High Seroprevalence of Human Metapneumovirus among Young Children in Israel

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Exposure to the newly discovered human metapneumovirus (HMPV) during the first 2 years of life was studied by longitudinal serological analysis in 40 healthy children in southern Israel. The seropositivity rate decreased to a minimum by age 13 months and increased to 52% by age 24 months. Evidence of new infection was detected in 13%, 23%, and 55% of children by ages 7, 13, and 24 months, respectively. The high exposure rates suggest that HMPV may be an important cause of community-acquired respiratory-tract infections in young children.

Respiratory viruses—including respiratory syncytial virus (RSV), influenza viruses, parainfluenza viruses, and adenoviruses—cause considerable disease burden and increased mortality among young children and elderly adults worldwide [1–3]. In particular, RSV is the leading cause of hospitalization for respiratory-tract infections (RTIs) in young children [2]. Despite extensive diagnostic investigations, the cause of many acute upper and lower RTIs remains unknown.

Recently, human metapneumovirus (HMPV), a newly discovered respiratory virus assigned to the Paramyxoviridae family, has been reported to cause RTIs in young children in The Netherlands [4]. The reported clinical manifestations were similar to those of RSV infection. Further serological analysis demonstrated that virtually all children in The Netherlands had been exposed to HMPV by age 5 years [4]. The virus has been subsequently identified in association with RTIs in children and adults in additional countries [5–9]. The widespread geographic distribution in temperate zones suggests that HMPV infection circulates worldwide. However, no reports have documented the occurrence of HMPV outside Western Europe, North America, and Australia.

We describe the rapid acquisition and high prevalence of anti-HMPV antibodies among young children in southern Israel. Longitudinal serological analysis was used to study exposure to HMPV during the first 2 years of life.

Patients, materials, and methods. One hundred sixty serum specimens obtained prospectively from 40 children at ages 2, 7, 13, and 24 months were tested for the presence and titer of anti-HMPV antibodies. These children were enrolled in a phase 2 study of a pneumococcal conjugate vaccine and were prospectively followed at the Pediatric Infectious Disease Unit of the Soroka University Medical Center (Ben-Gurion University of the Negev, Beer-Sheva, Israel). All of them were full-term, healthy, Jewish children from southern Israel who were recruited in 1998, at age 2 months. The study was approved by the institutional and national ethics committees and was performed in accordance with the human-experimentation guidelines of the Israeli Ministry of Health. Informed consent, including permission to test blood specimens for antibody to nonpneumococcal infectious diseases, was obtained from parents of all children.

Blood specimens were obtained at each visit. Serum aliquots were stored at −70°C until tested. Anti-HMPV antibodies were tested at the clinical virology laboratory of the Hadassah Medical Center (Jerusalem).

All specimens were coded before being transferred to the laboratory and were tested blindly. The serum specimens were analyzed for the presence of anti-HMPV antibodies by use of indirect immunofluorescence assay (IFA) using HMPV-infected Vero cells, as described elsewhere [4]. This assay was found to be specific for HMPV. Virus-specific guinea pig antiserum was used as a positive control (HMPV and specific antisera were provided by Prof. Albert Osterhaus, Erasmus Medical Center, Rotterdam, The Netherlands). Mock-infected cells and infected cells incubated with normal guinea pig serum or buffer were used as negative controls. For all the serum specimens that tested positive (IFA titer ≥32), the IFA titer was further determined by 2-fold end-point dilutions. All titers are expressed as reciprocal values.

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Comparison of antibody prevalence was made by use of Fisher’s exact test. $P < .05$ was considered to be significant.

**Results.** Analysis of the 160 serum specimens from the 40 children revealed that, by the age of 2 months, most children (32/40 [80%]) had anti-HMPV antibodies (figure 1). Antibody prevalence was significantly lower by ages 7 and 13 months (40% and 30%, respectively; $P < .001$, vs. antibody prevalence at 2 months) and increased by age 24 months (figure 1).

To evaluate the rate of new acquisition of anti-HMPV antibodies during the first 2 years of life, antibody titers were determined. Of the seropositive children, the proportion with titers $\geq 512$ at ages 7, 13, and 24 months was 0% (0/16), 25% (3/12), and 30% (6/21), respectively ($P = .017$). Of the 32 children who were positive at age 2 months, 18 (56%) became negative by age 7 months, 8 (25%) had lower titers at age 7 months than at age 2 months, 3 (9%) had higher titers at age 7 months than at age 2 months, and 3 (9%) had unchanged titers. In addition, of the 8 children who were seronegative at age 2 months, only 2 (25%) were positive at age 7 months. Thus, by age 7 months, of the 16 children who were seropositive, 5 had been newly exposed, and 11 probably still had maternal antibodies. Three (60%) of the 5 children who had been newly exposed by age 7 months remained positive at least by age 13 months. Ten (83%) of the 12 children who were seropositive at age 13 months were still positive at 24 age months.

On the basis of seroconversion rate or increased antibody titers, of 40 children, new exposures occurred in 5 (13%) by age 7 months, in 9 (23%) by age 13 months (an additional 10% of the group acquired the infection between age 7 months and 13 months), and in 22 (55%) by age 24 months. Individual longitudinal analysis identified several patterns among seropositive children between ages 2 months and 24 months: first, children who were seronegative at age 2 months and acquired antibodies at some point during the first 2 years of life (5/40 [13%]); second, children who were seropositive at age 2 months but lost their antibodies by age 24 months (16/40 [40%]); third, children who were seropositive at age 2 months but who demonstrated new exposure or, possibly, reinfection by age 24 months, by increasing or persisting titer (16/40 [40%]); and finally, at least 1 child in this group with a serologic pattern that could potentially result from reinfection (i.e., the child’s antibody titer was 32 at age 2 months, 256 at age 7 months, <32 at age 13 months, and 128 at age 24 months). This child was thus infected with HMPV between the age of 2 months and 7 months, with boosting of his antibody response between ages 13 months and 24 months. Three (8%) of the 40 children remained seronegative during the first 2 years of life.

**Discussion.** We have found a high prevalence of anti-HMPV antibodies among young children in southern Israel. By age 2 years, >50% of children have been exposed to the virus. Although the occurrence of HMPV infection was reported mainly in temperate zones, it is important to note that the children in the present study lived in southern Israel, a desert area characterized by a hot and dry climate during the summer and only a few days of rain (usually <20) during the winter. In addition, preliminary studies in our laboratory (data not shown) have shown 100% seroprevalence for HMPV among 25 8-year-old children who recently emigrated from Ethiopia. These findings support worldwide distribution of the virus.

One serologic study in The Netherlands examined children in various age categories who demonstrated increasing anti-HMPV seroprevalence between ages 6 months and 5 years [4].

![Figure 1. Seroprevalence and reciprocal titers of human metapneumovirus (HMPV) antibodies (Abs) in 40 children during the first 2 years of life. The indicated percentages were calculated for a total of 40 children. Nos. within boxes indicate percentages of children with each HMPV Ab titer.]
In our serologic analysis, we used a different approach to follow the longitudinal patterns of anti-HMPV antibody acquisition in 40 children. This approach allowed us to define the rate of HMPV infection during the first 2 years of life. The cumulative rate of new exposure showed that at least 13%, 23%, and 55% of the children became positive before ages 7, 13, and 24 months, respectively. Only a minority of the children (8%) remained seronegative during the first 2 years. The high prevalence at age 2 months most likely resulted from maternal transmission and possibly reflects the high HMPV seroprevalence among adults.

The duration of antibody detection ranged from <7 months to >18 months after infection, although we cannot exclude a boosting response as the reason antibody was detected >18 months after infection. The protective levels of antibodies and their role in long-term immunity remain to be determined.

A recent report has described reinfections by HMPV in an immunocompromised child [10]. Furthermore, phylogenetic studies have demonstrated significant sequence variation between different isolates, with >1 potential virus type [4, 5, 10, 11]. This result suggests that reinfections by different HMPV strains might occur among nonimmunocompromised children. One of the children in the present study exhibited a serologic pattern that could reflect reinfection; however, in the absence of virus isolation, this could not be proved. Direct analysis of virus isolates is needed to examine whether the epidemiology of HMPV in healthy individuals is similar to that of RSV, which is characterized by reinfections with different strains.

HMPV infection is reported to be associated with a wide spectrum of clinical manifestations, ranging from influenza-like illness to bronchiolitis and pneumonia [4–6, 9–11]; however, as yet, no reports with appropriate controls, which are critical to determine the clinical manifestation and the rate of asymptomatic infections, have been published. The high prevalence and infection rate revealed in the present study suggest that HMPV plays an important role in community-acquired RTIs in young children in Israel and could account for many of the undiagnosed infections. Direct virological studies are underway by our group to define the spectrum of the clinical manifestations and the relative contribution of HMPV to the burden of hospital admissions in young children.

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References