Published in final edited form as:

Pediatr Infect Dis J. 2012 July ; 31(7): 763–766. doi:10.1097/INF.0b013e3182539d92.

Viral Load and Acute Otitis Media Development after Human Metapneumovirus Upper Respiratory Tract Infection

Johanna Nokso-Koivisto, MD, PhD^{1,4}, Richard B. Pyles, PhD^{1,2}, Aaron L. Miller, MS¹, Janak A Patel, MD¹, Michael Loeffelholz, PhD³, and Tasnee Chonmaitree, MD^{1,3}

¹Department of Pediatrics, University of Texas Medical Branch, Galveston, TX

²Departments of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX

³Department of Pathology, University of Texas Medical Branch, Galveston, TX

Abstract

The role of human metapneumovirus (hMPV) in acute otitis media (AOM) complicating upper respiratory tract infection (URI) was studied. Nasopharyngeal specimens from 700 URI episodes in 200 children were evaluated; 47 (7%) were positive for hMPV, 25 (3.6%) with hMPV as the only virus. Overall, 24% of URI episodes with hMPV only were complicated by AOM, which was the lowest rate compared with other respiratory viruses. hMPV viral load was significantly higher in children with fever, but there was no difference in viral load in children with hMPV positive URI with or without AOM complication.

Keywords

Human metapneumovirus; upper respiratory tract infection; acute otitis media; viral load

INTRODUCTION

Human metapneumovirus (hMPV), which was first identified in 2001¹, is a respiratory virus that belongs to the Paramyxoviridae family, subfamily Pneumovirinae, along with RSV. Many studies have shown that hMPV is prevalent and infects almost all children by age 5 years causing respiratory infections ranging from upper respiratory tract infection (URI) to severe lower respiratory tract infections (LRI) in all age groups all over the world²⁻⁷.

Because of the difficulties in isolating this virus, molecular methods have been used for the diagnosis of hMPV infections. However, the higher sensitivity of respiratory viral nucleic acid amplification tests has made the interpretation of the positive results challenging. One way to correlate the presence of a specific virus with clinical disease is by virus

Corresponding author: Tasnee Chonmaitree Department of Pediatrics University of Texas Medical Branch 301 University Boulevard Galveston, TX 77555-0371 Phone: (409) 772-4876 Fax: (409) 747-1753 tchonmai@utmb.edu.

⁴Dr. Nokso-Koivisto's current address: Department of Otorhinolaryngology, Helsinki University Central Hospital, P.O.Box. 220, 00290 Helsinki, Finland

Conflicts of Interest Authors certify no conflicts of interest.

No reprints available.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

quantification; few studies have demonstrated the association of higher hMPV viral load and more severe LRI outcome^{3, 8, 9}.

Because hMPV is phylogenetically and clinically closely related to respiratory syncytial virus (RSV)^{1, 5}, it can be postulated that hMPV has the same tendency to induce acute otitis media (AOM) as RSV, an apparent ototropic virus¹⁰. During AOM, hMPV has been detected in 8% to 13% of NPS samples^{11, 12} and in 2.3% of middle ear fluids (MEF)¹². However, the role of hMPV in inducing AOM in children requires further study.

We have previously compared the rate of AOM complicating URI associated with various respiratory viruses¹⁰. In that study, relatively new viruses, including hMPV were not included. The aim of the present study was to determine the role of hMPV in AOM complicating URI and to determine if hMPV viral load is associated with AOM development after URI.

MATERIALS AND METHODS

Specimens and clinical data were obtained from a prospective, longitudinal study of children that aimed to determine the incidence and characteristics of URI complicated by AOM. The study was performed between January 2003 and March 2007 at the University of Texas Medical Branch in Galveston, Texas, USA, and was approved by the local Institutional Review Board. The study protocol has been described previously¹⁰. In brief, healthy children were enrolled at the age of 6 months to 3 years; they were followed for one year for occurrences of URI and AOM. The parents informed the study personnel when the child developed URI symptoms. Children were seen by a study physician, and followed closely for 4 weeks for the occurrence of AOM. At the study visit, otoscopic and physical examinations, and tympanometry were performed. AOM complicating URI was considered when AOM occurred within 28 days of the onset of URI. AOM was defined as 1) acute onset of symptoms, 2) signs of tympanic membrane inflammation and 3) the presence of fluid in the middle ear as documented by pneumatic otoscopy and/or tympanometry. Children were classified as otitis-prone if they had 4 OM episodes in one year, 3 in six months, 6 by age 6, or the first OM episode prior to age 6 months.

Respiratory specimens were collected for viral studies at the initial visit within 7 days of URI onset and when AOM was diagnosed. Nasal swabs were collected for viral culture and nasopharyngeal secretions (NPS) were collected for other viral studies using a suction catheter with mucus trap (Mucaid, Laboratoires Pharmaceutiques Vygon, Écouen, France). The catheter and trap were rinsed with 1mL of phosphate buffered saline (PBS) after collection to retrieve as much material as possible. The total secretion volume was recorded to provide the dilution factor of the original sample.

In the previous study, viral culture of nasal swab specimens was performed using standard methods¹⁰. NPSs collected during RSV season were also analyzed for RSV antigen detection by enzyme immunoassay (EIA). Culture and RSV-EIA –negative samples were analyzed by microarray PCR for RSV A and B, parainfluenza viruses 1-3 and influenza viruses A and B, and by real time polymerase chain reaction (PCR) for adenovirus, enterovirus, rhinovirus and coronavirus (OC43, 229E and NL63) (performed at the Medical College of Wisconsin, Milwaukee, USA).

Specific to this report, 727 available archived NPS specimens were tested for hMPV by qPCR. Nucleic acids were extracted from 200 µL of NPS samples using MagMAX® Total Nucleic Acid isolation kits (Ambion/Applied Biosystems, Austin, TX) in a Biosprint 96 (Qiagen, Valencia, CA). After extraction, the elution volume was diluted 1:1 with nuclease-free 0.1 mM EDTA (Ambion/Applied Biosystems, Austin, TX). The recovered RNA was

converted to cDNA using an iScript synthesis kit (Bio-Rad, Hercules, CA) in a reaction volume of 40 μ L. Generated cDNA was evaluated using a duplex qPCR assay with primers amplifying hMPV and RSV targets¹³. TaqMan probes (Sigma-Aldrich, St. Louis, MO, Integrated DNA Technologies, Coralville, IA) were used to track the specific amplification in the duplex. Each 25 μ L reaction contained: 12.5 μ L iQ supermix™ (Bio-Rad), 1 μ L (5 μ M) of hMPV and hRSV primers and 0.5 μ L (7.5 μ M) hMPV-FAM and RSV-TX Red probes, 4.5 μ L nuclease-free water and 3 μ L of cDNA template. qPCR was completed in a CFX™ real-time PCR machine (Bio-Rad). Viral genomic titers were extrapolated from standard curves of plasmids harboring the PCR targets generated in parallel for each run. Based on qPCR of known concentrations of positive material, the duplex assay is able to detect as few as 10 copies in 50% of the spiked samples providing a more sensitive assay than many of the other reports.

A parallel qPCR reaction for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH)¹⁴ was completed on every clinical sample to evaluate RNA and cDNA quality. Detection of hGAPDH at less than 500 copies /reaction from the NPS sample suggested inadequacy of the specimen and led to the exclusion of the sample from further analysis. Overall, 700 URI episodes were evaluable by this criterion, which represented 81% of the total 864 URI episodes for which specimens were collected for viral studies.

Viral load was calculated as RNA copies/ mL of the original specimen based on the original dilution of the specimens and subsequent assay dilutions associated with extraction and qPCR. Of 700 URI samples accurate viral load analyses of 58 NPS samples were not possible due to unknown NPS dilutions. As a result, viral load analysis was performed in NPS samples from 642 URI episodes.

Rates of AOM and fever were compared with hMPV viral load by Mann-Whitney rank sum test after natural logarithmic transformation of the viral load values.

RESULTS

Of 200 children who were studied for hMPV 49% were female, 59.5% were white, 28% black, 3% Asians and 9.5% were biracial; 45% were Hispanic, 55% non-Hispanic. Majority of the children (66%) did not attend daycare, 53% were breast fed, and 31% were exposed to cigarette smoke. Altogether, 56% of the children had experienced previous AOM episode(s) and 7% of all children were otitis-prone.

Of 700 URI episodes using all viral detection methods described above, respiratory viruses were detected in 534 (76%) episodes; 303 (43%) contained a single virus only. hMPV was detected from 48 (7%) samples; in 25 (3.6%) it was the sole virus. Other respiratory viruses detected were adenovirus in 163 (23%), rhinovirus in 143 (20%), RSV in 104 (15%), enterovirus in 100 (14%), coronavirus in 55 (8%), parainfluenza virus in 43 (6%) and influenza virus in 30 (4%) URI episodes. A total of 45 (23%) children with hMPV URI episodes were identified during the study period; three children had hMPV detected twice. hMPV was detected year-round except July; the highest incidence was from January to March. Median age at the time of hMPV infection was 17 months, which was among the youngest compared to URI caused by other viruses.

Children with hMPV alone had similar symptoms and signs of URI compared to all study children; children with hMPV seemed to have more fever, but the difference was not significant ($P=0.2$ by chi-square analysis).

Overall, 37% of URI episodes were complicated by AOM. hMPV was detected alone in 25 URI episodes, and 6 (24%) of these episodes were complicated by AOM, which was a lower

rate than any other virus. Among URIs associated with a single virus, the rate of AOM with other viruses was highest with adenovirus (48%), followed by coronavirus (46%), RSV (44%), enterovirus (32%), influenza (31%), parainfluenza (27%) and rhinovirus (27%).

Among 47 URI episodes positive for hMPV, the minimum viral load was 10 copies/mL in reaction and after calculating dilution factors, the median hMPV viral load was 3.2×10^7 copies/mL of undiluted NPS (mean 9.2×10^8 copies/mL, range $2.7 \times 10^4 - 1.0 \times 10^{10}$ copies/mL). Twenty-one (45%) of hMPV-positive samples had high hMPV viral load ($>10^8$ copies/mL). During 25 URI episodes with hMPV as the sole virus, the median viral load was significantly higher in children with fever (median 3.3×10^8 copies/mL) compared with those without (median 1.0×10^6 copies/mL) ($p=0.004$, Figure 1). However, no such relationship was found with AOM ($p = 0.47$, Figure 1). The distribution of hMPV viral load at the time of URI and the relationships between viral load and the presence of fever, and AOM is presented in Table 1 (Supplement Digital Content 1). hMPV viral load was not associated with any other signs or symptoms recorded at the visit (data not shown).

DISCUSSION

Our study is the first to compare the rate of AOM complicating hMPV-URI with the rates of AOM complicating URI due to various respiratory viruses. Data were from a prospective, longitudinal study of children at the peak age incidence of AOM. Among URI episodes due to single virus, we found hMPV to be associated with AOM in only 24% of cases, compared to 27-48% rates with other viruses. Interestingly, hMPV viral load during URI was not associated with AOM complication.

Although hMPV is closely related to RSV, hMPV seems to cause milder clinical disease^{5, 15}. The milder inflammatory disease caused by hMPV, compared to RSV may explain the lower rate of complications such as AOM as we reported here. Previously, other investigators reported high rate of concomitant AOM during URI due to hMPV, but these studies didn't use PCR to detect wide variety of respiratory viruses; therefore, there was a possibility that the cases could be hMPV co-infected with other viruses. Williams et al.⁷ reported 50% rate of AOM in 118 cases diagnosed retrospectively as hMPV-URI; they only used virus culture-negative samples for PCR detection targeting only hMPV but not other viruses. Heikkinen et al. reported 41% rate of AOM complication in 39 cases of hMPV-URI, including 61% rate in children younger than 3 years². Their study only used PCR to detect hMPV, rhinovirus and enterovirus; the rates for AOM complicating URI associated with other viruses were not reported. The strength of our present study is the use of comprehensive virologic studies, including PCR for broad spectrum of respiratory viruses and the rate of AOM complication was assessed and compared among URI episodes associated with a single virus only, taking out the co-founding factor of co-infections. One other possible reason for our low rate of AOM complicating hMPV-URI could be from the high sensitivity of our novel quantitative PCR assay, which may have detected more hMPV cases even with low viral load and milder disease. In any event, the role of hMPV in AOM has been established; hMPV have been detected from nasopharyngeal specimens of children with AOM, and from the middle ear fluids, with or without pathogenic bacteria^{12, 16}.

In the present study, we detected hMPV in 7% of URI episodes, which is within the previously reported range of 2-20%^{2-5, 8, 13, 17, 18}. We also show that the peak incidence in our local region is during winter months (January - March), which is consistent with study of Williams et al.⁷. In another study, large yearly variation in incidence of hMPV infections was observed; a high incidence of 25% was reported during the first study year, but a much lower rate, 4.7%, was observed the following season¹⁹. During our 4-year study period, there was no strong variation in yearly incidence of hMPV.

We found that hMPV viral load was significantly associated with the occurrence of fever; this is consistent with the observation by Martin et al¹⁷. More severe complications of the lower airway have been described in association with high hMPV load^{3, 8, 9}. However, we did not find a relationship between hMPV viral load and AOM complication. This may be either due to the relatively smaller number of children with AOM associated with hMPV in our study, or increasing viral load of hMPV may not cause more inflammation of the nasopharynx and Eustachian tube dysfunction, thereby limiting the rate of AOM complication.

In conclusion, hMPV was detected in 7% of URI episodes in children less than 4 years of age; and in 3.6% episodes as the only identified virus. The rate of AOM complicating hMPV-URI was lower than that of other viruses. Viral load was associated with the presence of fever, but not with AOM development or other signs or symptoms. Further studies are needed to investigate the mechanisms of differential rates of AOM associated with various respiratory viruses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Krystal Revai, Ron L Veselenak, Sangeeta Nair, M. Lizette Rangel, Kyralessa B. Ramirez, Syed Ahmad, Michelle Tran, Liliana Najera, Rafael Serna, Carolina Pillion and Ying Xiong for assistance with study subjects and specimen collection and processing. We also thank Antonella Casola and Roberto Garofalo for providing hMPV and RSV positive controls. Yimei Han is acknowledged for statistical analyses.

Source of Funding: Supported by the National Institute of Deafness and Other Communication Disorders [grant number R01 DC005841], and from the National Center for Research Resources [grant number UL1 RR029876], National Institutes of Health.

REFERENCES

1. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001; 7(6):719–724. [PubMed: 11385510]
2. Heikkinen T, Osterback R, Peltola V, Jartti T, Vainionpää R. Human metapneumovirus infections in children. *Emerg Infect Dis*. 2008; 14(1):101–106. [PubMed: 18258088]
3. Sarasini A, Percivalle E, Rovida F, Campanini G, Genini E, Torsellini M, et al. Detection and pathogenicity of human metapneumovirus respiratory infection in pediatric Italian patients during a winter–spring season. *J Clin Virol*. 2006; 35(1):59–68. [PubMed: 16023411]
4. Sloots TP, Mackay IM, Bialasiewicz S, Jacob KC, McQueen E, Harnett GB, et al. Human metapneumovirus, Australia, 2001–2004. *Emerg Infect Dis*. 2006; 12(8):1263–1266. [PubMed: 16965711]
5. van den Hoogen BG, van Doornum GJ, Fockens JC, Cornelissen JJ, Beyer WE, de Groot R, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. *J Infect Dis*. 2003; 188(10):1571–1577. [PubMed: 14624384]
6. Williams JV, Harris PA, Tollefson SJ, Halburnt-Rush LL, Pingsterhaus JM, Edwards KM, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med*. 2004; 350(5):443–450. [PubMed: 14749452]
7. Williams JV, Wang CK, Yang CF, Tollefson SJ, House FS, Heck JM, et al. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. *J Infect Dis*. 2006; 193(3):387–395. [PubMed: 16388486]

8. Gerna G, Sarasini A, Percivalle E, Campanini G, Rovida F, Marchi A, et al. Prospective study of human metapneumovirus infection: diagnosis, typing and virus quantification in nasopharyngeal secretions from pediatric patients. *J Clin Virol.* 2007; 40(3):236–240. [PubMed: 17855163]
9. Bosis S, Esposito S, Osterhaus AD, Tremolati E, Begliatti E, Tagliabue C, et al. Association between high nasopharyngeal viral load and disease severity in children with human metapneumovirus infection. *J Clin Virol.* 2008; 42(3):286–290. [PubMed: 18479963]
10. Chonmaitree T, Revai K, Grady JJ, Clos A, Patel JA, Nair S, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis.* 2008; 46(6):815–823. [PubMed: 18279042]
11. Schildgen O, Geikowski T, Glatzel T, Schuster J, Simon A. Frequency of human metapneumovirus in the upper respiratory tract of children with symptoms of an acute otitis media. *Eur J Pediatr.* 2005; 164(6):400–401. [PubMed: 15772811]
12. Suzuki A, Watanabe O, Okamoto M, Endo H, Yano H, Suetake M, et al. Detection of human metapneumovirus from children with acute otitis media. *Pediatr Infect Dis J.* 2005; 24(7):655–657. [PubMed: 15999017]
13. Bonroy C, Vankeerberghen A, Boel A, De Beenhouwer H. Use of a multiplex real-time PCR to study the incidence of human metapneumovirus and human respiratory syncytial virus infections during two winter seasons in a Belgian paediatric hospital. *Clin Microbiol Infect.* 2007; 13(5): 504–509. [PubMed: 17263836]
14. Bourne N, Pyles RB, Yi M, Veselenak RL, Davis MM, Lemon SM. Screening for hepatitis C virus antiviral activity with a cell-based secreted alkaline phosphatase reporter replicon system. *Antiviral Res.* 2005; 67(2):76–82. [PubMed: 15927278]
15. van den Hoogen BG, Osterhaus DM, Fouchier RA. Clinical impact and diagnosis of human metapneumovirus infection. *Pediatr Infect Dis J.* 2004; 23(1 Suppl):S25–32. [PubMed: 14730267]
16. Williams JV, Tollefson SJ, Nair S, Chonmaitree T. Association of human metapneumovirus with acute otitis media. *Int J Pediatr Otorhinolaryngol.* 2006; 70(7):1189–1193. [PubMed: 16427144]
17. Martin ET, Kuypers J, Heugel J, Englund JA. Clinical disease and viral load in children infected with respiratory syncytial virus or human metapneumovirus. *Diagn Microbiol Infect Dis.* 2008; 62(4):382–388. [PubMed: 18842376]
18. UtoKaparch S, Marchant D, Gosselink JV, McDonough JE, Thomas EE, Hogg JC, et al. The relationship between respiratory viral loads and diagnosis in children presenting to a pediatric hospital emergency department. *Pediatr Infect Dis J.* 2011; 30(2):e18–23. [PubMed: 20980931]
19. Caracciolo S, Minini C, Colombrita D, Rossi D, Miglietti N, Vettore E, et al. Human metapneumovirus infection in young children hospitalized with acute respiratory tract disease: virologic and clinical features. *Pediatr Infect Dis J.* 2008; 27(5):406–412. [PubMed: 18382388]

Figure 1.

The association between hMPV viral load and development of AOM after URI and fever during 25 URI episodes with hMPV only.

Above box plots show the percentiles and the median of hMPV viral load in children with AOM or fever (gray bars), and in children without AOM or fever (white bars). The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.