Identification of Parainfluenza virus 4 of human in Najaf / Iraq

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Abstract- Parainfluenza viruses (PIVs) are paramyxoviruses of the order Mononegavirales, the family Paramyxoviridae, and the subfamily Paramyxovirinae. Human PIVs (HPIVs) are currently divided into 5 serotypes—HPIV-1, HPIV-2, HPIV-3, HPIV-4a, and HPIV-4b—in 2 different genera: Respirovirus (HPIV-1 and HPIV-3) and Rubulavirus (HPIV-2 and HPIV-4). Detection by reverse transcription-PCR (RT-PCR) during in Najaf/ Iraq during 2012-2013. The HPIV-4 virus is identified by rapid test  and RT-PCR using a set of primers specific . A total of 320 cases from (nasal swab , blood ) of human including two groups (male and female), age group about to (25-40) years . Thirty three(33) sample gave the positive result with HPIV-4 virus specific primers. The RT-PCR procedure is rapid and sensitive, and could be used for the identification.

Index Terms- RT-PCR, PIVs, HPIV, HPIVs, HPIV-1, HPIV-2, HPIV-3, HPIV-4a, HPIV-4b, RSV, HCoV , HRV

1 INTRODUCTION

Human parainfluenza viruses are enveloped, negative strand RNA viruses belonging to the family Paramyxoviridae, and which cause respiratory tract infections. The two species human parainfluenza 1 (HPIV1) and human parainfluenza 3 (HPIV3) belong to the genus Respirovirus, whereas HPIV2 and HPIV4 belong to the genus Rubulavirus. Among the known human paramyxoviruses, the genome of HPIV4 has not yet been completely sequenced. The species HPIV4 is further divided into types HPIV4A and HPIV4B, based on antigenic differences demonstrated by hemadsorption inhibition and monoclonal antibody reactivity [1].

The seasonal patterns of HPIV-1, HPIV-2, and HPIV-3 are curiously interactive. HPIV-1 causes the largest, most defined outbreaks, which are marked by sharp biennial rises in croup cases in the autumn of odd-
numbered years. Outbreaks of infection with HPIV-2, although erratic, usually follow HPIV-1 outbreaks. Outbreaks of HPIV-3 infections occur yearly, mainly in spring and summer, and last longer than outbreaks of HPIV-1 and HPIV-2. Because HPIV-4 is infrequently isolated, infection with this pathogen is less well characterized [2].

Patients with HPIV infection typically present with a history of coryza and low-grade fever; they then develop the classic barking cough associated with croup. On physical examination, HPIV infection is associated with a broad range of findings, which may include fever, nasal congestion, pharyngeal erythema, nonproductive to minimally productive cough, inspiratory stridor, rhonchi, rales, and wheezing[3].

2 Material and Methods
2.1 Samples Collection
Three hundred twenty clinical samples were randomly collected from different areas of AL-Najaf province. Samples were collected during a period extended from 26 March 2012 up to 31 of April 2013.

2.2 Population Groups
Studied samples subject groups were distribution into (2) groups. This distribution was made depending on their age and clinical status of both gender.

2.3 Detection of Parainfluenza Virus 4(HPIV4)
Two different diagnostic procedures were used for detection of HPIV4 including, rapid device test, real time PCR.

2.4 Rapid Test
The CerTest parainfluenza virus Card is a one step colored chromatographic immunoassay for the qualitative detection of influenza type A and type B antigens. It can be used directly with nasal swabs or nasal wash or nasal aspirated specimens. Rapid test device was carried according to restriction manual of manufacturing company (CerTest-Spain). [4].

2.5 Real Time PCR Technique
2.5.1 RNA Extraction
Viral RNA was extracted by using Viral Nucleic Acid Extraction Kit (Primer Design Ltd PrecisionTM Viral RNA/DNA extraction kit) following the manufacturer's instructions directly from chicken egg allantoic fluids, virus-infected cell supernatants, plasma, serum, transport media
for nasal swab the concentration and the purity of the extracted total RNA were determined by measuring the absorbance ratio at wavelength 260 nm over 280 nm using a spectrophotometer.

2.5.2 cDNA Synthesis
Conversion of RNA isolated from above step to cDNA by the Power cDNA synthesis kit following the manufacturer's instructions directly.

2.5.3 cDNA Amplification
Amplification was carried out in the Laboratory of Veterinary hospital in Najaf. The viral RNA was extracted from 33 positive clinical samples by rapid test device (nasal swabs, plasma and serum) of population, using the primer design viral RNA kit (primer design UK) in accordance with the manufacturer’s instruction successfully amplified. All of the primers for the HA subtypes, NA subtypes was summarized in table(1).

Table(1): Primers of parainfluenza virus 4(HPIV4).Ref

<table>
<thead>
<tr>
<th>Parainfluenza Virus 4</th>
<th>PIV4-F1</th>
<th>CAA AYG ATT CAT CAC AGC AAA CAT TC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PIV4-R1</td>
<td>ATG TGG CCT GTA AGG AAA GCA</td>
</tr>
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</table>

2.5.4 Melting Curve Analysis
After completion of 45 cycles PCR amplification, the PCR products were melted by raising the temperature from 53°C to 95°C at a rate of 1°C/min. The Exicycler thermal block software displayed the data collected during melt curve analysis as -dF/dTv vs Temperature in figure(1). As a result melting temperatures were derived from melting peaks by melting curve analysis of the amplified DNA specimens.
3 Results

3.1 Rapid Test
Of a total (320) different clinical cases collected, only 33 cases were positive HPIV4 while (287) negative as detected by rapid test in figure(2 and 3).

Figure(2): Numbers of Cases Infected with HPIV4 by Rapid Test.

Figure(3): Rapid test device for detection of HPIV4.
3.2 Real Time PCR
Of a total 320 suspected of virus infected cases only 33 positive case were detected as HPIV4 virus infected with rapid test device. All the positive cases were underwent diagnosis with real-time – technique in figure(4).

![Real Time PCR for detection of HPIV4](image)

**Figure(4):** Real time PCR for detection of HPIV4

4 Discussion

4.1 Rapid Test

Rapid HPIV4 diagnostic tests are immunoassays that can identify the presence of viral nucleoprotein antigens in respiratory specimens of influenza by rapid diagnostic test. Potentially the test is of great benefit to the patient and public health. A rapid test is an easy and accurate test performed to diagnose test. A rapid test is performed in the health care practitioner's office. The present results are in agreement with other studies [5]. The present study findings are consistent with those of other studies [6]. while the studies of the 443 specimens in chine, at least one respiratory virus was detected in 366 specimens (83.6%). The most
frequently detected virus was RSV (169, 46.2% of positive patients), followed by HCoV (134, 36.6%), HRV (123, 33.6%), HMPV (66, 18%) and HPIVs (62, 16.9%) [6,7].

4.2 Real Time PCR

These viruses cause the majority of viral respiratory tract infections in male adults of HPIV4, then female, because the contact with other population also a significant cause of disease in immuno-compromised patients. A result which is in agreement with [8,9].

Even though HPIVs share common genetic and biochemical features, they differ in the age groups that they infect, seasonality, clinical manifestations. In this study, HPIV-3 was responsible for 94% (58/62) HPIVs infections, while HPIV-2 was responsible for only 4 cases and no HPIV-1 was detected. Most HPIV-3 infections occurred during a period of about 24 weeks during spring and summer. This result is consistent with previous studies, which showed that epidemics of HPIV-3 infection occurred in spring and summer [7]. In addition, 53% (31/58) of HPIV-3 infected patients were younger than 6 months and 79% (46/58) of HPIV-3 infections were in the first year of life [10,11].

The assay was found to be sensitive and specific. Previously, as-says using hybridization have been indicate to reduce sensitivity in comparison to a single target PCR. Real-time PCR was found to be more sensitive than cell culture on a range of different respiratory samples, which employed RT-PCR for the detection of viral infections. Conventional respiratory viral cell culture is limited by a lack of speed and therefore has little impact on patient care. Rapid immunological tests partly overcome this problem, but the low sensitivity requires cell culture to be performed on negative specimens [12].

5 CONCLUSION

In the light of the current study, it is concluded that: (1) Rapid test and real time PCR are important in the confirmation for detection of HPIV4. (2) The highest number of infected population is among male adults compared with other groups.
6 References


