Characterization of Human Metapneumovirus Infections in Israel

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Received 19 May 2005/Returned for modification 1 September 2005/Accepted 1 January 2006

Respiratory tract infections are a leading cause of morbidity and mortality worldwide. Even with the advancement of diagnostic tools, the causative agent of 20 to 30% of upper respiratory tract infections go undiagnosed. Recently, a newly identified human respiratory virus, human metapneumovirus (hMPV), was discovered in young children in The Netherlands. To study the prevalence of hMPV infections in Israeli children, respiratory specimens from 388 hospitalized children less than 5 years of age were evaluated for the presence of hMPV RNA, which was present in 42 (10.8%) of these samples. All hMPV-positive samples were negative for respiratory syncytial virus (RSV), influenza viruses (Flu) A and B, adenovirus, and parainfluenza viruses 1, 2, and 3. Conversely, hMPV RNA was not detected in 76 RSV-positive and 38 Flu A- or B-positive samples. Most hMPV activity was between the months February and April. Sequence analysis of 20 positive samples revealed that both of the hMPV genotypes (groups 1 and 2) have circulated in central Israel during the study period. Moreover, three of the four known hMPV subgroups (1A, 1B, and 2B) were detected among the tested samples. Seroprevalence of hMPV in 204 patients from the central part of Israel revealed that 100% of the children are hMPV seropositive by the age of 5 years old. We conclude that hMPV is a common respiratory pathogen in Israel, while mixed infections of hMPV with RSV or Flu in hospitalized children are apparently rare.

Human metapneumovirus (hMPV), a newly discovered virus, is an enveloped single-stranded negative-sense RNA virus (41). hMPV has been classified as a member of the *Paramyxoviridae* family and has been assigned to the subfamily *Pneumovirinae*, genus *metapneumovirus* (41). The *Paramyxoviridae* family also includes respiratory syncytial virus (RSV), measles virus, and mumps virus (26). As in the results seen with hMPV's close relative RSV, patients infected with hMPV present with symptoms ranging from upper respiratory tract infection to bronchiolitis and pneumonia often accompanied by high fever, myalgia, and vomiting (5, 7, 12, 23). hMPV has been shown to infect the majority of children by the age of 5 years. Moreover, reinfections have been observed in all age groups (13, 41). More recently, Semple et al. reported that children dually infected with hMPV and RSV present with severe bronchiolitis and increased risk of admission to a pediatric intensive care unit for mechanical ventilation (34).

Sequence analysis of hMPV isolates and infected patients revealed that there are two main genetic genotypes (1 and 2) (30, 41). Subsequently these two genotypes were found to represent four subgroups, 1A, 2A, 1B, and 2B (42). The presence of these two genotypes have been confirmed in many parts of the world (3, 4, 6, 43). More recently, Schildgen et al. have reported a new variant of hMPV isolated from a 6.5-year-old child in Germany (33).

hMPV infections have worldwide distribution. The virus has been detected in patient samples from countries all over the

world. hMPV has been reported from Canada (6), Australia (29), Denmark (44), Tunisia (14), France (15), Italy (28), Hong Kong (31), Japan (10), Brazil (8), the United States of America (11), Argentina (16), South Africa (20), Thailand (39), and Israel (46). The frequency of hMPV detection in patients with respiratory tract infections ranged from 2 to 25% (28, 36).

In Southern Israel, Wolf et al. reported the seroprevalence of hMPV among children less than 2 years of age to be 52% (46). To gain a better understanding of the epidemiology of hMPV in Israel, we evaluated the prevalence of hMPV in archived and prospective clinical samples from 388 hospitalized patients with defined respiratory clinical syndromes. Two patient populations were studied: one was negative to most common respiratory viruses for which laboratory diagnosis was sought (influenza A and B, RSV A and B, parainfluenza viruses 1, 2, 3, and adenovirus) and the other population was positive for either RSV or influenza viruses A or B. In addition, we performed phylogenetic analysis on hMPV-positive patient samples to understand which hMPV genotypes have circulated in Israel. Finally, we performed hMPV seroprevalence analysis on the sera of 204 individuals with a wide age distribution who had no clinical respiratory symptoms when the blood samples were obtained.

MATERIALS AND METHODS

Patients and samples. Clinical specimens from 388 hospitalized patients at several hospitals in the central part of Israel were tested for the presence of hMPV during two consecutive respiratory tract infection seasons (November 2002 to May 2003 and November 2003 to May 2004). Of the 388 patient samples checked, 274 (70.6%) were negative for all other respiratory viral pathogens routinely tested in our laboratory (RSV, influenza virus A and B, adenovirus, and parainfluenza 1, 2, and 3) and 114 (29.3%) patient samples were positive for either RSV (66.7%) or influenza viruses (33.3%). Of the 274 negative patient

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samples, 131 (47.8%) were collected in the 2002 to 2003 respiratory season and 143 (52.2%) were collected in the 2003 to 2004 respiratory season. Patient specimen types consisted of 238 (86.8%) nasal aspirates, 21 (7.7%) nose swabs, 9 (3.3%) throat swabs, 5 (1.8%) bronchial alveolar lavage samples, and 1 (0.4%) lung biopsy sample. The positive patient samples that were available from both respiratory seasons were 33 (29%) samples from 2002 to 2003 and 81 (71%) samples from the 2003 to 2004 season. Of 114 positive samples, there were 82 (72%) nasal aspirates, 16 (14%) nose swabs, 9 (7.9%) sputum samples, and 7 (6.1%) nose/throat swabs. Health care providers had been educated on the proper method of specimen collection, storage, and transport. Samples were kept at 4°C and transported within 48 h to Israel's Central Virology Laboratory. Nose and throat swabs were transported to the laboratory in M-199 medium supplemented with 200 µg/ml streptomycin, 100 U/ml penicillin, and 121.5 U/ml nystatin at 4°C. On the other hand, nasal aspirates and lung biopsy and bronchial alveolar lavage samples were transported in sterile leak-proof containers to the laboratory. All clinical samples were tested and reported negative for influenza viruses A and B, RSV A and B, and adenovirus by real-time PCR using *TaqMan* chemistry and tested for parainfluenza viruses 1, 2, and 3 by the shell vial method (18, 19). For hMPV molecular analysis, we used retrospective patient RNA samples from the 2002 to 2003 season which were stored at -70°C, while RNA patient samples from 2003 to 2004 season were analyzed immediately after RNA extraction.

Determination of hMPV seroprevalence by indirect immunofluorescent assay (IFA) was performed on 204 serum samples obtained during 2002 from hospitalized patients with no respiratory clinical symptoms. The distribution of patient samples in each of the groups was as follows: <1 year, 25; 1 to 4 years, 23; 5 to 14 years, 31; 15 to 19 years, 20; 20 to 49 years, 60; 50 to 59 years, 20; >60 years, 25. Patient serum was stored at -70°C.

Cell line and virus strain. Vero cell monolayers in T-25 flasks were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 200 µg/ml streptomycin, 100 U/ml penicillin, and 121.5 U/ml nystatin in a 37°C incubator. hMPV reference strain virus 200026583 (CAN 97-83) passage 3, which was used as a positive control, was graciously supplied by Dean D. Erdman from the Centers for Disease Control and Prevention, Atlanta, Ga. Vero cells (ATCC CCL8) were used to grow the hMPV reference strain. hMPV reference virus was vortexed for 30 s to homogenize samples, and 200 µl was used to inoculate 70% confluent Vero cells in duplicate. Inoculated cells were maintained in serum-free Dulbecco's modified Eagle's medium supplemented with 200 µg/ml streptomycin, 100 U/ml penicillin, 121.5 U/ml nystatin, and 2 µg/ml trypsin type IX (T-0.134; Sigma) and incubated at 34°C. Cell cultures were observed daily for 21 days for the appearance of cytopathic effects. Medium was replaced weekly.

IFA. IFA was performed as previously described (24, 41) with some modifications. Briefly, Vero cells infected with the hMPV reference strain were mixed with uninfected Vero cells at a ratio of 70% and 30%, respectively. The cells were then spotted on acetone-cleaned glass slides and left to dry at room temperature. The cells on each slide were then fixed with ice-cold acetone for 10 min and stored at -70°C until used. Patient's sera were serially diluted in phosphate-buffered saline and incubated with either hMPV-infected or uninfected (negative control) fixed Vero cells for 45 min at 37°C. The cells were then washed three times with phosphate-buffered saline and incubated with fluorescein isothiocyanate-conjugated AffiniPure goat anti-human immunoglobulin G, Fcγ fragment specific (Jackson ImmunoResearch Laboratories, Inc.) for 35 min at 37°C in a humidified box. Cells were then washed and dried, and mounting fluid was added. A Zeiss inverted fluorescent microscope was used to visualize all the samples. Patient samples with a titer greater than 1:32 was considered positive for hMPV antibodies as described by van den Hoogen et al. (41).

RNA extraction. Viral genomic RNA was extracted from patients' sample supernatants using either the QIAamp (QIAGEN GmbH, Hilden, Germany) RNA extraction kit during the 2002 to 2003 respiratory season or the High Pure viral RNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany) during the 2003 to 2004 respiratory season. Both kits were validated for viral RNA extraction from patient samples. In our hands, the efficiency for viral RNA extraction was similar for both kits used (data not shown). RNA was extracted by following the manufacturers' suggested protocols. Briefly, clinical samples were homogenized by vortexing for 30 s, and 140 µl was used to extract viral RNA by QIAGEN (QIAamp kit) or 200 µl was used to extract viral RNA by Roche (High Pure viral RNA). The RNA was eluted from the columns after several washes and stored at -70°C. A 5-µl aliquot was used for the one-step reverse-transcription (RT)-PCRs.

RT-PCR analysis and sequencing. Two different sets of primers were used in this study. Primer sequences encoding part of the L (polymerase) gene, which were generously provided by B. G. van den Hoogen from Erasmus University Medical Center, Rotterdam, The Netherlands, were used for the diagnosis of

patient clinical samples, for determining the presence of hMPV genome, and for phylogenetic analysis. The sequence of the forward (L6) and reverse (L7) primers used were 5'-CAT GCC CAC TAT AAA AGG TCA G-3' (nucleotide positions 11336 to 11357) and 5'-CAC CCC AGT CTT TCT TGA AA-3' (nucleotide positions 11506 to 11486), respectively. A positive control was prepared from the plasmid pCR 2.1, which contains a 171-bp-long insert derived from the hMPV L gene. The plasmid was kindly donated by B. G. van den Hoogen. The insert "RAP PCR fragment 10" was amplified using primers L6 and L7 after digestion of plasmid preps with HindIII and agarose gel purification. The purified fragment was diluted serially to serve as a marker for the sensitivity of the RT-PCR.

Primers sequences MPV01.2 (forward) and MPV02.2 (reverse) derived from the N gene, which were designed by Mackay et al. (25), were used for the phylogenetic analysis of hMPV-positive patient samples and to confirm hMPV patient samples positive by the L gene. The sequence of the forward primer for the N gene was 5'-AAC CGT GTA CTA AGT GAT GCA CTC-3' (MPV01.2), and the sequence of the reverse primer was 5'-CAT TGT TTG ACC GGC CCC ATA A-3' (MPV02.2). The resulting fragment length is 213 bp.

RT-PCR was performed using QIAGEN OneStep RT-PCR kit (QIAGEN GmbH, D-40724 Hilden, Germany). Briefly, 5 µl extracted RNA was added to a master mix composed of enzyme mix (mixture of heterodimeric recombinant reverse transcriptases Omniscript, Sensiscript, and HotStart *Taq* DNA polymerase), 400 µM concentrations of each deoxynucleoside triphosphates, 20 U RNase inhibitor (CPG, Lincoln Park, N.J.) and the hMPV primers sets at 20 pM final concentration (each). Amplification used the optimized profile provided by B. G. van den Hoogen in the thermal cycler (PTC-100; MJ Research, Watertown, MA): 42°C for 45 min, 95°C for 7 min, followed by 35 amplification cycles (denaturation at 95°C for 1 min, annealing at 45°C for 2 min, and synthesis at 72°C for 3 min) (personal communication). Amplification was completed with a prolonged synthesis at 72°C for 10 min. Amplicons were visualized by ethidium bromide staining following electrophoresis on a 2% agarose gel.

Real-time RT-PCR analysis. *TaqMan* chemistry was utilized to detect the hMPV genome in patient samples that were positive for either RSV or influenza viruses A and B. hMPV assays conditions were previously described by Maertzdorf et al. (27). The assay was at least as sensitive as the regular RT-PCR with gel detection.

Phylogenetic analysis. RT-PCR products were gel purified or purified directly from the RT-PCR mix and sequenced using ABI PRISM Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). Reaction mixtures were analyzed on Applied Biosystems model 373 DNA automatic sequencing systems. The Sequencher (Gencodes Corporation, Ann Arbor, MI) and University of Wisconsin GCG gene analysis programs (GCG analysis programs, Acleris, San Diego, Calif.) were used to compare nucleotide sequences. Phylogenetic trees were prepared by either (i) nearest neighbor analysis using Clustal X with 1,000 bootstraps (40) or (ii) maximum-likelihood phylogenetic analysis using the DnaM application of PHYLIP (<http://evolution.genetics.washington.edu/phylip/getme.html>; J. Felsenstein, Department of Genetics, University of Washington) with data bootstrapped 100 times and sequence order randomized thrice for each bootstrap. Trees were visualized using TreeView or NJ plot.

Nucleotide sequence accession number. Partial N and L gene sequences for individual hMPV isolates have been submitted to the EMBL database as a noncontiguous sequence. Sequences have been assigned accession numbers AM056029 to AM056048. The alignment of these sequences compared to isolate 00-1 (AF371337) for reference has been deposited in the EMBL alignment database as ALIGN_000919.

RESULTS

Incidence of hMPV infections among hospitalized children.

The Central Virology Laboratory (CVL) diagnostic testing primarily serves a population located in the central part of Israel. From November 2002 to November 2004, we received 613 respiratory specimens from hospitalized children for viral diagnosis. These specimens were first tested for common viral pathogens (RSV, influenza viruses A and B, adenovirus, and parainfluenza virus 1, 2, and 3) using various techniques (18, 19). Of the 613 patient samples, 274 (44.7%) were negative for all of the above respiratory viruses. In addition, of the 339 positive patient samples remaining, 114 were available for

FIG. 1. Histogram of monthly distribution of RT-PCR-positive clinical cases for hMPV RNA among hospitalized patients during the winter seasons 2002 to 2003 ($n = 25$) and 2003 to 2004 ($n = 17$).

hMPV testing. These samples were positive for either RSV (66.7%) or influenza viruses (33.3%).

Of the 274 negative samples, 42 (15.3%) were positive by RT-PCR for the hMPV L gene. Confirmation of the hMPV-positive samples was done with a different set of primers derived from the N gene. No discrepancy in hMPV-positive results was observed between the two sets of primers. Analyses of each of the two respiratory seasons revealed that 25 (19%) of the 131 samples analyzed during the 2002 to 2003 season and 17 (12%) of the 143 samples analyzed during 2003 to 2004 season were positive for hMPV. Interestingly, none of the patient samples positive for RSV or influenza viruses ($n = 114$) were positive for hMPV when tested by a real-time RT-PCR assay. This assay was at least as sensitive as the regular RT-PCR assay, and random repeat testing for RSV and influenza viruses for the positive samples was positive with cycle threshold values similar to those initially detected on the original sample, indicating that the RNA was not degraded. Since none of the 114 samples positive for either RSV or influenza were hMPV positive, we estimate that the rate of double infection was below 0.9%. Thus, in our study population, the percentage of positive hMPV RNA from the total specimens submitted to CVL ($n = 613$) for respiratory viral testing was estimated to be about 6.9%.

hMPV circulated in central Israel between November and April in each of the two seasons studied. Figure 1 shows the monthly distribution of positive hMPV samples. Increased hMPV incidence was observed during February/March for the 2002 to 2003 season, which corresponds to the RSV season in Israel. Main RSV activity was between the months of January and March 2003. On the other hand, hMPV activity was mainly during the months March and April for the 2003 to 2004 season, while the main RSV activity during this season was recorded earlier, between January and March 2004.

The age distribution among the hMPV-positive patients ($N = 42$) was as follows: <1 year, 7 (16.7%); 1 year, 25 (59.5%); 2 years, 5 (11.9%); 3 years, 4 (9.5%); 4 years, 1 (2.4%); 5 years, none. The male-to-female ratio within this group was 2:1.

hMPV phylogenetic analysis. Phylogenetic analysis was performed for the N (164 bp) and/or L gene (113 bp) from 20 of 42 (48%) hMPV RNA-positive samples isolated between January and December 2003. Sequences from all 20 isolates were highly similar (98 to 100%) to hMPV sequences in the EMBL/

FIG. 2. Phylogenetic comparison of hMPV N gene nucleotides detected during the respiratory seasons 2002 to 2003 and 2003 to 2004 with sequences from EMBL/GenBank database. The Clustal X nearest neighbor-joining method (number of bootstraps, 1,000) was used to compare 164-nucleotide sequences encoding the hMPV N gene with seven sequences from the EMBL/GenBank database. Subclades 1A, 1B, 2A, and 2B are indicated at the right hand side of the figure.

GenBank/DDBJ databases. The alignment of sequences from all 20 isolates compared to isolate 00-1 (AF371337) for reference has been deposited in EMBL alignment database as ALIGN_000919. Two main genotypes (groups 1 and 2), with two subgroups each, were reported in several areas of the world (3, 5, 21). Three of the four subgroups also circulated in Israel during 2003, as illustrated by the phylogenetic tree for N gene sequences from 12 randomly selected Israeli isolates and representative subgroup sequences obtained from the EMBL/GenBank/DDBJ databases (Fig. 2). The distribution was 25% subgroup 1A, 65% subgroup 1B, and 10% subgroup 2B. No representatives of subgroup 2A were observed among the 20 samples analyzed. Bootstrapped maximum likelihood phylogenetic trees were prepared for the 11 isolates in which both the L and N gene amplicons were sequenced (Fig. 3). The topology of the phylogenetic tree for the L gene was very similar to the tree of the N gene.

Seroprevalence of hMPV antibodies in healthy Israeli individuals. The seroprevalence of hMPV antibodies was determined by IFA in a cohort of 204 Israeli patients of different age groups from the central part of Israel (Fig. 4). Individuals investigated were hospitalized but did not have any respiratory symptoms at the time of blood sample collection. Children less than 1 year of age had low levels of hMPV antibodies (28%). This changed in the 5- to 14-year age group as hMPV seropositivity reached 100%. hMPV seropositivity declined with age to reach about 72% in the 20- to 49-year age group. This

FIG. 3. Phylogenetic analysis of hMPV-positive specimens detected in Israel. The nucleotide sequences of hMPV L gene (113 nucleotides) and the N gene (164 nucleotides) were determined. The maximum likelihood phylogenetic analysis using the Dnaml application of PHYLIP (100 bootstraps) was used to compare different Israeli sequences encoding hMPV L and N genes. Subgroup 1A, 1B, and 2B are indicated.

was followed by a gradual rise in seropositivity in the older individuals studied.

DISCUSSION

One of the biggest challenges in diagnostic microbiology is the determination of the causative agent of respiratory tract infections (RTI). Even with extensive diagnostic testing with highly sensitive assays such as PCR and real-time PCR, a substantial portion of RTI cannot be attributed to any known pathogen (17, 32, 36). Up to 40% of patients with respiratory illnesses during the winter months contain no identifiable viral pathogen (36). At CVL, where highly sensitive molecular diagnostic assays are utilized, 30 to 40% of hospitalized patients presenting with upper respiratory tract infections do not have a causative agent identified. In part, this may reflect the presence of human pathogens that have not been discovered yet. The discovery of hMPV in 2001 by van den Hoogen et al., has helped to identify a human pathogen that is causing some of the undiagnosed respiratory tract infections (41). In our

study, by using hMPV RNA detection assays, we have found hMPV to be associated with about 15% of the respiratory illness in hospitalized children negative for other common respiratory pathogens over the two-season study period. At Israel CVL, the incidence of hMPV RNA detection was 6.9% when determined from the total patient samples analyzed in the laboratory. Thus, during the two respiratory seasons studied in central Israel, hMPV is likely to have been the causative agent of at least 6.9% of the upper respiratory tract infections which led to hospitalization, although we cannot rule out other viral or bacterial pathogens as the actual causative agents of the illness. This rate would not change dramatically, since no double infections were detected in 114 (one-third) of the RSV- or influenza-positive patient samples available for analysis from central Israel. Dual infection did not appear to play a major role in the patient populations analyzed during 2002 to 2003 and 2003 to 2004 winter seasons in central Israel. Our data are consistent with the study reported by Lazar from Connecticut, who did not detect any dual infections of hMPV and RSV in their hospitalized patient population with severe or mild RSV infection (23). On the contrary, Semple et al. (34) reported a 10% rate of hMPV and RSV double infection in children hospitalized in the general wards and as high as 72% in infected infants admitted to the pediatric intensive care unit. The dramatic difference in the incidence of dual infection reported by Semple et al. and us could not be due to technical issues, since we used similar molecular techniques. Indeed, our evaluation of dual infections was with a highly sensitive real-time molecular assay that has a sensitivity and specificity at least similar to those of regular RT-PCR. Other groups which examined the rate of hMPV coinfection with other viruses among hospitalized children with acute RTI reported rates of 15 to 40% of the hMPV infections (1, 22). We have not detected hMPV coinfection with other viruses. However, we have not tested our samples for enteroviruses and rhinoviruses, which

FIG. 4. Age distribution of patients in central Israel (*n* = 204) seropositive for hMPV as determined by indirect immunofluorescence assay on samples obtained in 2002. The number of individuals in each age group is indicated on the top of each bar.

are common respiratory pathogens which only recently were recognized as contributing to acute expiratory wheezing in infants and children (22).

The data on the incidence of hMPV infections from other countries are inconsistent due to different calculation methods and year-to-year variation within a given region (35). The hMPV positivity rate ranged from 1.5% in Australia, 7.1% in Canada, 20% in the United States, and 25% in Italy (5, 28, 29, 45).

The clinical picture of hMPV-infected children was similar to what was reported in the world (31, 35, 37, 38). The Israeli children infected with hMPV presented with bronchiolitis symptoms similar to those caused by RSV. The symptoms of the hMPV-infected patients ranged from mild self-limiting respiratory illness, with cough, rhinorrhea, and wheezing as the major symptoms, to much more severe illness requiring mechanical ventilation.

Interestingly, there was a month difference between the hMPV seasons during the study period. During the season 2002 to 2003, the RSV and the hMPV season peaked at the same time between January and March 2002. However, during the 2003 to 2004, hMPV season peaked between March and April 2003, while RSV major activity was between the months January and March. Several countries reported different hMPV seasonality. In Canada, Boivin et al. reported hMPV activity in April (6), while a Dutch group reported hMPV activity during December (30).

In our study, patient's specimens were analyzed for the presence of hMPV mainly by RT-PCR to detect the hMPV L gene using conditions which were optimized by van den Hoogen et al. (41). Patient samples positive with the L gene were confirmed by a second PCR using a set of primers for the N gene as described by Mackay et al. (25). Sequence analysis of 20 patients' N and L hMPV genes revealed that the two main hMPV groups 1 and 2 circulated in Israel. Many studies reported the detection of hMPV group 1 as the only or the predominant serotype circulating (2, 3, 6, 28, 31). In our study population, hMPV group 1 also had the highest circulation rate (92% of the sequenced samples). Of the four subgroups reported (3, 4, 21), three were identified in central Israel (1A, 1B, and 2B). hMPV subgroup 1B had the highest circulation rate (65%), followed by 1A (25%) and 2B (10%), of the sequenced samples. No circulation of subgroup 2A was detected during the study period. However, subgroup 2A could be present in central Israel but at a frequency too low to have been detected in the small number of samples analyzed.

The similar topological clustering within the phylogenetic trees of N, P, M, and F genes of individual Canadian and Japanese hMPV strains (2, 21, 23) indicates parallel evolution of these genes. In general, this means that when the subgroup of an isolate is defined for one gene, the other genes will also belong to the same subgroup. The similar topological distribution of N and L genes in the bootstrapped phylogenetic trees of our isolates presented in Fig. 3, especially after collapsing branches with bootstrap values of less than 90%, indicates that a subgroup assignment for an hMPV isolate based on the N gene can be extrapolated to the L gene, as has been done for the P, M, and F genes (2, 21, 23). However, it should be noted that there appears to be less variability in the L gene subgroup 1B than in the corresponding N gene 1B subgroup (Fig. 3).

To determine the seroprevalence of hMPV in central Israel,

the serum of hospitalized Israeli patients admitted for symptoms other than respiratory tract-related illness was checked for hMPV antibodies (Fig. 4). The seroprevalence of hMPV in children less than 1 year of age was about 28%. This is similar to the 30% that was reported by Wolf et al. from children less than 13 months of age in the southern part of Israel (46). Moreover, a similar observation was reported by van den Hoogen et al., where the seropositivity of children less than 1 year of age from The Netherlands was about 25% (41). hMPV seroprevalence in Japanese children less than 5 years of age showed a pattern similar to that of the Israeli children (9). As the children's ages increase, hMPV seroprevalence increases reaching 100% within the age group of 5 to 14 years olds. The increase in the seroprevalence is similar to what was observed in The Netherlands (41). Unlike The Netherlands, where the percent seropositivity was at 100% in all age groups older than 5 years, in central Israel, the percent seropositivity after 14 years was less than 100%, ranging from a low of 72% in the age group of 20 to 49 to a high of 80% in individuals older than 60 years of age. The decrease in seroprevalence which occurs between 14 to 60 years might be due to a decline in immune responsiveness with age or due to reduced exposure to hMPV. This is similar to the pattern for RSV and may help explain why some adults are prone to reinfection, but the small sample size limits the interpretation of this finding.

In conclusion, this study is the first report that describes the viral genetic lineages and the prevalence of hMPV infections in central Israel. The discovery of hMPV and its incorporation to routine diagnostic procedures has allowed us to identify the cause of a significant number of respiratory tract infections. More upper RTI will be identified when sensitive diagnostic techniques are developed and routinely applied from testing common respiratory pathogens such as rhinoviruses, and coronavirus. However, there still remain a significant number of infections that have still not been associated with any known pathogen.

ACKNOWLEDGMENTS

We acknowledge the TaqMan Unit personnel for the work they have done on prescreening the respiratory samples for influenza viruses A and B, RSV A and B, and adenovirus. Many thanks to Dana Wolf from Hadassa University Hospital, Jerusalem, Israel for helping with developing the hMPV IFA assay. Many thanks to B. G. van den Hoogen, Dean Erdman, and Guy Bovin for assistance with initiating the hMPV study in Israel. We are indebted to Daniela Ram for critical review of the manuscript.

REFERENCES

1. Al-Sonboli, N., C. A. Hart, A. Al-Aeryani, S. M. Banajeh, N. Al-Aghbari, W. Dove, and L. E. Cuevas. 2005. Respiratory syncytial virus and human metapneumovirus in children with acute respiratory infections in Yemen. *Pediatr. Infect. Dis. J.* **24**:734–736.
2. Bastien, N., L. Liu, D. Ward, T. Taylor, and Y. Li. 2004. Genetic variability of the G glycoprotein gene of human metapneumovirus. *J. Clin. Microbiol.* **42**:3532–3537.
3. Bastien, N., S. Normand, T. Taylor, D. Ward, T. C. Peret, G. Boivin, L. J. Anderson, and Y. Li. 2003. Sequence analysis of the N, P, M and F genes of Canadian human metapneumovirus strains. *Virus Res.* **93**:51–62.
4. Biacchesi, S., M. H. Skiadopoulos, G. Boivin, C. T. Hanson, B. R. Murphy, P. L. Collins, and U. J. Buchholz. 2003. Genetic diversity between human metapneumovirus subgroups. *Virology* **315**:1–9.
5. Boivin, G., Y. Abed, G. Pelletier, L. Ruel, D. Moisan, S. Cote, T. C. Peret, D. D. Erdman, and L. J. Anderson. 2002. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. *J. Infect. Dis.* **186**:1330–1334.

6. Boivin, G., G. De Serres, S. Cote, R. Gilca, Y. Abed, L. Rochette, M. G. Bergeron, and P. Dery. 2003. Human metapneumovirus infections in hospitalized children. *Emerg. Infect. Dis.* **9**:634–640.
7. Cane, P. A., B. G. van den Hoogen, S. Chakrabarti, C. D. Fegan, and A. D. Osterhaus. 2003. Human metapneumovirus in a haematopoietic stem cell transplant recipient with fatal lower respiratory tract disease. *Bone Marrow Transplant.* **31**:309–310.
8. Cuevas, L. E., A. M. Nasser, W. Dove, R. Q. Gurgel, J. Greensill, and C. A. Hart. 2003. Human metapneumovirus and respiratory syncytial virus, Brazil. *Emerg. Infect. Dis.* **9**:1626–1628.
9. Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, H. Ishiko, and K. Kobayashi. 2004. Comparison of the seroprevalence of human metapneumovirus and human respiratory syncytial virus. *J. Med. Virol.* **72**:304–306.
10. Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, M. Yoshioka, X. Ma, and K. Kobayashi. 2003. Seroprevalence of human metapneumovirus in Japan. *J. Med. Virol.* **70**:281–283.
11. Esper, F., D. Boucher, C. Weibel, R. A. Martinello, and J. S. Kahn. 2003. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. *Pediatrics* **111**:1407–1410.
12. Esper, F., R. A. Martinello, D. Boucher, C. Weibel, D. Ferguson, M. L. Landry, and J. S. Kahn. 2004. A 1-year experience with human metapneumovirus in children aged <5 years. *J. Infect. Dis.* **189**:1388–1396.
13. Falsey, A. R., D. Erdman, L. J. Anderson, and E. E. Walsh. 2003. Human metapneumovirus infections in young and elderly adults. *J. Infect. Dis.* **187**:785–790.
14. Fodha, I., L. Legrand, A. Vabret, T. Jrad, N. Gueddiche, A. F. Trabelsi, and F. Freymuth. 2004. Detection of human metapneumovirus in two Tunisian children. *Ann. Trop. Paediatr.* **24**:275–276.
15. Freymouth, F., A. Vabret, L. Legrand, N. Eterradosi, F. Lafay-Delaire, J. Brouard, and B. Guillois. 2003. Presence of the new human metapneumovirus in French children with bronchiolitis. *Pediatr. Infect. Dis. J.* **22**:92–94.
16. Galiano, M., C. Videla, S. S. Puch, A. Martinez, M. Echavarría, and G. Carballal. 2004. Evidence of human metapneumovirus in children in Argentina. *J. Med. Virol.* **72**:299–303.
17. Grondahl, B., W. Puppe, A. Hoppe, I. Kuhne, J. A. Weigl, and H. J. Schmitt. 1999. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. *J. Clin. Microbiol.* **37**:1–7.
18. Hindiyyeh, M., V. Levy, R. Azar, N. Varsano, L. Regev, Y. Shalev, Z. Grossman, and E. Mendelson. 2005. Evaluation of a multiplex real-time reverse transcriptase PCR assay for detection and differentiation of influenza viruses A and B during the 2001–2002 influenza season in Israel. *J. Clin. Microbiol.* **43**:589–595.
19. Hu, A., M. Colella, J. S. Tam, R. Rappaport, and S. M. Cheng. 2003. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. *J. Clin. Microbiol.* **41**:149–154.
20. Ijpm, F. F., D. Beekhuis, M. F. Cotton, C. H. Pieper, J. L. Kimpen, B. G. van den Hoogen, G. J. van Doornum, and D. M. Osterhaus. 2004. Human metapneumovirus infection in hospital referred South African children. *J. Med. Virol.* **73**:486–493.
21. Ishiguro, N., T. Ebihara, R. Endo, X. Ma, H. Kikuta, H. Ishiko, and K. Kobayashi. 2004. High genetic diversity of the attachment (G) protein of human metapneumovirus. *J. Clin. Microbiol.* **42**:3406–3414.
22. Jartti, T., P. Lehtinen, T. Vuorinen, R. Osterback, B. van den Hoogen, A. D. Osterhaus, and O. Ruuskanen. 2004. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg. Infect. Dis.* **10**:1095–1101.
23. Lazar, I. 2004. Human metapneumovirus and severity of respiratory syncytial virus disease. *Emerg. Infect. Dis.* **10**:1318–1320.
24. Leventon-Kriss, S., L. Rannon, and R. Joffe. 1976. Fluorescence and neutralizing antibodies to herpes simplex virus in the cerebrospinal fluid of patients with central nervous system diseases. *Isr. J. Med. Sci.* **12**:553–559.
25. Mackay, I. M., K. C. Jacob, D. Woolhouse, K. Waller, M. W. Syrmis, D. M. Whitley, D. J. Siebert, M. Nissen, and T. P. Sloots. 2003. Molecular assays for detection of human metapneumovirus. *J. Clin. Microbiol.* **41**:100–105.
26. Mackie, P. L. 2003. The classification of viruses infecting the respiratory tract. *Paediatr. Respir. Rev.* **4**:84–90.
27. Maertzdorf, J., C. K. Wang, J. B. Brown, J. D. Quinto, M. Chu, M. de Graaf, B. G. van den Hoogen, R. Spaete, A. D. Osterhaus, and R. A. Fouchier. 2004. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J. Clin. Microbiol.* **42**:981–986.
28. Maggi, F., M. Pifferi, M. Vatteroni, C. Fornai, E. Tempestini, S. Anzilotti, L. Lanini, E. Andreoli, V. Ragazzo, M. Pistello, S. Specter, and M. Bendinelli. 2003. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infants in Italy. *J. Clin. Microbiol.* **41**:2987–2991.
29. Nissen, M. D., D. J. Siebert, I. M. Mackay, T. P. Sloots, and S. J. Withers. 2002. Evidence of human metapneumovirus in Australian children. *Med. J. Aust.* **176**:188.
30. Osterhaus, A., and R. Fouchier. 2003. Human metapneumovirus in the community. *Lancet* **361**:890–891.
31. Peiris, J. S., W. H. Tang, K. H. Chan, P. L. Khong, Y. Guan, Y. L. Lau, and S. S. Chiu. 2003. Children with respiratory disease associated with metapneumovirus in Hong Kong. *Emerg. Infect. Dis.* **9**:628–633.
32. Puppe, W., J. A. Weigl, G. Aron, B. Grondahl, H. J. Schmitt, H. G. Niesters, and J. Groen. 2004. Evaluation of a multiplex reverse transcriptase PCR ELISA for the detection of nine respiratory tract pathogens. *J. Clin. Virol.* **30**:165–174.
33. Schildgen, O., T. Geikowski, T. Glatzel, A. Simon, A. Wilkesmann, M. Roggendorf, S. Viazov, and B. Matz. 2004. New variant of the human metapneumovirus (HMPV) associated with an acute and severe exacerbation of asthma bronchiale. *J. Clin. Virol.* **31**:283–288.
34. Semple, M. G., A. Cowell, W. Dove, J. Greensill, P. S. McNamara, C. Halfhide, P. Shears, R. L. Smyth, and C. A. Hart. 2005. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J. Infect. Dis.* **191**:382–386.
35. Serafino, R. L., R. Q. Gurgel, W. Dove, C. A. Hart, and L. E. Cuevas. 2004. Respiratory syncytial virus and metapneumovirus in children over two seasons with a high incidence of respiratory infections in Brazil. *Ann. Trop. Paediatr.* **24**:213–217.
36. Stockton, J., I. Stephenson, D. Fleming, and M. Zambon. 2002. Human metapneumovirus as a cause of community-acquired respiratory illness. *Emerg. Infect. Dis.* **8**:897–901.
37. Suzuki, A., O. Watanabe, and H. Nishimura. 2003. Detection of human metapneumovirus from wheezing children in Japan. *Kansenshogaku Zasshi* **77**:467–468.
38. Takao, S., H. Shimozono, H. Kashiwa, Y. Shimazu, S. Fukuda, M. Kuwayama, and K. Miyazaki. 2003. Clinical study of pediatric cases of acute respiratory diseases associated with human metapneumovirus in Japan. *Jpn. J. Infect. Dis.* **56**:127–129.
39. Thanasugarn, W., R. Samransamruajkit, P. Vanapongtipagorn, N. Prapaphal, B. Van den Hoogen, A. D. Osterhaus, and Y. Poovorawan. 2003. Human metapneumovirus infection in Thai children. *Scand. J. Infect. Dis.* **35**:754–756.
40. Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**:4876–4882.
41. van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier, and A. D. Osterhaus. 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat. Med.* **7**:719–724.
42. van den Hoogen, B. G., S. Herfst, L. Sprong, P. A. Cane, E. Forleo-Neto, R. L. de Swart, A. D. Osterhaus, and R. A. Fouchier. 2004. Antigenic and genetic variability of human metapneumoviruses. *Emerg. Infect. Dis.* **10**:658–666.
43. Viazov, S., F. Ratjen, R. Scheidhauer, M. Fiedler, and M. Roggendorf. 2003. High prevalence of human metapneumovirus infection in young children and genetic heterogeneity of the viral isolates. *J. Clin. Microbiol.* **41**:3043–3045.
44. von Linstow, M. L., H. Henrik Larsen, J. Eugen-Olsen, A. Koch, T. Nordmann Winther, A. M. Meyer, H. Westh, B. Lundgren, M. Melbye, and B. Høgh. 2004. Human metapneumovirus and respiratory syncytial virus in hospitalized Danish children with acute respiratory tract infection. *Scand. J. Infect. Dis.* **36**:578–584.
45. Williams, J. V., P. A. Harris, S. J. Tollefson, L. L. Halburnt-Rush, J. M. Pingsterhaus, K. M. Edwards, P. F. Wright, and J. E. Crowe, Jr. 2004. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N. Engl. J. Med.* **350**:443–450.
46. Wolf, D. G., Z. Zakay-Rones, A. Fadeela, D. Greenberg, and R. Dagan. 2003. High seroprevalence of human metapneumovirus among young children in Israel. *J. Infect. Dis.* **188**:1865–1867.