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Seroepidemiologies of Human Metapneumovirus and Respiratory Syncytial Virus in Young Children, Determined with a New Recombinant Fusion Protein Enzyme-Linked Immunosorbent Assay

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We compared antibodies against human metapneumovirus (HMPV) and respiratory syncytial virus (RSV) in children. The antibody nadirs for both viruses were at 3 to 5 months, and the majority of children were seropositive for both by 2 years. There was no significant difference in the kinetics of maternal antibody decline or seroconversion relative to the two viruses.

Human metapneumovirus (HMPV) is a leading cause of lower respiratory tract illness (LRI) in children and adults (1–9). HMPV and respiratory syncytial virus (RSV) share a number of clinical and genetic similarities (1, 4, 10, 11). HMPV infection leads to significant morbidity in infants and other populations, including immunocompromised, high-risk, and elderly patients (7, 12–19). Interestingly, several studies have reported that the mean age of infants with LRI due to HMPV is higher than that of infants with RSV-associated LRI (1, 8, 10, 20–23). To test the hypothesis that this age discrepancy was due to a difference in maternally derived antibody titers, we determined seropositivity rates by age in a prospective collection of sera from young children.

Serum specimens were obtained from the Vanderbilt Vaccine Clinic, a clinic established for the purpose of evaluating investigational vaccines in young children and conducting viral surveillance (24, 25). Term infants were enrolled at birth and were followed until 5 years of age, although specimens were collected occasionally from older subjects. Serum was obtained and stored at −20°C until testing. Specimens used in the present study were each collected from a unique patient between December 1989 and August 2001 and were selected randomly to yield roughly similar numbers of specimens per 1-year age group. The Vanderbilt Institutional Review Board approved the study.

Human sera were tested for the presence of HMPV or RSV F protein-specific antibodies by enzyme-linked immunosorbent assay (ELISA). Soluble HMPV and RSV F proteins were expressed in Freestyle 293 cells (Invitrogen) and purified as described previously (26). ELISA methods were developed and conditions optimized using known negative and positive sera (not shown). Briefly, 100 ng/well of purified HMPV or RSV F protein was adsorbed onto 384-well polystyrene plates (Nunc) overnight in carbonate buffer (pH 9.8) at 4°C. Plates were blocked with 5% nonfat dried milk in phosphate-buffered saline (PBS) with 0.5% Tween 20 (PBS-T) for 2 h at room temperature. After the plates were washed with PBS-T, serial 4-fold dilutions of serum in duplicate were added, and the plates were incubated for 1 h at room temperature. Plates were washed with PBS-T, alkaline phosphatase-conjugated anti-human IgG (Southern Biotech) was added, and the plates were incubated for 1 h. Finally, plates were washed with PBS-T, and *p*-nitrophenyl phosphate substrate (Sigma) was added. The absorbance at 405 nm was read at 30 min and the mean of duplicate wells taken. Based on the baseline signal of the reference sera to each antigen, a specimen was designated positive if the ratio of the specimen absorbance compared to negative-control serum value was >2 at a dilution of ≥1:20 for HMPV F or ≥1:80 for RSV F. The ELISA endpoint titer assigned to each specimen was the reciprocal dilution that yielded a positive result. We used logistic regression to model the binary outcome of seropositivity as a function of age and virus, and we used linear regression to model the natural logarithm of titer as a function of age and virus. Restricted cubic splines were used to provide a smoothed estimate of the group means by age.

Two hundred eighty-two sera were included, with 144 (51%) collected from males. The racial distribution of the subjects was 145 (51%) white, 125 (44%) black, and 12 other. The age distribution of the children is shown in Table 1. Of children that were 60 months old or older, the mean age was 78 months (range, 60 to 122 months).

Of the 282 specimens, 219 (78%) were positive for HMPV and 219 (78%) were positive for RSV. Rates of seropositivity for both viruses were high for subjects <6 months old, and titers against both viruses initially decreased with age, reaching a nadir at 5 to 6 months. Seropositivity for HMPV and RSV increased with age after 6 months, reaching ≥80% in all subjects that were >2 years old. The probability of seropositivity varied by age for each virus (Fig. 1), but the probabilities of HMPV seropositivity and RSV seropositivity did not differ ($P = 0.12$). Similarly, the mean endpoint titers against both viruses were higher in subjects that were <6 months old and reached a nadir between 3 and 5 months. Serum titers against HMPV remained low until 13 months of age, when the mean log titer began to increase (Fig. 2). In contrast, antibody titers against RSV rose at an earlier age.

We compared titers of serum antibody against HMPV and RSV in a prospectively collected cohort of children. The nadirs for both viruses were between 3 and 5 months of age, consistent with the rate of decline for maternally derived antibodies. Thus, the

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TABLE 1 Number of serum samples from each age group

Child age group (mo)	No. of specimens
<3	8
3–5	9
6–8	16
9–11	9
12–23	48
24–35	52
36–47	48
48–59	54
>60	38
Total	282

waning titer of maternally derived antibody does not correlate with the observed higher mean age of infants with HMPV-associated LRI and does not explain the susceptibility of older infants to HMPV-associated LRI. Consistent with an older age for HMPV infection, the mean endpoint titer against HMPV remained low until 1 year of age. The delayed rise in seropositivity against HMPV compared to seropositivity against RSV reflects the observed older age for HMPV infection but fails to provide a biological explanation. Possible mechanisms include the protective serum antibody threshold against HMPV being lower than that against RSV or age-related differences in the contribution of immune response to disease. The serological data may also reflect differing transmission rates for HMPV and RSV or discordant ability of young infants to mount anti-F antibody responses to HMPV and RSV.

Our study has some limitations. Neutralizing antibodies might provide a better indication of protection against disease, but there was insufficient specimen remaining for this testing. Further, the clinical and demographic histories of these specimens were not available.

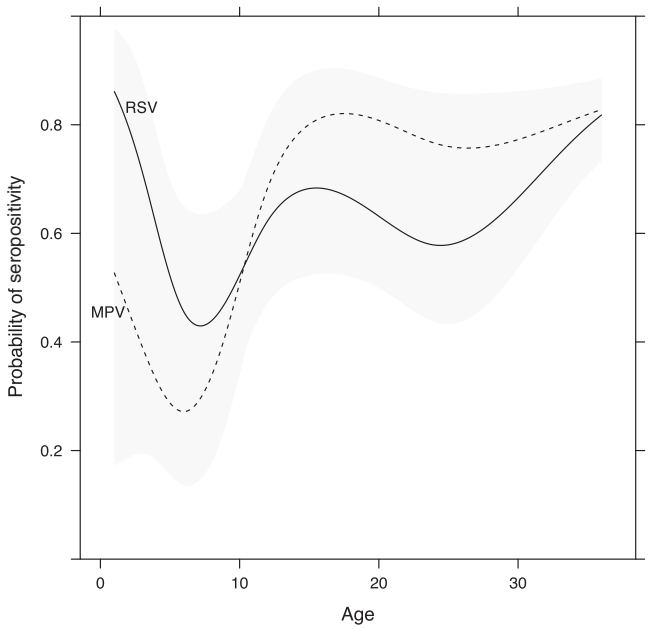

FIG 1 Estimated probabilities of seropositivity by virus and age (in months). Logistic regression was used to model seropositivity to HMPV (dotted) and RSV (solid) by age in months. The 95% confidence intervals (CIs) are indicated as shaded regions.

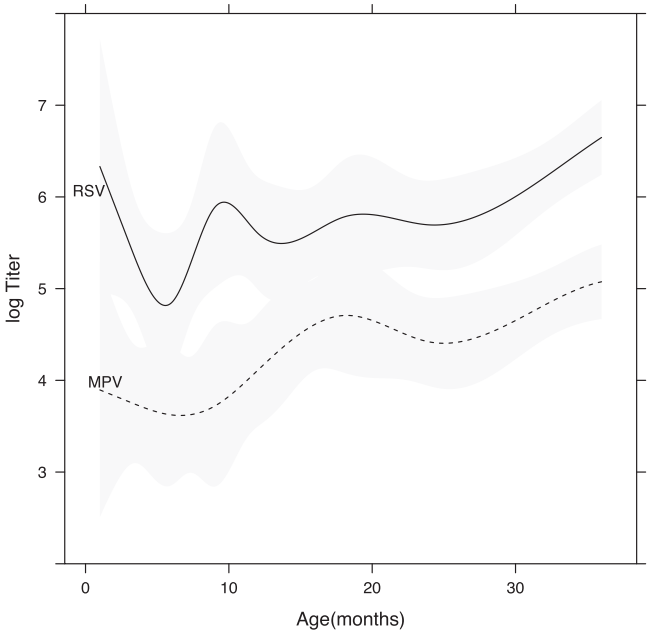

FIG 2 Log titer by virus and age. Linear regression was used to model the natural logarithm of endpoint titer for HMPV (dotted line) and RSV (solid line) by age. The 95% CIs are indicated as shaded regions.

Others have reported seroepidemiologic studies of HMPV using different methods and age groups (27–33). Only one report compared the seroepidemiologies of HMPV and RSV, but the results were grouped into age groups of 6 months to 5 years and 6 to 10 years (34). Our findings demonstrate that the kinetics of antibody titers against HMPV and RSV are similar during the first year of life. Further studies with humans and using animal models are needed to understand the reason for the reported difference between ages of LRI hospitalization of HMPV and RSV patients, which has implications for vaccine development.

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