

# Distribution and Trends of Human Parainfluenza Viruses in Hospitalised Children

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## Abstract

**Objective** To study the distribution of Human Parainfluenza viruses (HPIV) 1–4 and their trends in children ≤5 y of age, hospitalised at a tertiary care centre, Jaipur and co-infection with other respiratory viruses.

**Methods** Nasopharyngeal aspirate and throat swabs were collected and processed for extraction of nucleic acid using automated extraction system and real time RT-PCR was performed using primers and probes specific to HPIV 1–4 and other respiratory viruses on 743 samples.

**Results** Total positivity for Parainfluenza viruses 1–4 was found to be 69/743 (9.28 %), of which 50/533 (9.38 %) were boys and 19/210 (9.05 %) girls. Predominance of HPIV-3 was observed [41/743 (5.52%)] followed by HPIV-1 in 13/743 (1.75%), HPIV-4 in 10/743 (1.34%) and HPIV-2 in 5/743 (0.67%) patients. Maximum positivity was observed in age group 25–36 mo (12.98%) followed by 13–24 mo group (11.96%). HPIVs were found to be circulating round the year and each year. Co-infections with other respiratory viruses were observed in 22/69 (31.88%) of HPIV positive patients.

**Conclusions** All the four types of HPIV were found to be circulating in the index population during all the three years, predominantly during post monsoon and winter seasons. HPIV vaccination should be targeted for all types.

**Keywords** Human parainfluenza viruses 1–4 · Real time RT-PCR · Seasonal trends

## Introduction

Human Parainfluenza viruses (HPIVs) are important cause of upper and lower respiratory tract illnesses (LRTIs), causing morbidity and mortality in infants and young children. In the United States approximately five million LRTIs occur each year in children <5 y of age and HPIVs are isolated in up to one-third of these infections. HPIVs are of four types (HPIV 1–4). HPIV1–3 are important cause of respiratory infections in infants and children and are only second to respiratory syncytial virus (RSV) as a cause of hospitalisation for acute respiratory infections among children <5 y of age. While HPIV-4 has been reported in few studies only and the lack of epidemiological data prevents clear understanding of the complete picture. The clinical presentations of HPIV types are also not well known [1–4].

HPIV-1 is rare in infants younger than 1 mo, though it causes LRTI in young infants. About 60% of all HPIV-2 infections are seen in children <5 y of age, though the peak incidence is seen between 1 and 2 y of age. In the United States about 18,000 infants and children are hospitalised every year due to LRTI caused by HPIV-3. HPIV-4 mostly causes mild illnesses. However, few reports have indicated that it can cause severe infections in some settings [5].

Studies based on HPIVs infections are less in tropical countries and most of them do not demonstrate seasonal patterns [6, 7]. The exact reasons behind the different seasonality of the HPIVs are unknown, differences in ambient climate conditions has been proposed as one hypothesis [5]. The present study was undertaken to study the distribution and trends of different types of HPIVs in hospitalised children ≤5 y of age at a tertiary care hospital in Jaipur and co-infection with other respiratory viruses.

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## Material and Methods

Children with Acute Respiratory Infections (ARI) admitted in J. K. Lone Hospital, a pediatric hospital attached to Sawai Man Singh (SMS) Medical College, Jaipur were enrolled in the study over a period of 29 mo *i.e.*, from September 2012 through January 2015 and were tested with prior consent of the parent/guardian. The study was approved by the institutional ethics committee. A total of 751 parents were contacted and 743 (98.93%) parents gave consent for enrolment of their children in the study. The children were of  $\leq 5$  y of age, presenting with fever, cough, sore throat, nasal catarrh, shortness of breath and wheezing. Clinical findings observed were noted down as pneumonia and bronchiolitis.

The sample size for the study was calculated as 651 by using the formula  $n = 4 pq/l^2$  (where n = total number of samples; 4 is the factor to achieve the confidence level of 95%; p = known prevalence; q = 100 – p, and l = allowable permissible absolute error, set at 2%). A prevalence of 7% was reported by National Family Health Survey – 3 (NFHS- 3) for ARI in the age group under five in children of Rajasthan. A total of 743 samples were included in the study.

Nasopharyngeal aspirate and throat swab samples were collected from patients of ARI in viral transport medium (VTM), labeled and transported on ice at the earliest to Advanced Research Lab (ICMR Grade-1 Virology Lab) of SMS Medical College, Jaipur for further processing and storage of the samples.

Viral nucleic acid from samples was extracted using EasyMAG (Biomeurex) automated extractor according to the manufacturer's instructions. Briefly, the extraction was done from 400  $\mu$ l sample which was added to 1500  $\mu$ l lysis buffer and was incubated for 10 min off board. The samples were loaded into EasyMAG automated nucleic acid extractor and 100  $\mu$ l of magnetic silica was added to each sample and mixed well. Finally, the nucleic acid was eluted in a volume of 110  $\mu$ l.

Real time RT-PCR was performed by using primer probes for the detection of HPIVs 1–4 [8], Human *Rhinovirus* (HRV), Human *Adenovirus* (HAdV) [8], Human *Metapneumovirus* (HMPV A/B), Human *Bocavirus* (HBoV) [9], *Influenza A* (FLU A) [10], *Enterovirus* (EV) [11], *Respiratory Syncytial virus* (RSV A/B) [12], *Influenza B* (FLU B) [13] and Human *Coronavirus HKU-1* (HCoV HKU1) [14].

Statistical analysis of HPIVs positive vs. HPIVs negative samples with clinical characteristics was done by using Chi square test to study the *p* value.

## Results

Among 743 samples tested for Parainfluenza virus (1–4) infection, 533 (71.74%) were of boys and 210 (28.26%) of girls. The total positivity for Parainfluenza viruses 1–4 was found to be 69/743 (9.28 %), of which 50/533 (9.38 %) belonged to boys and 19/210 (9.05 %) to girls (Table 1). Mono infections with HPIVs were observed in 47/69 (68.12%) and co-

**Table 1** Demographic and clinical characteristics of the study population

| Patient details                 | HPIV-1<br>(a)   | HPIV-2<br>(b)   | HPIV-3<br>(c)  | HPIV-4<br>(d)  | Total HPIVs Positive<br>(a + b + c + d) |
|---------------------------------|-----------------|-----------------|----------------|----------------|-----------------------------------------|
| <b>Age in months</b>            |                 |                 |                |                |                                         |
| 1–12                            | 06/437 (1.37%)  | 2/437 (0.46%)   | 23/437 (5.26%) | 4/437 (0.91%)  | 35/437 (08.00%)                         |
| 13–24                           | 04/117 (3.42%)  | 00/117 (00.00%) | 08/117 (6.84%) | 02/117 (1.71%) | 14/117 (11.96%)                         |
| 25–36                           | 03/77 (3.90%)   | 02/77 (2.60%)   | 05/77 (6.49%)  | 00/77 (00.00%) | 10/77 (12.98%)                          |
| 37–48                           | 00/44 (00.00%)  | 00/44 (00.00%)  | 03/44 (06.80%) | 02/44 (4.54%)  | 05/44 (11.36%)                          |
| 49–60                           | 00/68 (00.00%)  | 01/68 (1.47%)   | 02/68 (2.94%)  | 02/68 (2.94%)  | 05/68 (07.35%)                          |
| Total                           | 13/743 (1.75%)  | 5/743 (0.67%)   | 41/743 (5.52%) | 10/743 (1.34%) | 69/743 (9.28 %)                         |
| <b>Gender</b>                   |                 |                 |                |                |                                         |
| Male                            | 10/533 (1.87%)  | 4/533 (0.75%)   | 27/533 (5.06%) | 9/533 (1.69%)  | 50/533 (9.38 %)                         |
| Female                          | 3/210 (1.43%)   | 1/210 (0.47%)   | 14/210 (6.66%) | 1/210 (0.47%)  | 19/210 (9.05 %)                         |
| <b>Clinical characteristics</b> |                 |                 |                |                |                                         |
| Cough                           | 13/13 (100.0%)  | 05/05 (100.0%)  | 41/41 (100.0%) | 10/10 (100.0%) | 69/69(100.0%)                           |
| Fever                           | 13/13 (100.0%)  | 05/05 (100.0%)  | 38/41 (92.68%) | 10/10 (100.0%) | 66/69(95.65%)                           |
| Shortness of breath             | 08/13 (61.53%)  | 03/05 (60.0%)   | 22/41 (53.66%) | 05/10 (50.0%)  | 38/69(55.07%)                           |
| Sore throat                     | 03/13 (23.07%)  | 02/05 (40.0%)   | 09/41 (21.95%) | 01/10 (10.0%)  | 15/69(21.74%)                           |
| Nasal catarrh                   | 02/13 (15.38 %) | 02/05 (40.0%)   | 06/41 (14.63%) | 02/10 (20.0%)  | 12/69(17.39%)                           |
| Wheezing                        | 00/13 (0.00%)   | 01/05 (10.0%)   | 00/41 (0.00%)  | 00/10 (0.00%)  | 1/69(1.45%)                             |
| Pneumonia                       | 02/13 (15.38 %) | 01/05 (10.0%)   | 08/41 (19.51%) | 00/10 (0.00%)  | 11/69(15.94%)                           |

infections with other respiratory viruses were observed in 22/69 (31.88%) of HPIV-3 positive patients. HPIV-3 was predominantly associated with co-infections. The details of co-infections are mentioned in Table 2. Pneumonia was observed in 11/69 (15.94%,  $p = 0.03$ ) of HPIV-3 positive cases (Table 3) and five of these HPIV-3 positive pneumonia patients had co-infection with other respiratory viruses. HPIVs were not detected in patients with bronchiolitis.

Co-infections of HPIV with other respiratory viruses were predominantly observed in 1–12 mo age group 12/22 (54.54%). Details of co-infections with other respiratory viruses is given in Table 2. Rhinovirus was the most commonly associated virus in co-infections with HPIVs 9/22 (40.90%). Boys infected with co-infections, 17/22 (77.27%) were higher than girls, 5/22 (22.73%). Among patients with co-infections, cough was observed in 22/22 (100.0%), nasal catarrh in 4/22 (18.18%), shortness of breath in 14/22 (63.63%), pneumonia in 5/22 (22.72%) and sore throat in 1/22 (4.54%) patients. No

co-infections were observed in bronchiolitis and wheezing patients.

The occurrence of HPIVs varied in different age groups. Maximum positivity was observed in age group 13–48 mo [29/69 (42.02%)]. HPIV-3 predominated in all age groups except 49–60 mo age group in which HPIV-3 was equally distributed with HPIV-4 (Table 1).

During the study period of 29 mo, all the four types of HPIVs were found to be circulating with predominance of HPIV-3. Among the four types of HPIVs, HPIV-3 was found to be circulating during all the months of the year with exception to few months; HPIV-1 and 2 during post monsoon and winters and HPIV-4 during winters. Details are given in Fig. 1.

## Discussion

The present study was undertaken to observe the distribution and trends of Human Parainfluenza viruses 1–4 in hospitalised children  $\leq 5$  y of age along with co-infection with other respiratory viruses. HPIVs are the second most important cause of LRTIs in infants after RSV causing a significant amount of disease burden globally [2]. During the study period of 29 mo, significant positivity of HPIVs was detected (9.28%). A detection rate of 3–17% for HPIVs has been reported among hospitalised ARI pediatric patients from various parts of the world [1, 7, 15, 16], and 0% - 16.27% from India [17, 18].

In the present study, among the four types of HPIVs, type 3 (5.52%) was predominant followed by HPIV-1 (1.75%) and HPIV-4 (1.34%). Study from China also reported predominance of HPIV-3 followed by HPIV-1 but lower for HPIV-4 [1]. Predominance of HPIV-3 has also been reported by other studies from India, [18] United States [19] and China [16, 20]. HPIVs were found to be circulating in all the three years of the present study. HPIV-3 was present throughout the year while HPIV-1 and 2 were positive post monsoon and in winters and HPIV-4 only in winters. Whereas a study from Delhi reported HPIVs only in the first year in a study of 2 y [18] and Fry et al. from USA reported biennial activity of HPIV-1 and HPIV-2 [21]. Competitive interaction between HPIV-3 and HPIV-1 has been reported by a study from USA [21] but, same was not observed in index study. Studies based on detection of HPIV-4 are very few; as a result seasonal variation is not well known [1, 21] and only 1.34% positivity was observed in the present study for HPIV-4, with peak activity in winters. Different geographical locations and climatic factors may influence the circulating patterns of different HPIVs. Ambient climatic conditions like temperature and humidity may favour the propagation of particular HPIV type at a particular time which may vary in different places [2]. Malnutrition, overcrowding, vitamin A deficiency and improper breast feeding have also been reported to predispose to HPIV infections [2].

**Table 2** Details of co-infections with other respiratory viruses in the study population

| Co-infections                      | Total          |
|------------------------------------|----------------|
| HPIV-1 with co-infections          | 3/69 (4.34%)   |
| HPIV-1 + HMPV A/B                  | 2/69 (2.89%)   |
| HPIV-1 + RSV A/B+ HRV*             | 1/69 (1.45%)   |
| HPIV-2 with co-infections          | 1/69 (1.45%)   |
| HPIV-2 + FLU B                     | 1/69 (1.45%)   |
| HPIV-3 with co-infections          | 13/69 (18.84%) |
| HPIV-3 + HRV                       | 2/69 (2.89%)   |
| HPIV-3 + FLU A                     | 2/69 (2.89%)   |
| HPIV-3 + HAAdV                     | 2/69 (2.89%)   |
| HPIV-3 + HBoV+ HRV                 | 1/69 (1.45%)   |
| HPIV-3 + HBoV+ HRV*                | 1/69 (1.45%)   |
| HPIV-3 + EV                        | 1/69 (1.45%)   |
| HPIV-3 + RSV A/B*                  | 1/69 (1.45%)   |
| HPIV-3 + HRV + HAAdV*              | 1/69 (1.45%)   |
| HPIV-3 + HBoV*                     | 1/69 (1.45%)   |
| HPIV-3 + HAAdV + HMPV A/B          | 1/69 (1.45%)   |
| HPIV-4 with co-infections          | 4/69 (5.79%)   |
| HPIV-4 + HRV                       | 3/69 (4.34%)   |
| HPIV-4 + HAAdV                     | 1/69 (1.45%)   |
| HPIV-4 & HPIV-1 with co-infections | 1/69 (1.45%)   |
| HPIV-4 + HPIV-1 + HBoV+ HCoV HKU1  | 1/69 (1.45%)   |
| Total                              | 22/69 (31.88%) |

HPIV-1 Human Parainfluenza virus-1; HPIV-2 Human Parainfluenza virus-2; HPIV-3 Human Parainfluenza virus-3; HPIV-4 Human Parainfluenza virus-4; HMPV A/B Human Metapneumovirus; RSV A/B Respiratory Syncytial virus; HRV Human Rhinovirus; FLU B Influenza B; FLU A Influenza A; HAAdV Human Adenovirus; HBoV Human Bocavirus; EV Enterovirus; HCoV HKU1 Human Coronavirus HKU-1

\*Pneumonia co-infection

**Table 3** Statistical analysis of HPIVs positive vs. HPIVs negative samples with clinical characteristics

| Total number of cases enrolled for HPIVs - 743 |                         |                          |                                           |                    |
|------------------------------------------------|-------------------------|--------------------------|-------------------------------------------|--------------------|
| Clinical characteristics                       | HPIVs positive (n = 69) | HPIVs negative (n = 674) | Chi square value (degrees of freedom = 1) | Two-tailed P value |
| Cough                                          | 69                      | 658                      | 1.674                                     | 0.1957             |
| Fever                                          | 66                      | 613                      | 1.758                                     | 0.1848             |
| Shortness of breath                            | 38                      | 387                      | 0.141                                     | 0.7076             |
| Sore throat                                    | 15                      | 114                      | 1.016                                     | 0.3135             |
| Nasal Catarrh                                  | 12                      | 114                      | 0.010                                     | 0.9198             |
| Wheezing                                       | 1                       | 10                       | 0.001                                     | 0.9820             |
| Pneumonia                                      | 11                      | 189                      | 4.658                                     | 0.0309*            |

\**p* value <0.05 significant

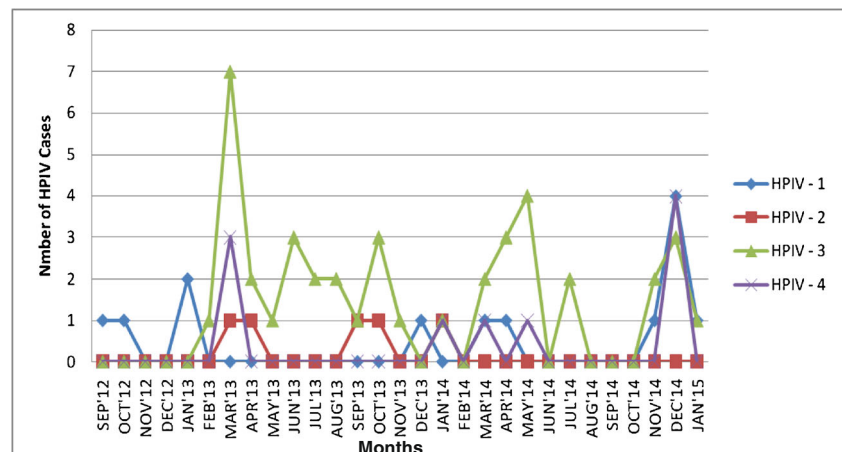
HPIV infections may occur throughout the life since immunity to the virus is incomplete, however low or nil positivity has been reported in 1st mo of life due to maternal antibodies, highest positivity was observed in children of 13–48 mo in the index study as reported earlier too [2].

In the present study, pneumonia was observed in 15.94% of HPIV positive patients, of which HPIV-3 accounted for maximum cases, as reported earlier also [2]. All the four HPIVs positive patients presented with similar spectrum of signs and symptoms making it difficult to correlate the HPIV type with a particular sign and symptom as reported earlier also [1, 20]; but this could be due to the fact that only hospitalised patients were included in the index study where difference in severity may not be appreciable. However, none of the index HPIV positive patients presented with bronchiolitis which is an important serious manifestation of LRTI [2]; however, this could be due to the fact that bronchiolitis was observed in very few patients enrolled in the present study. According to Wang et al. [22] and Stankowa et al. [23], more severe respiratory illnesses were caused by HPIV-3 than by types 1 and 2, whereas, no significant differences in the severity of the illnesses among different HPIV types was reported from Taipei [16].

In the present study, co-infections were detected in significant number (31.88%) of HPIV positive cases. All the four types of HPIVs were associated with co-infections with other respiratory viruses, as also observed in China [1]. Moreover, clinical manifestations in patients infected only with HPIV and those co-infected with other viruses were similar as also reported from China [1], whereas a study from Delhi reported more serious illness in patients with co-infections [13]. Co-infections were mostly detected in children up to 1 y of age. Immature immune system in children may predispose them to potential pathogens. No difference was found in occurrence of Parainfluenza viruses in boys vs. girls in the present study though predominance of Parainfluenza viruses in boys has been reported from China [1].

Limitation of the index study is that only hospitalised patients'  $\leq 5$  y were included while inclusion of outdoor patients may have provided a better comparison in terms of correlating HPIV types with severity of disease. Moreover inclusion of samples from other regions in Rajasthan may further help in understanding the trends in the state so as to formulate policy for vaccination in the future for better prevention and control of HPIVs infection. Testing for bacterial infections would have also helped in understanding etiology of ARI in the state.

**Fig. 1** Trends of different types of HPIVs



However, the study highlights the trends of HPIV infection which can be of great help in planning vaccination strategies. Vaccination may help decrease the severity of illness in HPIV infected patients even if it may not help prevent infection [3, 14].

## Conclusions

All the four types of HPIV were found to be circulating in index population during all the three years, predominantly during post monsoon and winter seasons. HPIV vaccination should be targeted for all types. It was not possible to differentiate the four types and co-infection with other viruses based on signs and symptoms.

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## Compliance with Ethical Standards

**Conflict of Interest** None.

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