

Original Research Article

Seroprevalence of Anti-human Metapneumovirus Antibodies in Hospitalized Children in Suleimani City/Iraq.

Abstract:

Background: Human Metapneumovirus (hMPV) is an important respiratory viral pathogen among children, and it is one of the causes of pediatric hospital admissions due to acute respiratory tract infections.

Objective: This study was done to predict the seroprevalence of anti-hMPV antibodies among hospitalized children presenting with acute respiratory tract infections in Suleimani Governorate, Kurdistan Region/Iraq.

Place and duration: This study was done at the department of microbiology, school of medicine, Suleimani university, between April 2011 and March 2012.

Methods: Indirect immunofluorescent assay (IIFA) was performed to detect serum anti-hMPV antibodies (IgM and IgG antibodies) from three hundred hospitalized children less than 5 years of age with acute respiratory tract infections.

Results: IgM anti-hMPV antibodies were positive in thirty six (12%) out of three hundred children. The highest seroprevalence was found in the age group <1 year, while the lowest in the age group 4 to < 5 years. No significant sexual difference was found among seropositive children. The IgM anti – hMPV seropositive children were suffering from pneumonia, bronchiolitis, or other less severe acute respiratory tract infections like acute bronchitis and croup in frequencies of sixteen (44%), 10 (28%), and 10 (28%). The IgG anti-hMPV antibodies were positive in two hundred and twenty five (75%) out of the three hundred children, and there was a gradual increase in percentage of seropositivity with increasing age.

Conclusion: hMPV is an important viral respiratory pathogen among hospitalized children in Suleimani Governorate/Kurdistan/Iraq, and most of the children had experienced hMPV infection by the age of five years.

Keyword: Suleimani city, hospitalized children, indirect immunofluorescence assay, human metapneumovirus, acute respiratory tract infection.

Introduction

The hMPV was first identified in Netherland by Van den Hoogen BG, et al. in 2001, the isolated new paramyxovirus was identified as a new member of Metapneumovirus genus based on virological data, sequence homology and gene constellation[1] . The virus had been overlooked previously because the growth of clinical isolates *in vitro* is slow, has a delayed cytopathic effect and requires added trypsin [2].

The viral genome of hMPV is similar to that of respiratory syncytial virus (RSV) [3] and children who are infected with hMPV have clinical features similar to those infections caused by RSV ranging from acute upper respiratory tract infections to severe acute lower respiratory tract infections like bronchiolitis and pneumonia [4].

Van den Hoogen BG, et al. also studied the serological manifestation of children in Netherland and noticed that by the age of 5 years, virtually all children have been exposed to hMPV [1].

Generally, serum IgM anti-hMPV antibodies appears within few days after infection and remain detectable for 1 to 2 weeks later and therefore they represent markers of recent infections, while IgG emerges later and stay for longer duration and therefore they are good markers of previous hMPV infections [5]. Seroepidemiological studies showed that the prevalence of hMPV infections may differ between geographic locations [6].

In Iraq, the seroepidemiology of hMPV infections has not been studied before. The aim of the present study was to measure IgG and IgM anti-hMPV antibodies in hospitalized children who were admitted to Pediatric Teaching Hospital in Suleimani city/Kurdistan Region/Iraq with acute respiratory tract infections.

Materials and Methods

The current study enrolled three hundred hospitalized children whose ages were less than five years. The children were admitted to Pediatric Teaching Hospital in Suleimani city due to respiratory tract infections of undiagnosed etiology. Both sexes were included

and were chosen by systematic random sampling, one hundred ninety three of children were males and one hundred and seven were females. The escort and parents of diseased children signed informed consent for giving permission for blood collection from their children in this study. The duration of sample collection extended for one year between April 2011 and March 2012.

All sera were tested for IgG anti-hMPV antibodies using indirect immunofluorescent assay (IIFA) by applying ready-to-use slides (Millipore Company/USA) coated with epithelial cells infected with hMPV. In addition to slides, IgG dilution buffer, Fluorescein – labeled anti- IgG antibodies conjugate, PBS – Tween washing buffer, and mounting medium were also used in the procedure. Briefly, the procedure for detection of IgG anti-hMPV antibodies was done by diluting the patients' sera with IgG sample buffer diluent with different dilutions of sera were tested and the 1:101 dilution was the best, therefore this dilution was used, then 25 µl of each diluted sera were added to each reaction field of hMPV slide. The slides were incubated at 37°C for 1 hour then washed with washing buffer. IgG conjugate was then added and another incubation and washing step followed by addition of mounting medium and covering with cover slip. The presence of green cytoplasmic fluorescence in the cells indicated positive sera. The fluorescence was detected with the fluorescence microscope by using FITC filter. The procedure for detection of IgM anti-hMPV antibodies was done in the same manner as for detection of IgG anti-hMPV antibodies. The positive IgM anti-hMPV antibodies were shown as green cytoplasmic fluorescence similar to the corresponding IgG anti-hMPV antibodies slides.

The Statistical Package for Social Science (SPSS, Chicago, IL, USA), version 16 was used for data entry and analysis. Chi-square test (X^2) and Fisher's exact test were used to test the association between categorical variables. P value of ≤ 0.05 was considered as statistically significant.

Results

Indirect immunofluorescence assay was performed to detect IgM anti-hMPV antibodies, the IIFA was an achievable technique (Figures 1 and 2).

Seroprevalence of IgM anti-hMPV antibodies among children revealed that positivity was found in thirty six (12%) out of the three hundred hospitalized children and the results in different age groups showed that positive sera were found in eleven (31%) in age group <1 year, 9 (25%) in age group 1 to < 2 years, 7 (19%) in age group 2 to < 3 years, 6 (17%) in age group 3 to < 4 years, and 3 (8%) in age group 4 to < 5 years. The highest seroprevalence is found in the age group 1 to < 2 years, while the lowest in the age group 4 to < 5 years. The results were statistically not significant ($P < 0.02760$) (Figure 3).

Out of the 36 IgM anti-hMPV seropositive children, 24 (67%) were males and 12 (33%) were females, the male to female ratio was 2:1; while for the 264 seronegative children, 166 (63%) were males and 98 (37%) were females; the differences in gender between the two groups were not statistically significant ($p = 0.7156$).

Children with positive IgM anti-hMPV antibodies were considered as recent infection with the virus. The IgM anti-hMPV seropositive children were suffering from pneumonia, bronchiolitis, or other respiratory tract infections in frequencies of sixteen (44%), 10 (28%), and 10 (28%), while the remaining two hundred and sixty four IgM anti-hMPV seronegative children showed the corresponding clinical manifestations in frequencies of sixty three (24%), sixty nine (26%), and one hundred and thirty two (50%) respectively. These results among the IgM anti-hMPV seropositive and the seronegative groups were statistically significant ($P \text{ value} < 0.05$) (Table 1).

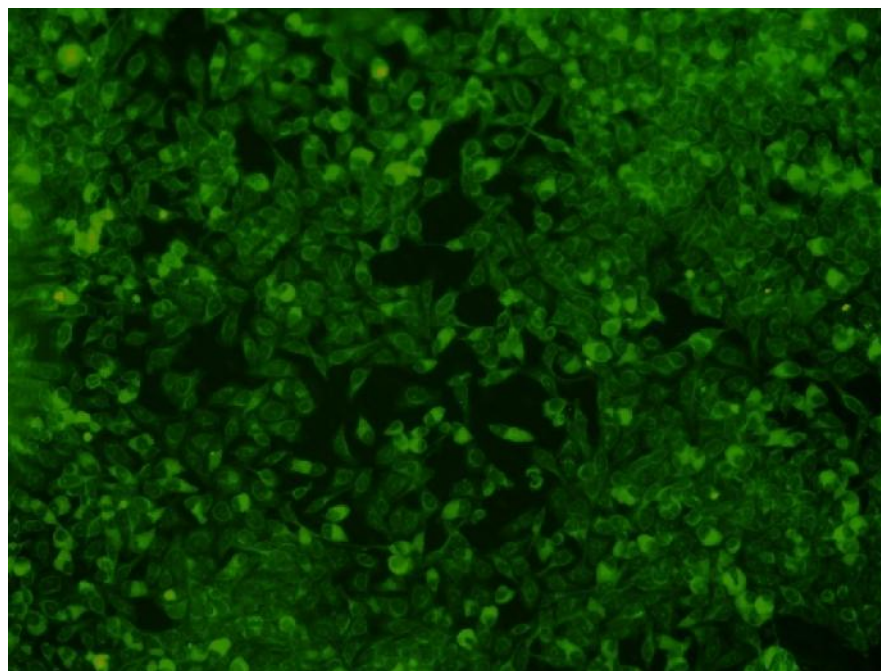


Figure (1): Positive IIFA for the detection of IgM anti-hMPV antibodies in the sera of patients with respiratory tract infections. The cells show cytoplasmic fluorescence after staining with FITC.

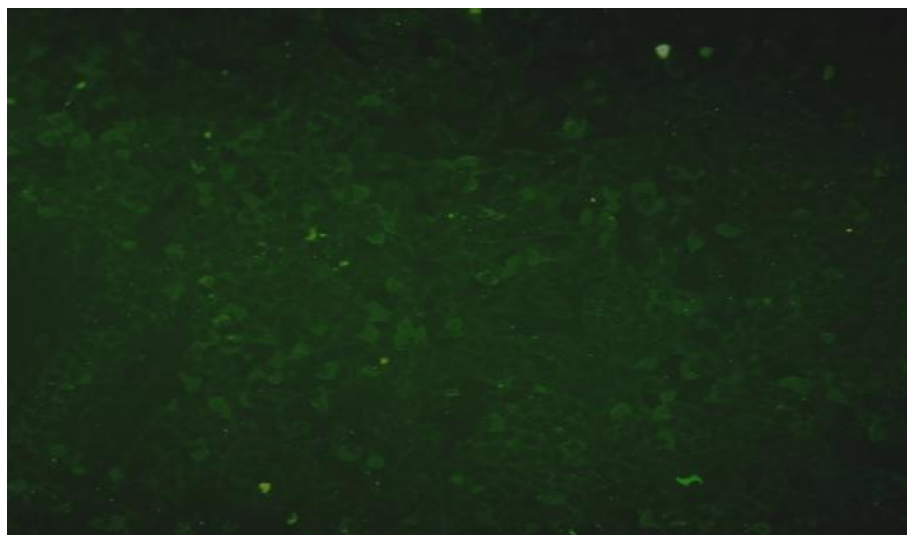


Figure (2): Negative IIFA for the detection of IgM anti-hMPV antibodies (or for the detection of IgG anti-hMPV antibodies) in the sera of patients with respiratory tract infections.

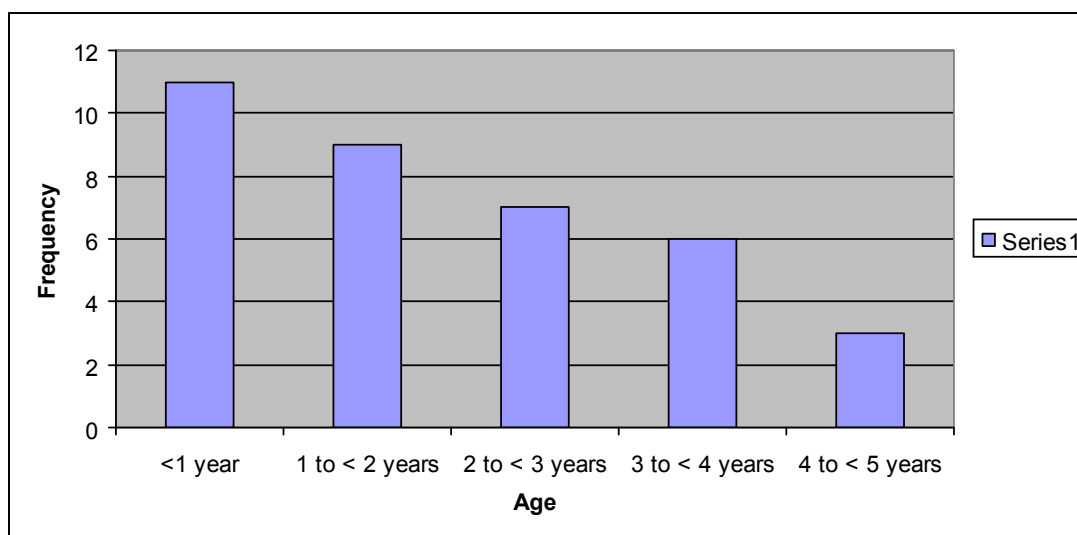


Figure (3): Column chart illustrating seroprevalence of IgM anti-hMPV antibodies among children enrolled in the study.

Table (1): The frequencies and percentages of respiratory infections among seropositive and seronegative IgM anti-hMPV antibodies in patients enrolled in the study.

	Frequency (Percentage)		
	Pneumonia	Bronchiolitis	Other acute respiratory infections
Seropositive IgM Anti-hMPV Children	16 (44%)	10 (28%)	10 (28%)
Seronegative IgM Anti-hMPV children	63 (24%)	69 (26%)	132 (50%)

IIFA was also used to detect IgG anti-hMPV antibodies in the sera of patients enrolled in the study. The results showed that two hundred twenty five (75%) out of three hundred hospitalized children in the study were positive, (Figures 4, 5).

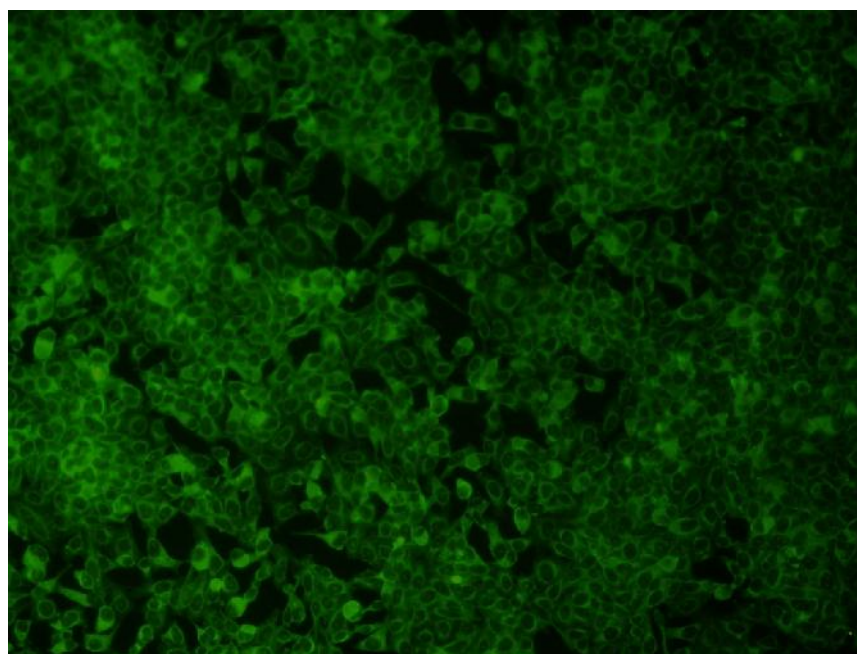


Figure (4): Positive IIFA for the detection of IgG anti-hMPV antibodies in the serum of patient with respiratory tract infection. The cells show cytoplasmic fluorescence after staining with FITC.

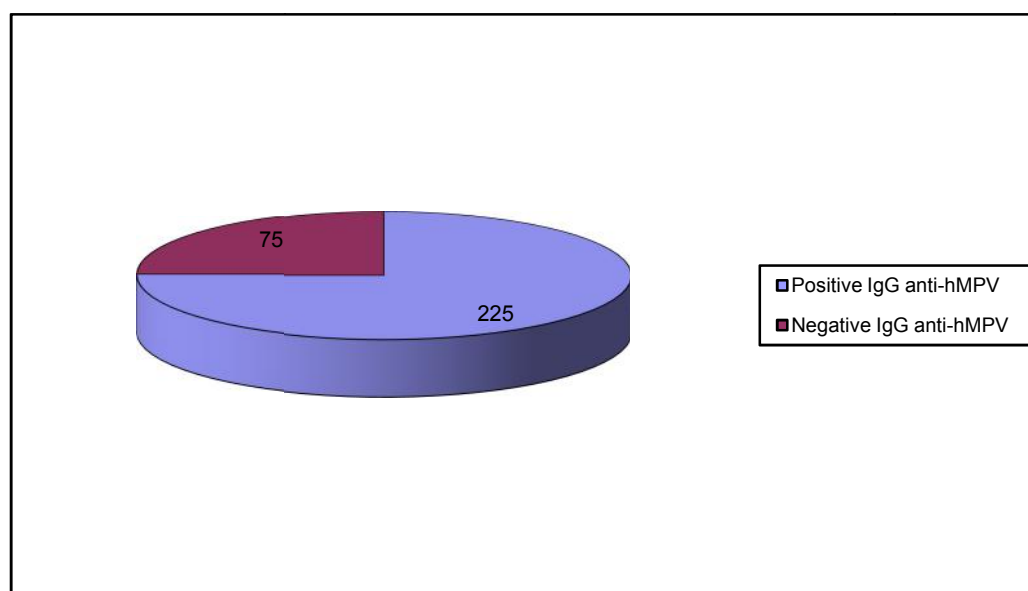


Figure (5): Pie chart demonstrating the frequencies of positive and negative IgG anti-hMPV antibodies among children in the study.

Positive IgG anti-hMPV antibodies were found in forty six (64.7%) patients in age group < 1 year, forty nine (68%) patients in age group 1 to < 2 years, forty seven (77%)

patients in age group 2 to < 3 years, forty (83.3%) patients in age group 3 to < 4 years, forty three (89.6%) patients in age group 4 to < 5 years. The results of IgG anti-hMPV antibodies among different age groups were statistically significant ($P < 0.05$). The results clarified the presence of gradual increase in the percentage of positive IgG anti-hMPV antibodies in children enrolled in the study (Figure 6).

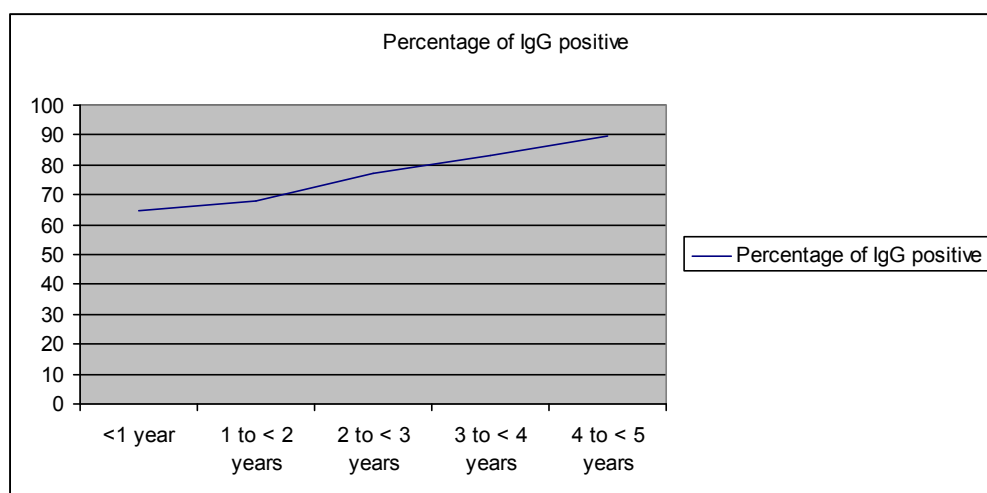


Figure (6): Percentages of positive IgG anti-hMPV antibodies among children in the study in different age groups.

Sixteen children out of thirty six IgM seropositive showed IgG seropositivity, while the remaining twenty IgM seropositive children were IgG seronegative (table 2).

Table (2): The seropositivity of IgM and IgG anti-hMPV antibodies in patients enrolled in the study.

Antibodies	Frequency	(%)
Only IgM	20	7
Only IgG	209	70
IgM and IgG	16	5
Total IgM	36	12

Total IgG

225

75

Discussion

We applied IIFA to detect anti-hMPV antibodies in the sera of hospitalized children with acute respiratory tract infections (ARTIs). The present study showed that IIFA could detect IgM antibodies in children, who were acutely infected with hMPV while negative sera showed absence of fluorescence in the slides, and the best results were obtained at a serum dilution of 1:101; the same dilution was also chosen for detection of IgG anti-hMPV antibodies. Other researchers mostly used home – made IIFA and ELISA techniques for detection of serum antibodies specific to hMPV [7-10]. They performed these techniques using home – made IIFA slides or home – made ELISA microplates, both of which were difficult to be done locally due to the lack of the materials and equipments for preparing antigens of hMPV.

The current study showed that 12% of hospitalized children of less than five years of age showed serological evidence of recent infections with hMPV. The high positive was an indication that hMPV was important pathogen circulating in children with ARTIs in Suleimani city; this result was higher than that reported elsewhere [11,12].

The highest seroprevalence was found in age group 1 to < 2 years, while the lowest in age group 4 to < 5 years. These findings might reflect the weaning in maternal antibodies and increase in susceptibility to infection in age group 1 to < 2 years, while reinfection is less frequently associated with hospital admission.

Pneumonia was the most encountered hMPV infection among IgM-positive children though bronchiolitis and other less severe ARTIs were also reported. These results reflected the wide range of respiratory diseases related to hMPV among children [5].

The present study also showed that 75% of all children were positive for anti-hMPV antibodies; this finding indicated that hMPV was a common respiratory pathogen in children [1, 13-16].

Some variations in seroprevalence of hMPV in children less than 5 years of age may be due to different geographic areas, different viral strains, or differences in viral

exposure. In the first study of hMPV in Netherland almost all children experienced hMPV infections by the age of five years [1].

The results revealed steady increase in seroprevalence of IgG positive children with increasing age. This reflected the ubiquitous nature of hMPV in community [6]. Results in children under 1 year of age had IgG seroprevalence of 62.5% may be due to the presence of maternal antibodies, while the presence of the same antibodies in 1 to <2 years of age represented early hMPV infections.

The results showed that nearly half of IgM positive children were also IgG positive. This finding might indicate re-infections with the hMPV, while the remaining IgM positive and IgG negative might represent primary infection with hMPV, however, there was no indication about the timing of IgG titer elevation after the primary infection [5].

In the current study, we showed that IIFA was a useful and rapid test for the diagnosis of hMPV infections. However, the development of rapid tests for diagnosis of hMPV infections that are easier to be performed in clinical laboratory settings is necessary for the appropriate management of patients.

Conclusion

It is the first record of hMPV seroprevalence in Iraq. IIFA is an applicable technique for detection IgM and IgG anti-hMPV antibodies. ARTIs that are caused by hMPV include pneumonia, bronchiolitis, and other less severe respiratory infections. hMPV is an important respiratory pathogen among hospitalized children with ARTIs, and more than two thirds of children experienced hMPV infections by the age of five years.

Consent

Authors declare that written informed consent was obtained from the patient's families.

Ethical Approval

This study was approved by the ethical committee of the medical school, Suleimani University.

References

1. Van den Hoogen BG, De Jong JC, Groen J, Kuiken T, De Groot R, Fouchier RAM, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med*. 2001; 7:719–724.
2. Stéphane Biacchesi, Quynh N. Pham, Mario H. Skiadopoulos, Brian R. Murphy, Peter L. Collins, and Ursula J. Buchholz. Modification of the trypsin-dependent cleavage activation site of the human metapneumovirus fusion protein to be trypsin independent does not increase replication or spread in rodents or nonhuman primates. *Virology*. 2006; 80:5798–5806.
3. Boivin G, De Serres G, Côté S, Gilca R, Abed Y, Rochette L, et al. Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis* 2003; 9:634–40.
4. Heikkinen, T., R. Osterback, V. Peltola, T. Jartti and R. Vainionpää. Human metapneumovirus infections in children. *Emerg Infect Dis* 2008; 14(1): 101-106.
5. Ebihara, T., R. Endo, H. Kikuta, N. Ishiguro, H. Ishiko, M. Hara, Y. Takahashi, and K. Kobayashi. 2004. Human metapneumovirus infection in Japanese children. *J Clin Microbiol* 42:126-132.
6. Lu G, Gonzalez R, Guo L, Wu C, Wu J, Vernet G, et al. Large-scale seroprevalence analysis of human metapneumovirus and human respiratory syncytial virus infections in Beijing, China *Virology* 2011; 8: 62.
7. Falsey AR, Erdman D, Anderson LJ, Walsh EE. Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 2003;187:785
8. Hamelin ME, Boivin G. Development and validation of an enzyme-linked immunosorbent assay for human metapneumovirus serology based on a recombinant viral protein. *Clin Diagn Lab Immunol* 2005; 12:249-53.
9. Leung J, Esper F, Weibel C, Kahn JS. Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. *J Clin Microbiol* 2005; 43:1213-19.

- 321 10. Liu L, Bastien N, Sidaway F, Chan E, Li Y. Seroprevalence of human
322 metapneumovirus (hMPV) in the Canadian province of Saskatchewan analyzed
323 by a recombinant nucleocapsid protein-based enzyme-linked immunosorbent
324 assay. *J Med Virol* 2007;79:308-13.
- 325 11. Guy Boivin, Gaston De Serres, Stéphanie Côté, Rodica Gilca, Yacine Abed,
326 Louis Rochette, et al. Human Metapneumovirus Infections in Hospitalized
327 Children. *Emerg Infect Dis* 2003; 9(6): 634–40.
- 328 12. John V. Williams, Kathryn M. Edwards, Geoffrey A. Weinberg, Marie R. Griffin,
329 Caroline B. Hall, Yuwei Zhu, et al. Population-based incidence of human
330 metapneumovirus infection among hospitalized children. *J Infect Dis*. 2010; 201
331 (12): 1890-98.
- 332 13. Pavlin JA, Hickey AC, Ulbrandt N, Chan YP, Endy TP, Boukhvalova MS, et al.
333 Human metapneumovirus reinfection among children in Thailand determined by
334 ELISA using purified soluble fusion protein. *J Infect Dis* 2008; 198:836–42.
- 335 14. Zhao X, Zhang B, Duan ZJ, Xie ZP, Gao HC, Xiao NG, et al. Molecular
336 epidemiological and clinical feature of human metapneumovirus in children with
337 acute lower respiratory tract infection in Changsha area from 2007 to 2011.
338 *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*. 2012; 26(2):99-101.
- 339 15. Noyola DE, Alpuche-Solis AG, Herrera-Diaz A, Soria-Guerra RE, Sanchez-
340 Alvarado J, Lopez-Revilla R Human metapneumovirus infections in Mexico:
341 epidemiological and clinical characteristics. *J Med Microbiol* 2005; 54:969–74.
- 342 16. TL Wang, GM Zheng, ZY Jiang, LF Tang, HF Tang, ZM Chen. HK. Human
343 metapneumovirus Infection in hospitalized children with acute lower Respiratory
344 tract infection in Hangzhou, China *J Paediatr* 2013; 18:6-11.