Human Metapneumovirus Reinfection among Children in Thailand Determined by ELISA Using Purified Soluble Fusion Protein

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Background. Human metapneumovirus (hMPV) is a newly discovered paramyxovirus that causes acute respiratory illness. Despite apparent near-universal exposure during early childhood, immunity is transient.

Methods. An indirect screening enzyme-linked immunosorbent assay using a recombinant soluble fusion (F) glycoprotein derived from hMPV was used to test for anti-F IgG in 1380 pairs of acute- and convalescent-stage serum samples collected from children in Kamphaeng Phet, Thailand.

Results. Of the 1380 serum sample pairs tested, 1376 (99.7%) showed evidence of prior infection with hMPV. Sixty-six paired specimens demonstrated a 4-fold rise in titer, for an overall reinfection rate of 4.9%. Two children demonstrated evidence of an initial infection. Forty-eight of the 68 new infections or reinfections occurred in 2000, accounting for 13.2% of all nonflaviviral febrile illnesses in the study population in that year. Of 68 positive cases, 85.3% complained of cough and 66.2% complained of rhinorrhea, compared with 61.4% and 49.0% of negative cases, respectively (P < .01). All positive samples were also tested for an increase in titer of antibodies to respiratory syncytial virus F, and 27% exhibited a 4-fold rise.

Conclusion. These results demonstrate that hMPV reinfections cause illness at a rate equal to that seen for initial infections. hMPV may have a more significant impact in older children than previously realized and may be the cause of significant outbreaks in this population.

Human metapneumovirus (hMPV) is a recently discovered paramyxovirus that causes acute respiratory illness [1] and has been documented worldwide, including in Thailand [2–5]. Serological surveys have demonstrated that >90% of those >5 years old have antibodies to hMPV [1, 6]. Despite this apparent near-universal exposure during early childhood, reinfection with hMPV occurs, similar to that seen for respiratory syncytial virus (RSV) [7]. The largest epidemiological study to examine cases of reinfection revealed hMPV to be the cause of ~4.5% of respiratory illnesses in 984 adult volunteers [8]. In addition, reinfection among children has also been documented in case reports and as incidental findings in larger prospective studies [9–14].

To test retrospectively for viral infection, serological assays can be performed using acute- and convalescent-stage patient serum samples. For hMPV, tests have included immunofluorescence assays using hMPV-infected cells [11, 15] or recombinant baculovirus–infected cells expressing the fusion (F) protein [16] as well as ELISAs using hMPV-infected cell lysates [17, 18], lysates from recombinant baculovirus–infected cells expressing nucleoprotein [19], purified nucleoprotein.
from a prokaryotic expression system [20], and a recombinant vesicular stomatitis virus expressing hMPV F protein [6]. However, to our knowledge, no serological studies have been conducted using purified hMPV glycoproteins from a eukaryotic or human cell line system.

The membrane-anchored envelope glycoproteins of paramyxoviruses are the principle antigenic determinant to which virus-neutralizing antibodies are directed. The F protein of hMPV is highly conserved among viral isolates and is a major immunological determinant [21, 22]. Purified soluble F (sF) protein has been produced and is immunogenic in animal models [23, 24]. Expression of hMPV F protein in a eukaryotic system should produce an antigen that is closely related to native protein and that would be ideal for a sensitive serological assay. One concern in serological tests is cross-reactivity to related proteins. RSV is the human paramyxovirus most closely related to hMPV genetically, and currently all human data demonstrate a lack of cross-reactivity between conserved hMPV proteins and RSV antibodies to viral surface proteins [6, 20, 25].

A retrospective study in Bangladesh identified hMPV as the most common respiratory virus in children with cough and fever, on the basis of an increase in titer between acute- and convalescent-stage samples in 107 serum sample pairs tested [17]. This is one of a limited number of studies that has examined evidence of hMPV infection in older children, with 42 of those tested >5 years old. Here, using a purified F protein as antigen in an indirect ELISA, we determined seroprevalence and reinfection rates for hMPV using archived serum samples from 1380 febrile children aged 7–16 in Thailand.

METHODS

Subjects. The Armed Forces Research Institute of Medical Sciences and the University of Massachusetts Medical School (UMMS) conducted a prospective study of dengue virus transmission and disease in children attending primary school in Kamphaeng Phet Province, Thailand, from 1998 to 2002 [26, 27]. They enrolled children from 12 elementary schools and performed active case surveillance from 1 June to 15 November each year, concurrent with peak dengue transmission. Village health workers investigated any school absence to determine whether the child had a history of fever within 7 days of the absence or an oral temperature of \( \geq 38^\circ\text{C} \). If the criteria were met, health workers administered a symptom questionnaire and obtained blood samples then and \(~14 \) days later. The Walter Reed Institute of Research Human Use Review Committee, the UMMS Human Subjects Committee, and the Thai Ethical Review Committee, Ministry of Public Health, Nonthaburi, Thailand, approved the protocol under which the samples were collected. The Uniformed Services University of the Health Sciences Institutional Review Board approved the use of the serum samples for the present study as an exempt protocol.

Protein production. LLC-MK2 cells were infected with hMPV virus strain CAN97–83 of the A2 genotype (provided by K. Yim, Virion Systems). RNA was extracted using the RNAsin Mini Kit (Qiagen), in accordance with the manufacturer’s instructions, and cDNA was created. The soluble version of F is a truncate of F protein with an S peptide tag that lacks the transmembrane domain and was used as cDNA and the following oligonucleotides: 5’-CCGCTCGAGATGTCTTGAGGAAAGTGGTGATCAT-3’ and 5’-GGATCCCTAGCTGCCATGTCGGCGATTTAGCAGGCGGTTCCTTTTCTGTGACTGCTTGAGATTCTG-3’. The construct was cloned into pCRII-Blunt-TOPO (Invitrogen) and further subcloned into phCMV1 (Genentics) as XhoI-BamHI fragments. Transfection of HEK293T cells with the phCMV1 sF\(_{A2}\) vector resulted in high expression levels and secretion of sF\(_{A2}\) in the culture supernatant. A stable HEK293T cell line secreting sF\(_{A2}\) was generated using geneticin selection and cloned by limited dilution. HEK293T cells expressing S peptide–tagged sF\(_{A2}\) were grown to confluency in 1700-cm\(^2\) tissue culture roller bottles in Dulbecco’s modified Eagle medium (Quality Biological) supplemented with 10% Cosmic Calf Serum (HyClone), 2 mmol/L l-glutamine, 100 U/mL penicillin and streptomycin, and 10 \( \mu \)g/mL gentamicin (Quality Biological), and then the medium was replaced with Opti-MEM Reduced-Serum Medium (Invitrogen) and cultured for 72 h at 37°C with CO\(_2\). The supernatant was clarified by centrifugation, supplemented with 0.1 mol/L l-arginine (Sigma-Aldrich) and 0.1% Triton X-100, and filtered through a low-protein-binding membrane before being passed over an XK 26 column (Amersham Pharmacia Biotech) with 20 mL of S-protein agarose (Novagen) at a flow rate of \(~5\) mL/min. The column was washed with PBS with 0.1% Triton X-100 and 0.1 mol/L l-arginine and then with PBS with 0.5 mol/L NaCl, 0.5% Triton X-100, 0.1 mol/L l-arginine, and 0.02 mol/L Tris. The sF protein was eluted with 0.2 mol/L l-arginine and 0.2 mol/L citric acid (pH 2) and neutralized with HEPES (pH 8). The eluate was then concentrated using 30-kDa Centricron centrifugal filter units (Millipore) and buffer-exchanged into PBS with 0.2 mol/L l-arginine and 0.01% Triton X-100. Protein concentrations were determined by SDS-PAGE and Coomassie staining with SimplyBlue SafeStain (Invitrogen), and densitometry analysis was performed using Scion Image software and comparison with known protein standards. The concentrated sF\(_{A2}\) glycoprotein had a molecular size of \(~58\) kDa.

Because of the large number of serum samples to be tested and the need for additional protein antigen, we also obtained sF protein made from strains NL/1/00, an A1 genotype, and NL/1/99, a B1 genotype (MedImmune), as described by Ulbrandt et al. [24]. All 3 versions of hMPV sF protein (sF\(_{A1}\), sF\(_{A2}\),sF\(_{B1}\)) and RSV sF protein (MedImmune) bound to positive human control serum samples in Western blot analysis.

Western blot analysis. Purified proteins were separated by SDS-PAGE on NuPAGE Novex 4%–12% Bis-Tris gel (Invitrogen), transferred to nitrocellulose membranes, and then blocked

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with 5% milk in PBS with 0.5% Tween 20 (PBS-T; Sigma-Aldrich) overnight at 4°C or for 1–2 h at room temperature. The membranes were incubated for 1 h at room temperature with antisera at a dilution of 1:2,500. Membranes were washed 4 times with PBS-T and incubated with horseradish peroxidase–conjugated ImmunoPure goat anti–human IgG (Pierce Biotechnology) at a dilution of 1:2,500 for 1 h at room temperature. The membranes were washed again 4 times with PBS-T and visualized by chemiluminescence (Super-Signal West Pico chemiluminescent substrate; Pierce Biotechnology).

ELISA. The 96-well Immulon 2 microtiter plates (Dynex Technologies) were coated with purified sF protein from hMPV or RSV in 50 mmol/L NaHCO₃ and 20 mmol/L Na₂CO₃ buffer (pH 9.6) at a concentration of 50 ng of protein per well and incubated overnight at 4°C. The plates were washed with PBS-T and blocked overnight at 4°C or for 1 h at 37°C with PBS-T with 5% bovine serum albumin (BSA; Sigma-Aldrich) added. The plates were washed with PBS-T, and dilutions of serum in PBS-T containing 1% BSA were added and incubated for 1 h at 37°C. The plates were washed and incubated for 1 h at 37°C with alkaline phosphatase–conjugated goat anti–human IgG (Jackson ImmunoResearch) diluted 1:500. The plates were washed again with the same solution, and antibodies were detected by adding 100 μL of p-nitrophenyl phosphate substrate (Pierce Biotechnology) to each well, incubating for 30 min at room temperature, and then adding 50 μL of 2N NaOH to each well to stop the reaction. The optical density (OD) at 405 nm was measured using a VersaMax microtiter plate reader (Molecular Devices).

Paired acute- and convalescent-phase serum samples were assayed in duplicate on the same 96-well microtiter plate at 3 screening dilutions (1:100, 1:500, and 1:5000) using the sFₐ₁ genotype as the antigen against a standard dilution of hMPV antisera. Serial dilutions of known hMPV-positive serum samples (reference samples provided by J. Kahn, Yale University) were run on every ELISA plate, and a standard curve was generated. The positive cutoff was considered to be the first OD value on the standard curve that was 1.5 times the previous dilution. All OD values used for screening and definitive testing were on the linear portion of the standard curve.

A serum sample pair was considered negative if both the acute- and the convalescent-stage sample tested negative at the 1:100 dilution and the results were confirmed with repeated serial 2-fold dilutions starting at 1:64. A serum sample pair was considered to indicate a previous infection but not a recent reinfection if both the acute- and the convalescent-stage sample had equivalent ELISA titers at all positive dilutions. If both samples were positive at the 1:5000 dilution, they were retested to confirm the same antibody titers at serial 2-fold dilutions to 1:262,144. A serum sample pair was considered to indicate a possible reinfection if the convalescent-stage titer was positive at the 1:5000 dilution and there was a ≥2-fold rise in OD value between the acute- and the convalescent-stage titer at 1:5000. A serum sample pair was considered to indicate a possible new infection when the acute-stage sample showed no evidence of antibody and the convalescent-stage titer was positive at the 1:5000 dilution. Samples for all possible reinfections and new infections were retested in serial 2-fold dilutions. For the retest, a positive antibody response to hMPV was defined as a paired serum sample for which there was a ≥4-fold increase in the level of IgG antibodies between the acute- and the convalescent-phase serum sample.

All confirmed positive samples were also tested for evidence of increasing titers of antibody to hMPV sFₐ₁ and sFₐ₂ and RSV sF protein in serial 2-fold dilutions, with a ≥4-fold increase in titer considered positive. For the RSV control, we used serial dilutions of concentrated human RSV antibody (provided by J. Blanco, Virion Systems) on each plate to generate a standard curve.

Table 1. Characteristics of children with samples in the serum bank, of those tested, and of those negative and positive for human metapneumovirus (hMPV).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Untested children (n = 1177)</th>
<th>Tested children (n = 1380)</th>
<th>Negative (n = 1312)</th>
<th>Positive (n = 68)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled children</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, range, years</td>
<td>6–15</td>
<td>7–16</td>
<td>7–16</td>
<td>7–14</td>
<td></td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>10.1 ± 1.5</td>
<td>9.9 ± 1.6</td>
<td>9.9 ± 1.6</td>
<td>10 ± 1.6</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Sex, no. male/no. female (ratio)</td>
<td>594/583 (1:02)</td>
<td>700/680 (1:03)</td>
<td>664/648 (1:02)</td>
<td>36/32 (1:1.13)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Clinical symptomsab</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough, no. (%)</td>
<td>840/1162 (72.3)</td>
<td>860/1375 (62.5)</td>
<td>802/1307 (61.4)</td>
<td>58/68 (85.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Rhinorrhea, no. (%)</td>
<td>661/1160 (57.0)</td>
<td>683/1371 (49.8)</td>
<td>638/1303 (49.0)</td>
<td>45/68 (66.2)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Temperature, mean, °C</td>
<td>38.2</td>
<td>38.1</td>
<td>38.1</td>
<td>37.8</td>
<td>.016</td>
</tr>
</tbody>
</table>

*For age and sex, P values are for both comparisons between untested and tested children and comparisons between positive and negative samples. For clinical symptoms, P values are only for comparisons between all tested children and those with positive samples. The χ² test was used for categorical data, and the t test was used for continuous variables.

ab Data on some variables were missing for some children, as indicated in the totals for cough and rhinorrhea.

c Temperature at the time of the health care worker visit.
Means were compared using Student’s t test, and proportions were compared using the χ² test.

RESULTS

Study population demographics. In the initial dengue study, acute- and convalescent-stage serum samples were tested for evidence of recent infection with dengue and Japanese encephalitis viruses, and 2557 serum sample pairs were negative. For these samples, the subjects ranged in age from 7 to 16 years (mean, 9 years 11 months), 50.6% were male, and 73.9% reported cough, rhinorrhea, or both; their average temperature at the acute-stage visit was 38.1°C. We tested 1380 (54%) of the samples for evidence of past and present hMPV exposure on the basis of sample and reagent availability. These samples were from 1049 children; 254 children had 2–6 febrile illnesses during the study period. All of the samples obtained during 1999 and 2000 were tested, and one-third from 2001 and one-half from 2002 were randomly selected and tested. We also tested the available samples from 1998—from 1 June to 29 July, 18–22 September, and 21–29 October 1998. There were no significant differences in sex or age between tested and untested subjects (table 1).

Serosurvey. Of the 1049 patients tested, all but 3 (99.7%) had evidence of prior exposure to hMPV. One child had a fever twice in 1998 and tested negative for prior exposure to hMPV both times. Of the 1380 pairs of acute- and convalescent-stage serum samples tested, 74 met our screening criteria for a possible new infection or recurrent infection and an additional 21 for which both the acute- and the convalescent-stage samples were positive at the 1:5000 dilution. Of the 74 serum sample pairs indicating a possible infection, 64 were positive for reinfection and 2 were positive for new infection. Of the 21 serum sample pairs with indeterminate screening results, 2 were positive for reinfection, for a total of 66 presumptive reinfections (4.8%) and 2 new infections (0.1%). A total of 66 children accounted for the 68 positive cases, with 2 children demonstrating reinfections in 1998 and again in 2000. Table 2 lists the results by year. Because the samples were screened and retested using protein provided by MedImmune (sFAl), all of the samples representing nonexposure, new infections, or reinfections were then retested using sFA2 under the same assay conditions, and the same results were obtained (data not shown).

The acute-stage serum samples that were negative for hMPV sF protein by ELISA and the paired samples that showed a possible new infection were further tested by Western blotting. A representative example of each is shown in figure 1. In each case, the acute-stage samples were negative for the 3 subtypes of hMPV F protein and were positive for antibody to RSV F protein. The 2 new infections (figure 1A) demonstrated antibody to all 3 F subtypes in the convalescent-stage samples. Although we cannot exclude the possibility that antibody was present at levels below the limit of detection in the acute-stage samples, it appears that 4 sample pairs had acute-stage samples that were negative for exposure and that 2 of these demonstrated a new infection with hMPV in the convalescent-stage sample, confirming the ELISA results.

Table 2. Results of the human serosurvey.

<table>
<thead>
<tr>
<th>Year</th>
<th>Paired serum samples tested/total collected (%)</th>
<th>Paired samples with a ≥4-fold rise in titer, a no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>390/1083 (36.0)</td>
<td>14 (3.6)</td>
</tr>
<tr>
<td>1999</td>
<td>326/341 (95.6)</td>
<td>1 (0.3)</td>
</tr>
<tr>
<td>2000</td>
<td>364/372 (97.8)</td>
<td>48 (13.2)</td>
</tr>
<tr>
<td>2001</td>
<td>157/461 (34.1)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>2002</td>
<td>143/300 (47.7)</td>
<td>3 (2.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1380/2557 (54.0)</td>
<td>68 (4.9)</td>
</tr>
</tbody>
</table>

a Paired serum samples with a ≥4-fold rise in the titer of IgG to human metapneumovirus soluble fusion protein between acute- and convalescent-stage serum samples.

b One case was a presumptive new infection with a negative acute-stage serum sample.

Figure 1. Western blot analysis of serum samples presumed to be negative for human metapneumovirus (hMPV) and serum samples demonstrating new infection. Purified hMPV soluble fusion (sF) proteins sFA1, sFA2, and sFB1, and respiratory syncytial virus (RSV) sF were resolved on 4%–12% Nu-PAGE gel and transferred to nitrocellulose membranes. The membranes were probed with human serum samples as follows: acute- and convalescent-stage samples from a patient after the acute-stage sample tested negative but the convalescent-stage sample tested positive for hMPV sF antibody by ELISA (A) or an RSV-positive convalescent-stage sample from a patient for whom both the acute- and convalescent-stage samples were negative for hMPV sF by ELISA (B). Bound antibodies were reacted with a peroxidase-conjugated goat anti–human IgG and visualized by chemiluminescence.
If A and B represent different serological subtypes, ELISA results could be negative if a patient were infected with lineage B and if sF lineage A were used as the antigen. Leung et al. [6] demonstrated no difference in ELISA reactivity using as antigens cell lysates infected with recombinant vesicular stomatitis virus expressing fusion protein from A or B lineage. To verify that our assay was also equally sensitive for the 2 lineages, we used sFB1 protein as an antigen to test 12 serum sample pairs with evidence of reinfection and 48 with evidence only of prior exposure, and the results were identical to those obtained with sFA1 protein (data not shown).

Nevertheless, antibodies can cross-react to shared epitopes, and we considered the possibility of potential cross-reactivity to RSV, because the RSV F glycoprotein is 30%–43% homologous to hMPV F [28]. Therefore, we used RSV F to assay the 68 hMPV reinfection–positive serum sample pairs. Of these, only 18 (27%) of the sample pairs had a ≥4-fold rise in titers of antibodies to the RSV antigen, and only 3 of these 18 had a greater titer increase in response to RSV than to hMPV (data not shown). Although we cannot rule out cross-reactivity, it is also possible that at least some of the samples positive for both hMPV and RSV reinfection may represent coinfections with >1 virus.

Epidemiology of hMPV infection in the study population. We found the majority of reinfections in 1998 and 2000. We were not able to test a representative sample of the study period in 1998, but 48 of the 68 new infections or reinfections occurred in 2000, accounting for 13.2% of all nonflaviviral febrile illnesses in the study population in that year. Thirty-one of these cases occurred in a discrete 2-week time period (figure 2), suggesting a localized outbreak. Twenty of these cases were from 3 of 12 participating schools, with another 3 schools accounting for the remaining 11 cases. Although clinical information on the patients in the present study was limited and a majority of all children in the study had respiratory symptoms, a significantly higher proportion of hMPV-positive patients complained of rhinorrhea or cough compared with all of the patients tested (table 1), a finding consistent with respiratory disease.

DISCUSSION

This is the first report on the seroepidemiology of hMPV infection in Thailand and the first study to examine reinfection rates among children systematically. Using our ELISA with recombinant F glycoprotein as an antigen to detect hMPV antibodies, we found a seroprevalence in this population of nearly 100%. The reinfection rate (average, 4.9% per year) was similar to that seen in adults [18]. We believe that this rate is probably an underestimate, for a number of technical reasons. First, we used very conservative criteria to determine cutoffs for possible reinfections in the initial screening. Many serum sample pairs had higher OD values for the convalescent-stage than the acute-stage sample, but the convalescent-stage samples either did not meet the criterion of being positive at the 1:5000 dilution or did not have an OD reading ≥2 times that of the acute-stage sample at
this dilution (e.g., 257 convalescent-stage samples had an OD reading between 1.5 and 2 times that of the acute-stage samples). Second, blood samples were obtained only from children with a history of fever. Several published reports quote rates of fever in hMPV infections near 50% or lower [13, 14, 17, 18, 29, 30]. Especially given that these illnesses are almost entirely reinfections, it is possible that there were other cases of hMPV infection that were symptomatic but did not meet the fever criterion. Third, it is possible that the peak season for hMPV infection is during the winter months, as discussed below, and we did not have samples from this time of year. Finally, patients may mount such a rapid IgG response to a reinfection that the ability to detect a change between acute- and convalescent-stage titers is diminished. The average length of time between the onset of illness and the collection of the acute-stage sample was 1.2 days among all of the tested patients. Because IgG levels typically increase within 2 days after reexposure [31], this could occur before symptoms are even apparent, resulting in increased titers in the acute-stage sample. In another study, 2 of 7 patients with known hMPV reinfections, as demonstrated by viral isolation, did not develop a 4-fold rise in titer between acute- and convalescent-stage samples [10].

We could not confirm that elevations of antibody reactive to hMPV F glycoprotein correlated with actual reinfections with the virus, because we did not have respiratory samples. Indeed, we did see that 27% of all hMPV reinfection–positive samples reacted to RSV F. Although all existing human data demonstrate a lack of cross-reactivity between conserved hMPV proteins and RSV antibodies [6, 20, 25], cross-reactivity to RSV could be causing some of the hMPV-positive results. It may be possible to compare serum samples from patients with known hMPV infections with those from patients with known RSV infections to determine their ability to bind to and neutralize recombinant viruses expressing the hMPV F protein. We also did not conduct parallel testing using hMPV-infected cell lysates as the antigen in ELISA, and such testing could help confirm our results.

There are many reports of coinfections with various respiratory viruses, including hMPV. Although most investigators have reported low rates of coinfection, some have reported that 16%–30% of hMPV-positive samples harbored a coinfection with other viral or bacterial pathogens [32–34]. Therefore, although it is unlikely that all specimens positive for both RSV and hMPV represent true coinfections, some of them may. It is also important to document the potential for cross-reactivity or nonspecific antibody increases as serological tests are developed for pneumoviruses.

Two genotypes of hMPV have been documented, and some studies suggest that they may represent 2 serotypes on the basis of neutralization capability [35]. However, we have found 100% concordance using ELISA with both the A and B lineages of hMPV F, demonstrating significant cross-reactivity between the 2 F glycoproteins. If they are separate serotypes, they cannot be distinguished by reactivity to the F glycoprotein, and, because F is the major antigenic determinant of virus-neutralizing antibodies, our results do not support a serotype distinction between the hMPV genotypes.

Epidemics of hMPV infection seem to peak in the winter to spring in temperate regions [2, 36], although there are reports of summer outbreaks [37]. There are few published studies regarding transmission of hMPV infection in tropical areas, but one study in Hong Kong demonstrated hMPV activity only from January through July [38]. In another study in New Delhi, most hMPV infections were detected from December through February [39], and a study of samples collected during July and August in central India found that 5 of 26 were positive [40]. In a retrospective serosurvey in Bangladesh, all cases of hMPV infection occurred from January through July, with a peak in April [17]. Interestingly, in one study in Thailand, all 6 cases of hMPV infection detected during a 2-year period occurred between August and November [5]. Here we also demonstrate active infection throughout the summer and fall, with an outbreak occurring in August. However, given the usually reported pattern, if samples had been obtained during the months from winter through spring, infection or reinfection with hMPV may have accounted for an even greater percentage of illnesses in this population.

Taken together, our findings demonstrate that recombinant hMPV F is an antigenic glycoprotein that is useful for the detection of antibodies to hMPV. This is the first study to use purified recombinant F in an ELISA for screening and to determine infection rates among a human cohort. We have obtained evidence that reinfection with hMPV is a significant cause of illness in older pediatric populations. Further studies are required to define clearly the role hMPV infection plays in this population.

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