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## Authors' Reply

Our group has recently reported one of the largest series of hMPV-infected infants (N:101) described to date.<sup>1</sup> As Wilkesmann et al. comment in their letter,<sup>2</sup> the severity of the disease associated with hMPV was similar to RSV in our patients, and no differences could be shown either in terms of length of hospital stay, oxygen-therapy requirements, infiltrate/atelectasis, fever frequency, nor in the patient's prematurity or underlying disease history. We did not use a matched pairs approach, but a random sample of 95 hospitalized infants and documented as RSV-infected because our population was very homogeneous, including only infants less than 2 years of age.

Although data from other reports suggest that dual infection with hMPV and other respiratory viruses is rare,<sup>3,4</sup> the high co-infection rate found in our patients (29.7%) appears to suggest quite the opposite, the coinfection rate being among the highest reported to date.<sup>5-7</sup> Our results are probably related to the exhaustive virological research performed in all specimens: molecular virological diagnosis was performed by two different multiplex reverse transcription-nested polymerase chain reaction (RT-PCR) assays. The first one was designed for specific detection of respiratory syncytial virus (RSV) types A and B, adenoviruses, and influenza A, B, and C viral genomes. The second assay was designed to detect parainfluenza viruses (types 1-4), human coronavirus 229E and OC43, as well as for generic detection of enterovirus and rhinovirus polyprotein genes. Both assays were performed in all specimens, as previously described.<sup>8,9</sup> Detection of human metapneumovirus (HMPV) in respiratory secretions from every patient was performed using two independent RT-nested PCR assays which were designed in two different genes: one gene encoding for the matrix protein (M), and another gene encoding for the viral polymerase (L), as described elsewhere.<sup>10</sup> Positive results were considered confirmed when in two different aliquots, analyzed in alternate days, the PCR result was concordant. The high rate of coinfections (44.3%) reported by Wilkesmann et al.<sup>2</sup> confirm our results, although it is quite surprising, being done that they only have investigated the presence of RSV, influenza A, B by an antigen ELISA and cell culture. In our study, adenovirus, identified by PCR, was the second most frequent virus in hMPV co-infections. Moreover, in other series recently published by our group,<sup>11</sup> rhinovirus was the second most frequent virus identified in hospitalized children and in 39.5% of cases dual or multiple infection were detected. Our results suggest that the inclusion of multiplex RT-PCR into diagnostic testing strategies would provide help in identifying the true relevance of the multiple viral infections, as well as the characteristics and severity associated with this condition.

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## REFERENCES

- Garcia-Garcia ML, Calvo C, Perez-Brena P, De Cea JM, Acosta B, Casas I. Prevalence and clinical characteristics of human metapneumovirus infections in hospitalized infants in Spain. Pediatr Pulmonol 2006;41:863–871.
- Wilkesmann A, Schildgen O, Eis-Hübinger E, Geikowski T, Glatzl T, Lentze M, Bode U, Simon A. Human metapneumovirus infections cause similar symptoms and clinical severity as respiratory syncytial virus infections. Eur J Pediatr 2006;165:467–475.
- Vicente D, Cilla G, Montes M, Pérez-Trallero E. Human metapneumovirus and community-acquired respiratory illness in children. Emerg Infect Dis 2003;9:602–603.
- Esper F, Boucher D, Weibel C, Martinello RA, Kahn JS. Human metapneumovirus infection in the United States: Clinical manifestations associated with a newly emerging respiratory infection in children. Pediatrics 2003;111:1407–1410.
- 5. Greensill J, McNamara P, Dove W, Flanagan B, Smyth RL, Hart CA. Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis 2003;9:372–375.
- Maggi F, Pifferi M, Vatteroni M, Fornai C, Tempestini E, Anzilotti S, Lanini L, Andreoli E, Ragazzo V, Pistello M, Specter S, Bendinelli M. Human metapneumovirus associated with respiratory tract infections in a 3-year study of nasal swabs from infant in Italy. J Clin Microbiol 2003;41:2987–2991.
- Cuevas LE, Nasser AM, Dove W, Gurgel RQ, Greensill J, Hart CA. Human metapneumovirus and respiratory syncytial virus, Brazil. Emerg Infect Dis 2003;9:1626–1628.
- Coiras MT, Perez-Breña P, García ML, Casas I. Simultaneous detection of influenza A, B, and C viruses, respiratory syncytial virus, and adenoviruses in clinical simples by multiplex reverse transcription nested-PCR assay. J Med Virol 2003;69:132–144.
- Corias MT, Aguilar JC, García ML, Casas I, Perez-Brena P. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 2004;72:484–495.
- López-Huertas MR, Casas I, Acosta-Herrera B, García ML, Corias MT, Perez-Brena P. Two RT-PCR based assays to detect human metapneumovirus in nasopharyngeal aspirates. J Virol Methods 2005;129:1–7.
- Calvo Rey C, García-García ML, Casas Flecha I, Sánchez Mateos M, Rodrigo García G, De Cea Crespo JM, Pérez-Breña P. Role of rhinovirus in respiratory tract infections in hospitalized children. An Pediatr (Barc) 2006;65:205–210.

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