Fluctuations in antibody titers against enterovirus D68 in pediatric sera collected in a community before, during, and after a possible outbreak

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Short Communication

Fluctuations in antibody titers against enterovirus D68 in pediatric sera collected in a community before, during, and after a possible outbreak

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Summary

Previously, we reported a hospital-based epidemiological study on enterovirus (EV)-D68 infection among children during the autumn of 2015, which indirectly inferred an outbreak in Sendai, Japan. In the present study, we collected sera from children (aged 0–6 years; without symptoms of infectious diseases) in the Sendai community during four sample collection periods (1 year before, 6 months before, immediately after, and 1 year after the possible outbreak period). Immunity levels against EV-D68 infection in the children were analyzed by assaying the neutralization antibody titers of the sera. Further, it was confirmed that the immunity levels considerably increased during the possible outbreak period and gradually waned over 1 year in the absence of another outbreak. These results provide background information that supports the results of our previous hospital-based surveillance.
Enteroviruses (EVs) are positive-sense single-stranded RNA viruses belonging to the Picornaviridae family. Based on molecular and biological characteristics, they are classified into more than 110 serotypes, including human EV-A, EV-B, EV-C, and EV-D, which in turn includes D68 (1). EV-D68 is further classified into lineages 1, 2, and 3 on the basis of nucleotide sequences of the VP1 capsid coding region (2). EV-D68 has been considered a causative agent of mild-to-severe acute respiratory infections since its initial discovery in 1962 in the United States (3). In recent years, EV-D68 infections have been reported worldwide (4-10); however, most reports are based only on the detection of the viral gene in respiratory specimens from patients using PCR, and the results are rarely supported by sero-epidemiological evidences at community level, with a few exceptions (11, 12). We previously reported the detection of EV-D68 among 16 children with respiratory symptoms who visited an outpatient clinic at a secondary care hospital (Sendai Medical Center, Miyagino Ward, Sendai, Japan) during weeks 36–39 of 2015 (13), suggesting the occurrence of an outbreak. However, data were inconclusive enough to prove the occurrence of a community-wide outbreak because the study was based only on an indirect observation of the community through a limited number of patient visits to the hospital. Reportedly, most cases of EV-D68 infection presenting in a clinic were symptomatically mild and managed as outpatients (14), suggesting that several cases with low severity exist in communities without patient visits to a clinic during the possible outbreak period.

The present study aimed to utilize sero-epidemiological analysis to provide a direct observation of the outbreak, considering that traces of the outbreak still remain in the community. In case of a possible outbreak, the anti-EV-D68 antibody titers in the pediatric sera obtained from the Sendai community would fluctuate before, during, and after the outbreak period. The retrospective epidemiological study was conducted using sera collected at a private pediatric outpatient clinic located within the hospital’s medical zone. Pediatric sera were collected and stored as historical specimens for future retrospective epidemiological investigations, with informed consent obtained from their guardians. Sera were primarily collected from patients without any symptom of infectious diseases for use in case management, and the excess specimens remaining after examinations were stored at −20°C in a serum library. The pediatric serum samples were subjected to antibody titration against EV-D68 using microneutralization assay, as previously reported (15). The SMC-15-Sendai895 strain of the virus, which was isolated during the possible outbreak period in 2015 and classified into lineage 2 and clade B by nucleotide sequencing of the VP1 region of the viral gene, was used as a
challenge virus.

After passing the D’Agostino and Pearson omnibus normality test, data were subjected to one-way analysis of variance for comparison between the four sample collection periods (Figure 1). Kruskal–Wallis test was used to compare patient ages during the four sample collection periods (Table 1). $P < 0.05$ was considered significant. Statistical analyses were conducted using Prism software (GraphPad, San Diego, CA, USA).

A total of 103 serum samples of children aged 0–6 years were randomly retrieved from the serum library based on the four sample collection periods, age, and sex. The sample distribution was as follows: 17 (16.5%) samples collected 1 year before (weeks 40–52 of 2014), 30 (29.1%) samples collected 6 months before (weeks 3–35 of 2015), 31 (30.1%) samples collected immediately after (weeks 40–52 of 2015), and 25 (24.3%) samples collected 1 year after (weeks 40–52 of 2016) the possible outbreak in 2015 (Table 1). Age range 0–6 years was selected for analysis because the virus was detected in this range in a previous study (14). Three serum samples were used as negative controls.

Pediatric sera demonstrated positive antibody titers, varying between 3 and 10 log2 titers; however, the negative controls were below the detection limit. The geometric mean titers (GMTs) fluctuated across the four sample collection periods (Figure 1). Similar GMTs were observed between sera collected 1 year before [GMT: 3.8; 95% confidence interval (CI): 3.0–4.8] and 6 months before (GMT: 3.6; 95% CI: 3.2–4.1) the possible outbreak period; however, GMTs immediately after the possible outbreak period (GMT: 6.8; 95% CI: 6.2–7.4; $P < 0.0001$) were higher. The titer became significantly lower 1 year after the outbreak (GMT: 5.6; 95% CI: 5.0–6.3; $P < 0.005$) but remained significantly higher than that during the first two sample collection periods.

Increase in GMTs from that obtained in pediatric sera during the first two sample collection periods to that obtained during the third sample collection period indicates EV-D68 was circulating among children in the community immediately after the outbreak and strongly supports an outbreak suggested by results of our previous hospital-based surveillance. The GMTs obtained in pediatric sera during the fourth sample collection period were higher than those obtained during the first two sample collection periods but lower than those obtained during the third sample collection period. The herd immunity among children in the community may have gradually waned in 1 year after the outbreak. However, the waning of antibody titer over time at the individual level is of interest to be studied. Despite similar ongoing levels of...
surveillance in the community, no significant outbreak has occurred since the third sample collection period. If we assume that EV-D68 had been continuously introduced from external sources into the community even after the outbreak, then our findings may indicate a herd immunity level that can suppress the outbreak of an EV-D68 infection. In summary, the present study demonstrated fluctuating antibody levels against EV-D68 infection, which increased immediately after the possible outbreak and gradually waned in 1 year after the outbreak, in children of the Sendai community; these results provide us with background information that support the results of our previous hospital-based surveillance.

Ethics

Pediatric sera used in this study were collected under the opt-out policy based on the ethical guidelines of the Japanese government on medical research and under the approval of the Ethics Committee of the Sendai Medical Center.

Conflict of interest

None to declare

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Table 1. Data describing sera used in this study based on period of collection

| sample collection period (year and weeks) | 2014  | 2015  | 2016  |
|-----------------------------------------|-------|-------|-------|
|                                         | 40–52 | 3–35  | 40–52 | 40–52 |
| Samples (n)                             | 17    | 30    | 31    | 25    | -     |
| Age in months, mean (Range)             | 54 (36–60) | 48 (24–72) | 48 (36–72) | 48 (24–72) | 0.35$^a$ |
| Male (%)                                | 8 (47.1) | 16 (53.3) | 15 (48.4) | 12 (48.0) | 0.89$^b$ |

$^a$Kruskal–Wallis test, $^b$Chi-squared test.
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Figure 1.

Scatter plots of neutralization antibody (NAb) titers of the sera against enterovirus (EV)-D68 for each of the four sample collection periods. Titers are expressed in log₂ base. One-way analysis of variance was used for all comparisons. P values associated with each comparison are indicated. ns: not significant, NC: negative control.