# Summary and conclusion

Human metapneumovirus is an enveloped, negative sense, RNA virus, from the family *Paramyxoviridae*, subfamily *Pneumovirinae* and genus Metapneumovirus, that has been proven to be variable genitically and antigenically with two subgroups (A and B) and their sublineages.

Human metapneumovirus has been reported in 2001 to be a human respiratory tract virus causing a wide spectrum of illness ranging from asymptomatic infection to bronchiolitis and pneumonia. It gives a clinical picture that is indistinguishable from clinical pictures of other respiratory tract infection viruses especially that of RSV to which the hMPV is highly related pathophysiologically.

The prevalence of the virus is of a wide range, it has been reported in the elderly and adults as well as children, yet its highest rates are among children under 5 years of age with a big chance for reinfection in all age groups. It has been reported that the hMPV was the second most frequently detected respiratory virus in children of 0 to 2 years of age, after RSV. 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. hMPV has been found to be circulating all year long and it circulates predominately in the late winter and spring, and the peak of activity at any given location often coincides with or follows the peak of RSV activity.

Treatment programs and vaccine candidates are under trial to produce preventive and prophylactic measures against the hMPV, especially in children, the elderly and the immunocompomised group of patients.

Laboratory diagnosis of hMPV infection has been limited because of the difficulty in growing the virus and the lack of readily available diagnostic reagents. Isolation in conventional cell cultures can take 2 weeks or more, and cytopathic effects can be difficult to recognize. Shell vial centrifugation cultures can provide results within 2 days, but high-quality antibodies have not been commercially available.

Direct immunofluorescence (DFA) staining of clinical specimens, with results available within 2 to 4 h, is commonly used in clinical virology laboratories for the rapid diagnosis of respiratory viruses.

PCR assays are generally more sensitive than other methods for detection of viruses, can be automated, and are suitable for high-volume testing in reference laboratories. Thus, reverse transcription-PCR (RT-PCR) is the most widely reported test.

- This study was performed to rapidly diagnose hMPV infection using DFA test in respiratory secretions and to compare this test by duplex real time RT- PCR to evaluate the contribution of hMPV as a cause of respiratory infections in infants below 2 years old and to define clinical features and seasonal patterns of the virus.
- This study was done at Benha University Hospital during the period from January to June 2008 on 48 patients (30 females and 18 males).

Their ages ranged from 1 month to 2 years with the mean age of 6.8 month.

- Respiratory specimens were collected from the total study population (a nasopharyngeal aspiration and a nasopharyngeal swab samples from every patient). Direct immunofluorescent assay was done to detect hMPV F protein and N antigens for all respiratory secretion samples. Duplex real time RT- PCR was done for all patients for detection of hMPV F and M genes by LightCycler 1.5(Roche) system using Light cycler-RNA Amplification Kit SYBR Green I.
- From the 48 patients, 30 (62.5%) were diagnosed as bronchiolitis, 15 (31.3%) were diagnosed as bronchopneumonia, 2 (4.2%) were diagnosed as acute bronchitis and 1 (2.1%) was diagnosed as bronchial asthma.
- Real time PCR could detect human metapneumovirus in 12 (25 %) patients out of 48.
- The results of the DFA test showed that among fourty eight patients, a human metapneumovirus infection was detected in 27.1% (13/48) of patients by direct immunofluorescent assay, whereas in 72.9% (35/48) of patients were negatives.
- The present study shows that 12 cases were positive for hMPV by both methods of identification (direct immunofluorescence assay test and RT- real time PCR), one case was positive by DFA and negative by RT- real time PCR.
- Considering the RT-Real time PCR as a reference or standard method, the sensitivity of DFA was 100% while the specificity of was 97.2%.

- In diagnosis of hMPV infection there was a very good agreement between results of DFA and RT-real time PCR (Kappa=0.94).
- It is noted that 75% of hMPV positive cases occur in the bronchiolitis group, 25% in the bronchopneumonia group but this is statistically insignificant.
- It is noted that infants below three months age represent (7/12) 58.34% of positive cases. Infants whom ages ranged from seven to nine months and ten to twelve months represent 25% and 8.33% of positive cases respectively. Infants above one year old represent only (1/12) 8.33% of positive cases.
- The present study shows that 50 % of positive cases occur in males and 50 % occur in females.
- The present study shows that the peak of hMPV activity was during spring as 33.3% of positive cases were in April and 25% were in March.
- Real time RT- PCR took less time than DFA (approximately 2 hours including the automated extraction procedures). DFA takes approximately 4 hours.