infectivity of the virus and correlate with immune protection against that EV serotype. However, on some occasions the neutralizing antibody response can also remain low and be even transient, especially if the infection is caused by a low dose of the virus (24). Therefore, the prevalence of neutralizing antibodies in this kind of cross-sectional retrospective surveys may underestimate the true number of past infections making it difficult to assess the proportion of diabetes cases which could be causally linked to CBVs.

Based on the current findings the neutralizing antibody levels against CBV1 and CBV6 were lower than those against other CBV types, and they did not increase by age as clearly as antibodies against other serotypes, suggesting that a part of CBV1 and CBV6 infections may have remained undiagnosed. This kind of variation in neutralizing antibody responses against different serotypes has also been described after vaccinations with live poliovirus vaccine (poliovirus type 1 and 3 induce lower antibody responses than poliovirus type 2). Such a low neutralizing antibody response may also have biological significance making the virus able to evade the host’s immune response. This is clearly seen in patients with antibody deficiencies since they are suffering from severe and chronic EV infections. This kind of scenario has been proposed for human parechoviruses (HPeVs) which are close relatives of EVs (25).

In our previous study in the prospective DIPP birth-cohort CBV3 was observed to have a strong protective effect against type 1 diabetes (14). Further analyses indicated that this effect could be mediated by immunological cross-protection against CBV1 infections attenuating its diabetogenic effect. However, this kind of protective effect of CBV3 was not detected in the current study. One possible explanation is related to the chronological order of CBV1 and CBV3 infections. In the DIPP series we were able to identify the exact timing of infections by measuring antibodies in
longitudinal follow-up samples, and the protective effect of CBV3 was related to early CBV3 infections which occurred before CBV1 (14). Accordingly, the possible protective effect of early CBV3 infections may have remained unrecognized in the current study since this “serological scar” may have been faded by antibodies induced by the later CBV3 infections occurring after CBV1.

The prevalence of CBV antibodies was lower in Finland than in other counties supporting our previous studies indicating a relatively low frequency of EV infections in Finland (26). In contrast to EV infections, the incidence of type 1 diabetes is exceptionally high in Finland being the highest in the world (about 60/100,000 children/year) (27). The frequency of EV infections has also declined over the past decades both in Finland and in Sweden while the incidence of type 1 diabetes has increased in both countries (17). Based on these findings we have earlier launched the “polio hypothesis” which claims that a low frequency of EV infections in the background population increases the risk of EV-induced β-cell damage (28). This hypothesis is based on an analogous experience from another EV disease, paralytic poliomyelitis, which is the well-known complication of poliovirus infection and similarly associated with low frequency of poliovirus infections in the population (28). Polio hypothesis has recently been supported by mouse studies showing that the absence of maternal EV antibodies increases the risk for severe outcomes of an EV infection in offspring (29). However, this relationship was not absolute since e.g. Sweden had a relatively high frequency of infections although it has also a high incidence of T1D.

In conclusion, the current study supports the idea that the group of B coxsackieviruses may include diabetogenic virus types and indicates that CBV1 may be one of them. However, based on the present data it is difficult to predict how large fraction of type 1 diabetes could be causally related to CBV infections. The experience from other EV diseases such as polio suggests that a very small proportion of infected individuals will ever develop the disease leading to a scenario where the antibody prevalence can be almost similar in case and control subjects in this kind of cross-sectional
studies (30). Therefore, prospective studies would be needed to confirm the risk effect of CBV infections and to evaluate their etiologic fraction. In any case, the present observations together with previous studies showing that CBV1 is frequent (31) and able to cause insulitis and islet cell damage in young babies (32) as well as pancreatitis, transient diabetes (33, 34) and persisting infection in mice (2) make it an attractive target for further studies addressing the possible role of EVs in the pathogenesis of type 1 diabetes.

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S.O. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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The members of the VirDiab study group have participated in the planning of the study and all authors have participated in the review and edition of the manuscript. In addition, S.O. organized laboratory work and data analyses and wrote the manuscript. S.T. and A.S.K. organized virus
antibody analyses and participated in the data analysis. J.I. was responsible for the HLA genotyping and M.K. for the autoantibody analyses. P.K. and M.T.S. were responsible for the recruitment of study subjects in Finland and K.T. in England. H.Hu. carried out the statistical analysis of the data. H.H. was the coordinator of the VirDiab project, and responsible for the overall scientific management of the project.

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Competing Interests

H.H and M.K. are both minor (<5%) shareholders and members of the board of Vactech Ltd., which develops vaccines against picornaviruses. All other authors have no conflicts of interests.
<table>
<thead>
<tr>
<th></th>
<th>T1D</th>
<th>Controls</th>
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</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>249</td>
<td>249</td>
</tr>
<tr>
<td><strong>Recruitment period</strong></td>
<td>May 2001-January</td>
<td>May 2001-April 2005</td>
</tr>
<tr>
<td><strong>Mean age (mean years and range)</strong></td>
<td>9.0 (1.1-22.7)</td>
<td>9.0 (1.0-23.5)</td>
</tr>
<tr>
<td><strong>Gender (% males)</strong></td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td><strong>Time of sampling</strong></td>
<td>3 (0-31)</td>
<td>68 (-512-514)</td>
</tr>
<tr>
<td>(mean days and range since T1D diagnosis in case child)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>HLA-DQB genotype (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DR3</td>
<td>20.6</td>
<td>12.3</td>
</tr>
<tr>
<td>DR3/DR4</td>
<td>27.0</td>
<td>8.8</td>
</tr>
<tr>
<td>DR4</td>
<td>37.0</td>
<td>46.8(^1)</td>
</tr>
<tr>
<td>x</td>
<td>15.3</td>
<td>32.2</td>
</tr>
<tr>
<td><strong>Autoantibody positive (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA</td>
<td>95.0</td>
<td>5.8</td>
</tr>
<tr>
<td>IAA</td>
<td>36.3</td>
<td>0.6</td>
</tr>
<tr>
<td>GADA</td>
<td>59.4</td>
<td>0.0</td>
</tr>
<tr>
<td>IA-2A</td>
<td>74.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

\(^1\)The high frequency of HLA-DR4 in the control group is due to the Finnish control children who were matched with cases also for HLA-DR and were participating in the prospective DIPP study recruiting children with type 1 diabetes—associated HLA-DR alleles (15).
TABLE 2

Neutralizing antibodies against different CBV serotypes in patients with type 1 diabetes and control subjects. Neutralizing antibody-positive were samples having a titer ≥4 by plaque assay.

<table>
<thead>
<tr>
<th>Antibody prevalence (%)</th>
<th>T1D (N=249)</th>
<th>Controls (N=249)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBV1-all</td>
<td>28.5</td>
<td>18.5</td>
<td>1.7</td>
<td>1.06-2.74</td>
<td>0.03</td>
</tr>
<tr>
<td>CBV1-ATCC</td>
<td>24.4</td>
<td>15.9</td>
<td>1.7</td>
<td>1.02-2.92</td>
<td>0.04</td>
</tr>
<tr>
<td>CBV1-wt</td>
<td>24.9</td>
<td>17.3</td>
<td>1.8</td>
<td>1.05-3.00</td>
<td>0.03</td>
</tr>
<tr>
<td>CBV2-ATCC</td>
<td>44.9</td>
<td>42.7</td>
<td>1.1</td>
<td>0.73-1.72</td>
<td>0.59</td>
</tr>
<tr>
<td>CBV3-all</td>
<td>37.3</td>
<td>40.6</td>
<td>0.8</td>
<td>0.55-1.20</td>
<td>0.29</td>
</tr>
<tr>
<td>CBV3-ATCC</td>
<td>37.2</td>
<td>40.3</td>
<td>0.8</td>
<td>0.56-1.24</td>
<td>0.38</td>
</tr>
<tr>
<td>CBV3-wt</td>
<td>30.0</td>
<td>33.7</td>
<td>0.8</td>
<td>0.54-1.32</td>
<td>0.45</td>
</tr>
<tr>
<td>CBV4-ATCC</td>
<td>35.5</td>
<td>38.4</td>
<td>0.9</td>
<td>0.52-1.46</td>
<td>0.60</td>
</tr>
<tr>
<td>CBV5-ATCC</td>
<td>38.0</td>
<td>34.5</td>
<td>1.1</td>
<td>0.69-1.9</td>
<td>0.61</td>
</tr>
<tr>
<td>CBV6-ATCC</td>
<td>10.3</td>
<td>11.6</td>
<td>0.8</td>
<td>0.42-1.45</td>
<td>0.44</td>
</tr>
</tbody>
</table>

ATCC=prototype virus strain from American Type Culture Collection;  wt=wild type virus strain
CBV-all= antibodies against either ATCC or wt strain
FIG. 1.

Proportion of seropositive children (%)
FIG. 2.

A

Proportion of seropositive children (%)

Age (years)

<2 3-4 5-6 7-8 9-10 11-12 13-14 15-

B

Proportion of seropositive children (%)

Age (years)

<2 3-4 5-6 7-8 9-10 11-12 13-14 15-
FIG. 3.
FIG. 1. Prevalence of neutralizing antibodies against different CBV serotypes in various countries (neutralizing antibody titer ≥4 by plaque assay). CBV1 (black bars), CBV2 (horizontally striped bars), CBV3 (white dotted bars), CBV4 (white bars), CBV5 (black dotted bars), CBV6 (skew striped bars).

FIG. 2. Proportion of children positive for CBV antibodies in different age groups. (neutralizing antibody titer ≥4 by plaque assay). Children positive for at least one CBV serotype (A); Children positive for individual CBV serotypes (B). White diamond presents children positive for CBV1, black diamond for CBV1wt, black circle for CBV2, black triangle for CBV3, white triangle for CBV3wt, white circle for CBV4, black square for CBV5 and white square for CBV6.

FIG. 3. Relative proportion of low and high antibody titers among children seropositive for different CBV serotypes. Altogether 249 patients and 249 control subjects were analyzed for the presence of different titers of antibodies against ATCC CBV strains. Black bars represent titer of 1/4, skew striped bars titer 1/16, dotted bars titer 1/64, horizontally striped bars titer 1/256 and white bars titer 1/1024. Statistical significances when the distribution of CBV1 antibody titers was compared to that of other serotypes: CBV1 vs. CBV2: \( P=0.0001 \); CBV1 vs. CBV3: \( P=0.0001 \); CBV1 vs. CBV4: \( P=0.0001 \); CBV1 vs. CBV5: \( P=0.008 \); CBV1 vs. CBV6: \( P=0.0001 \).
References


