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

Tariq A. G. Aziz[1*], Nabil Salmo[2] and Ali H. Bayati[3]

[1]Department of Microbiology, School of Medicine, Suleimani University. Suleimani, Iraq.
[2]Department of Pathology and Forensic Pathology, School of Medicine, Suleimani University, Suleimani, Iraq.
[3]Community Health Department, Suleimani Technical College, Suleimani Polytechnic University, Suleimani, Iraq.

Authors' contributions

This work was carried out in collaboration between all authors. Author TAGA designed the study, supervised the laboratory work and reviewed the manuscript. Author NS supplied the necessary equipment for this work and participated in the interpretation of the IIFA. Author AHB participated in laboratory work, data analysis, drafting of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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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 old 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 old, while the

**Corresponding author: Email: dr.taga@yahoo.com;*

lowest in the age group 4 to <5 years old. No significant gender 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.

Keywords: Hospitalized children; indirect immunofluorescence assay; human metapneumovirus; acute respiratory tract infection.

1. INTRODUCTION

The hMPV was first identified in Netherland, and soon after it was recognized as a new member of Metapneumovirus genus based on virological data, nucleotide 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 remains longer 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.

2. MATERIALS AND METHODS

The current study enrolled three hundred hospitalized children up to five years old. The children were admitted to Pediatric Teaching Hospital in Suleimani city due to respiratory tract infections of undiagnosed etiology. Both genders were included and were chosen by systematic random sampling, the inclusion criteria involved children less than five years old and were admitted to the pediatric hospital due to respiratory tract infections (RTI), while exclusion criteria included children equal to, or more than, five years, whatever the cause of

their admission, and children who were admitted to the hospital due to causes rather than RTI. One hundred ninety three of children were males and one hundred and seven were females. The child's parent signed informed consent for giving permission for blood collection from their children in this study. The duration of sample collection ranging from April 2011 to 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, phosphate buffer saline (PBS) – Tween washing buffer (dilution 1:10), and mounting medium (Euroimmun Company/Germany) 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 demonstrated to be optimal, therefore this dilution was used, then 25µl of each diluted sera were added to each reaction field of hMPV slide. The slides were incubated at 37ºC 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 fluorescein isothiocyanate (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.

3. RESULTS

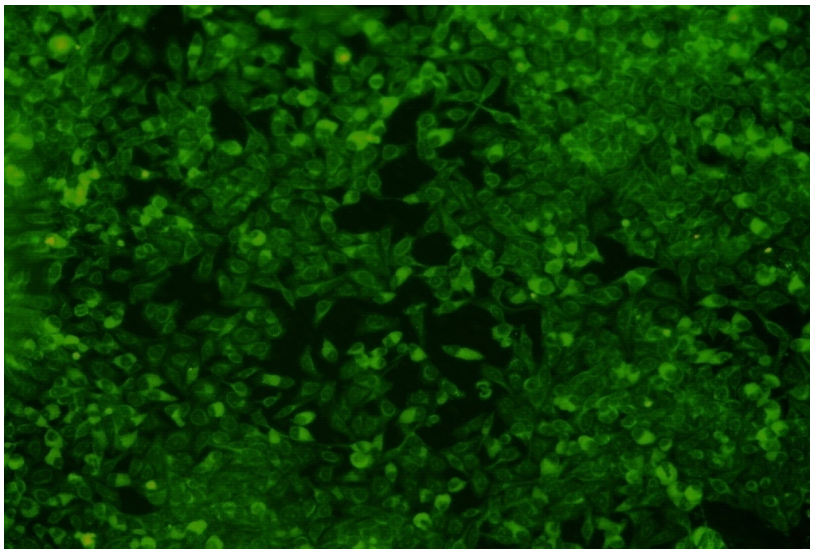
Indirect immunofluorescence assay was performed to detect IgM anti-hMPV antibodies, the IIFA was an achievable technique (Figs. 1A and B).

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 old, 9 (25%) in age group 1 to <2 years old, 7 (19%) in age group 2 to <3 years old, 6 (17%) in age group 3 to <4 years old, and 3 (8%) in age group 4 to <5 years old. The highest seroprevalence is found in the age group 1 to <2 years old, while the lowest in the age group 4 to < 5 years old. The results were statistically not significant (P <0. 0.2760) (Fig. 2).

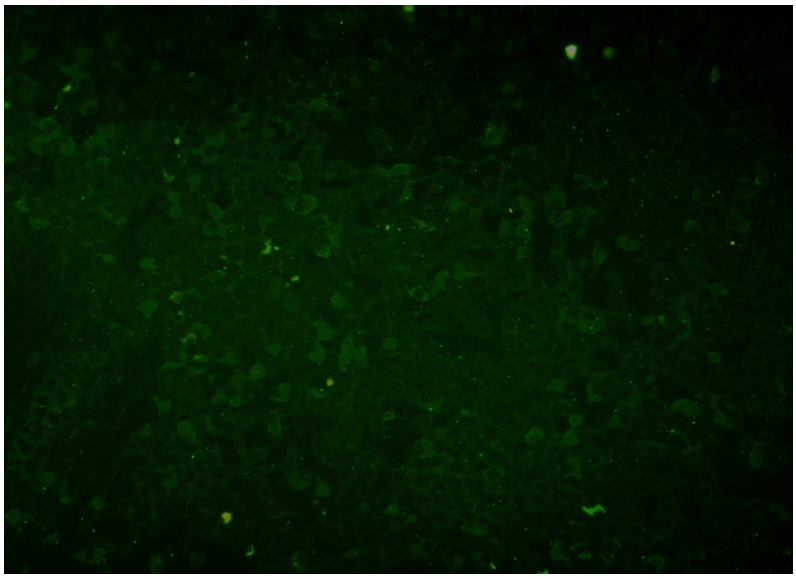
Out of the 36 IgM anti–hMPV seropositive children, 24 (67%) were males and 12 (33%) were females, the male to female ratio was 2:1; while for the 264 seronegative children, 166 (63%) were males and 98 (37%) were females; the differences in gender between the two groups were not statistically significant (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 value <0.05) (Table 1).



A



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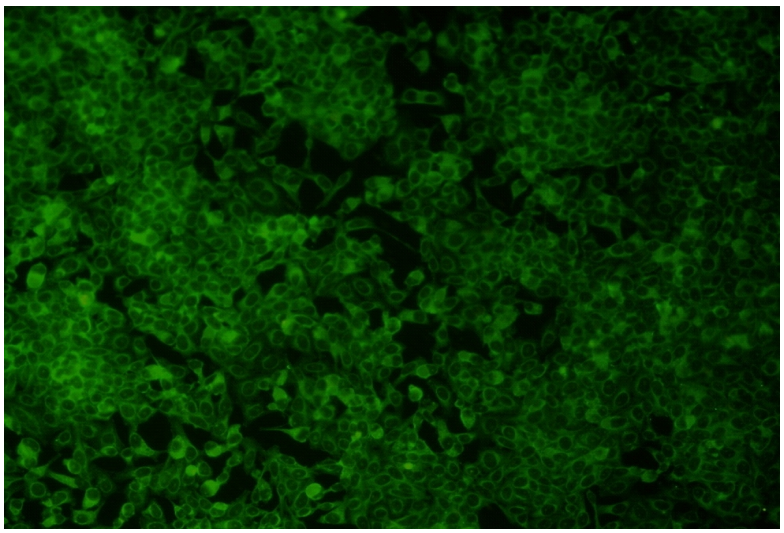

C

Fig. 1A. 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. B. 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. C. 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

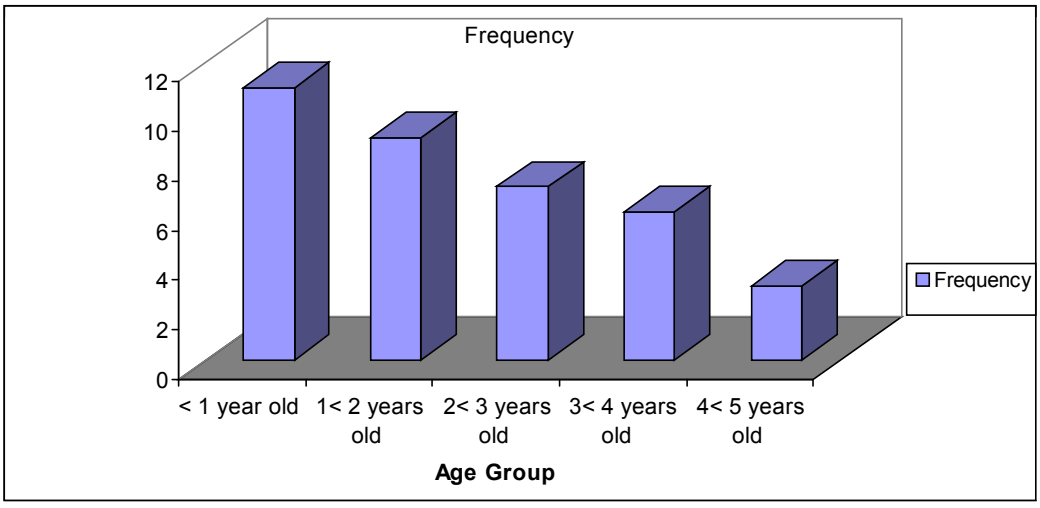

Fig. 2. Seroprevalence of IgM anti-hMPV antibodies among children enrolled in the study

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 (Fig. 1*C*). The results showed that two hundred twenty five (75%) out of three hundred hospitalized children in the study were positive.

Positive IgG anti-hMPV antibodies were found in forty six (64.7%) patients in age group <1 year old, forty nine (68%) patients in age group 1 to <2 years old, forty seven (77%) patients in age group 2 to <3 years old, forty (83.3%) patients in age group 3 to <4 years old, forty three (89.6%) patients in age group 4 to <5 years old (Fig. 3). 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 (Fig. 4).

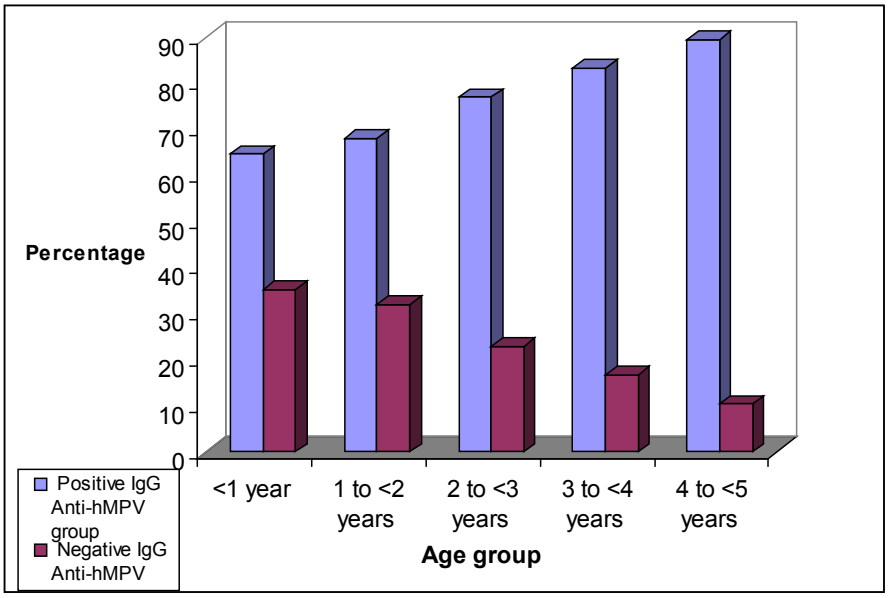

Fig. 3. The Percentages of positive and negative IgG anti-hMPV antibodies among children in the study

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

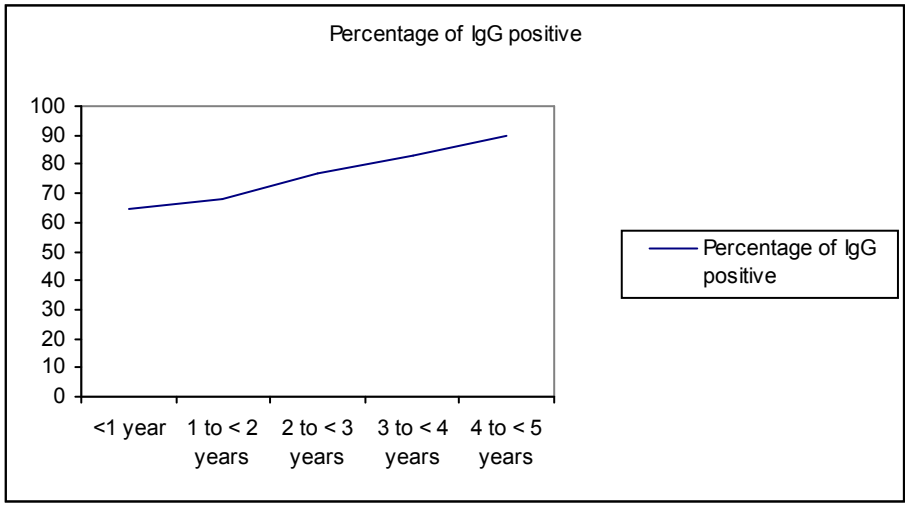

Fig. 4. Percentage of positive IgG anti-hMPV antibodies among patients enrolled in the study

Table 2. 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

The results of this study showed that the peak of hMPV infections is during February, while the lowest frequency of infections was present in July and Augest, (Fig. 5).

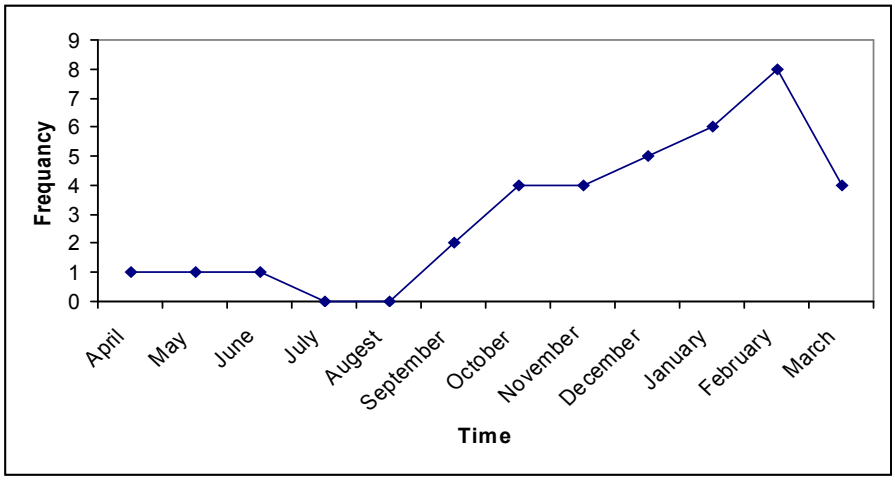

Fig. 5. Seasonal distributions of hMPV infections among hospitalized children in the study group

4. DISCUSSION

We applied IIFA to detect anti-hMPV antibodies in the sera of hospitalized children with acute respiratory tract infections (ARTIs). 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 old, while the lowest in age group 4 to <5 years old. These findings might reflect the weaning in maternal antibodies and increase in susceptibility to infection in age group1 to <2 years old, 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.

The autumn – winter was the most common period for HMPV infections in the current study, while summer was the least season of HMPV infections. Other studies reported variable seasonal predominance. In a study done in China, spring was the main season [14], in

Jordan the peak was reported in March [17], whereas in India hMPV infection rate peaked in spring-summer period of 2008-2009 and 2009-2010, while hMPV circulated predominantly during winter-spring period of 2010-2011 [18],

5. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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. Virol J. 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, Osterback R, Peltola V, Jartti T, Vainionpaa R. Human metapneumovirus infections in children. Emerg Infect Dis. 2008;14(1):101-106.
5. Ebihara T, Endo R, Kikuta H, Ishiguro N, Ishiko H, Hara M, Takahashi Y, Kobayashi K. Human metapneumovirus infection in Japanese children. J Clin Microbiol. 2004;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 Virol J. 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.
10. Liu L, Bastien N, Sidaway F, Chan E, Li Y. Seroprevalence of human metapneumovirus (hMPV) in the Canadian province of Saskatchewan analyzed by a recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. J Med Virol. 2007;79:308-13.
11. Guy Boivin, Gaston De Serres, Stéphanie Côté, Rodica Gilca, Yacine Abed, Louis Rochette, et al. Human Metapneumovirus Infections in Hospitalized Children. Emerg Infect Dis. 2003;9(6):634–40.
12. Jhon V. Williams, Kathryn M. Edwards, Geoffrey A. Weinberg, Marie R. Griffin, Caroline B. Hall, Yuwei Zhu, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. J Infect Dis. 2010;201(12):1890-98.
13. Pavlin JA, Hickey AC, Ulbrandt N, Chan YP, Endy TP, Boukhvalova MS, et al. Human metapneumovirus reinfection among children in Thailand determined by ELISA using purified soluble fusion protein. J Infect Dis. 2008;198:836–42.
14. Zhao X, Zhang B, Duan ZJ, Xie ZP, Gao HC, Xiao NG, et al. Molecular epidemiological and clinical feature of human metapneumovirus in children with acute lower respiratory tract infection in Changsha area from 2007 to 2011. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi. 2012;26(2):99-101.
15. Noyola DE, Alpuche-Solis AG, Herrera-Diaz A, Soria-Guerra RE, Sanchez-Alvarado J, Lopez-Revilla R Human metapneumovirus infections in Mexico: Epidemiological and clinical characteristics. J Med Microbiol. 2005;54:969–74.
16. TL Wang, GM Zheng, ZY Jiang, LF Tang, HF Tang, ZM Chen. HK. Human metapneumovirus Infection in hospitalized children with acute lower Respiratory tract infection in Hangzhou, China J Paediatr. 2013;18:6-11.
17. Ali SA1, Williams JV, Chen Q, Faori S, Shehabi A, Jundi EA, Khuri-Bulos N, Halasa N. Human metapneumovirus in hospitalized children in Amman, Jordan.J Med Virol. 2010;82(6):1012-6.
18. Banerjee S1, Sullender WM, Ahuja RK, Broor S. Seroepidemiological study of human metapneumovirus in New Delhi, India. Indian J Med Microbiol. 2011;29(4):363-7.

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