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 hander twenty clinical samples were randomly collected from different areas of AL-Najaf province..Samples were collected during a period extended from 26 March 2012 upto 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 manufactur-ing 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 absorb-ance ratio at wavelength 260 nm over 280 nm using a spectropho-tometer.

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

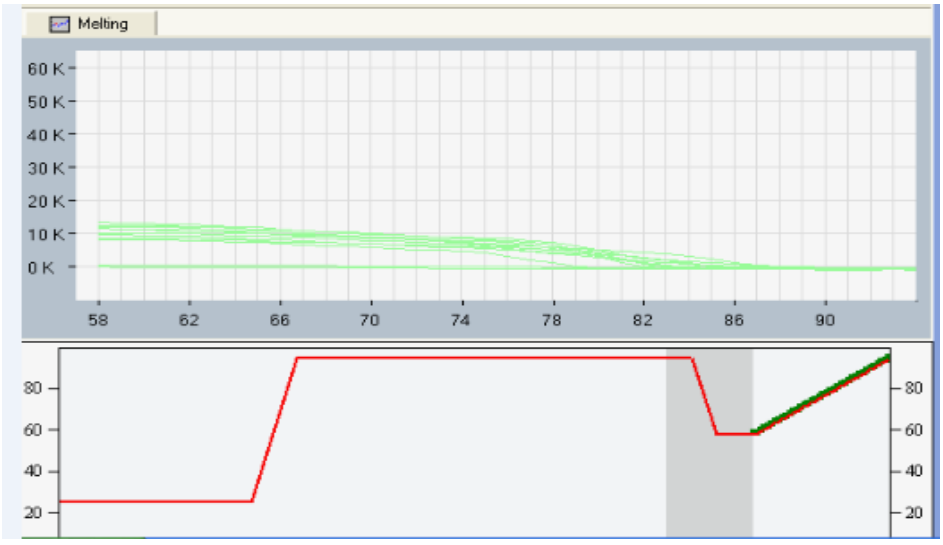
Amplification was carried out in the Laboratory of Veterinary hospi-tal in Najaf. The viral RNA was extracted from 33 positive clinical samples by rapid test device (nasal swabs, , plasma and serum) of population, using the primer design viral RNA kit (primer design UK) in accordance with the manufacturer's 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

Parainfluenza Virus 4	
PIV4-F1	CAA AYG ATC CAC AGC AAA GAT TC
PIV4-R1	ATG TGG CCT GTA AGG AAA GCA

2.5.4 Melting Curve Analysis

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

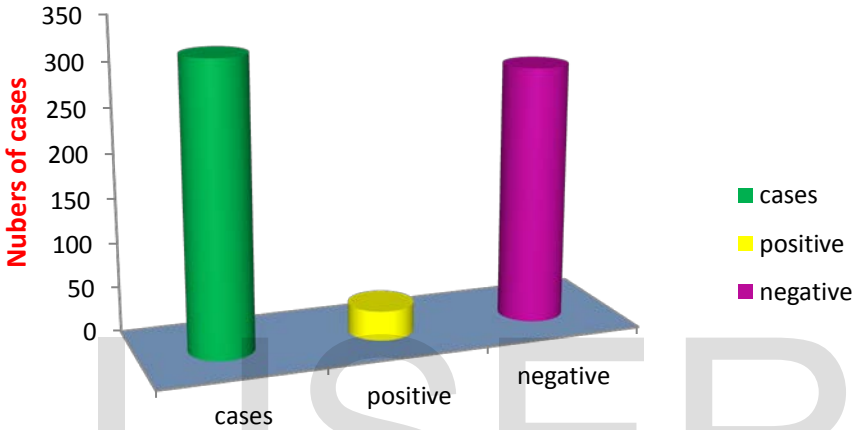


Figure(1): Melting Temperatures

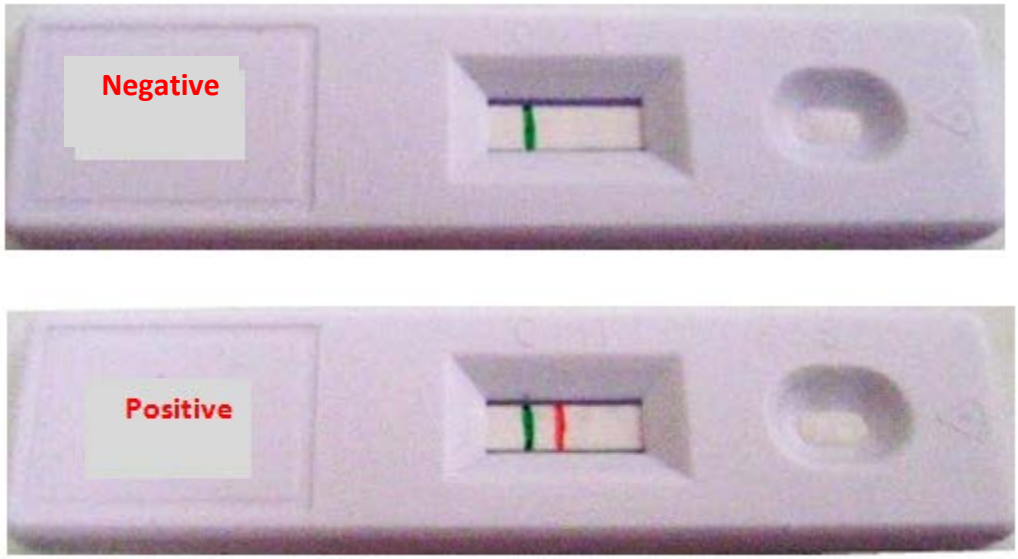
3 Results

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).



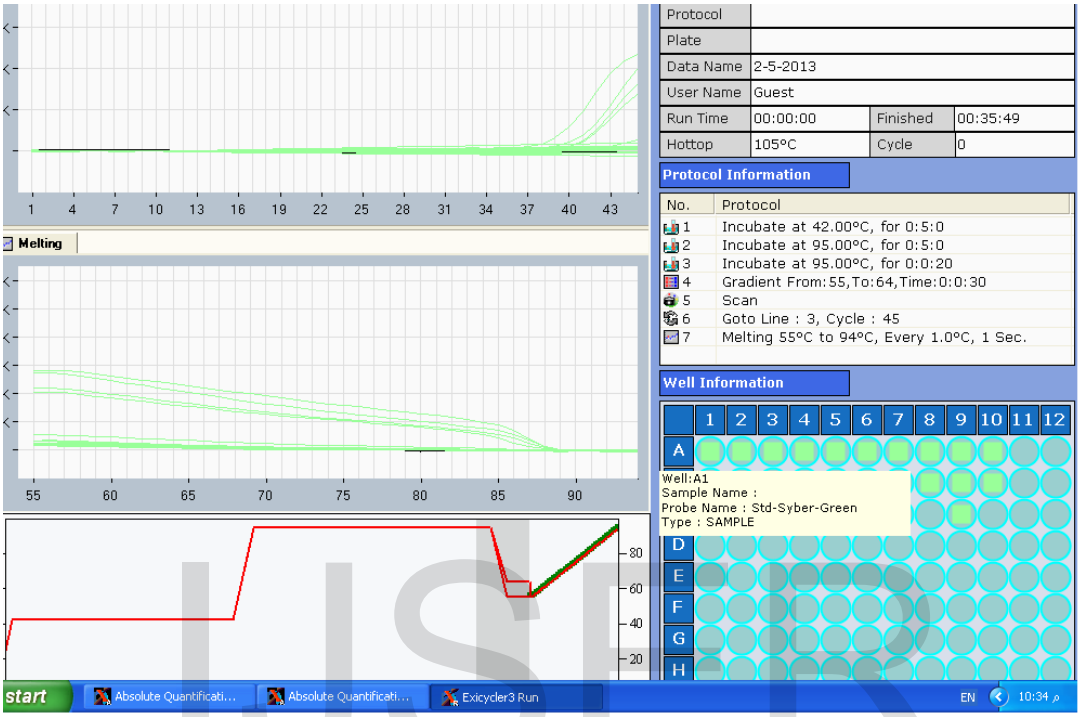
Figure(2): Numbers of Cases Infected with HPIV4 by Rapid Test .



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 undergone diagnosis with real -time – technique in figure(4).



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. Poten-tially 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 stud-ies 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 agree-ment 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 infec-tions. Conventional respiratory viral cell culture is limited by a lack of speed and therefore has little impact on patient care. Rapid im-munological tests partly overcome this problem, but the low sensi-tivity 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

[1] R.A. Lamb, G.D. Parks," Paramyxoviridae: the viruses and their replication". In *Fields Virology* 5th ed; Knipe, D.M. Howley, P. M. Griffin, D. E. Lamb, R. A. Straus, S. E. Martin, M.A. Roizman, B., Eds.; Wolters Kluwer Lippincott Williams & Wilkins: Philadelphia, PA, USA, 2007; Vol. 1, pp. 1449-1496.

[2] F.E. Lee, J. Treanor," Viral infections. In: Mason RJ, Broaddus CV, Martin TR, et al. Murray & Nadel's Textbook of Respiratory Medicine".5th ed. Philadelphia, Pa: Saunders Elsevier; 2010:chap 31.

[3] G.A. Weinberg, K.M. Edwards," Parainfluenza viral disease. In: Goldman L, Schafer AI", eds. *Cecil Medicine*. 24th ed. Philadelphia, Pa: Saunders Elsevier; 2011:chap 371.

[4] A.Weinberg, M.L.Walker ," Clinical and Diagnostic Labor-atory Immunology", 12(3), 367-370. 2005.

[5] S.K.Lau, W.To, P.W.Tse, A.K.Chan, P.C.Woo, H.Tsoi, "Human parainfluenza virus 4 outbreak and the role of diagnostic Tests". J Clin Microbiol. 2005;43:4515–21.

[6]N.Mao,Y.Ji,Z.Xie,H.Wang,,J.An,X.Zhang,Y.Zhang,Z.Zhu,A.Cui,S.Xu ,K.Shen,C.LiU, "Respiratory Tract Infection among Children and Genetic Analysis of HPIV-3 Strains in Beijing, China "2012.

[7]S.H.Kim,J.H. Huh,S.Y. Bae,J.S. Kim,S.Y. Yoon, "Epidemiology of respiratory viral infection in 2004–2006.". Korean J Lab Med 26: 351–357.2006.

[8] M.M. Fe, A.J.Monteiro, F.E.Moura ,"Parainfluenza virus infections in a tropical city: clinical and epidemiological aspects". Braz J Infect Dis 12: 192–197. 2008.

[9] H.Bando, K.Kondo, M. Kawano, H. Komada, M. Tsurudome, M. Nishio, Y. Ito," Molecularcloning and sequence analysis of human parainfluenza type 4A virus HN gene: its irregularitieson structure and activities". *Virology* **1990**, *175*, 307-12

[10] K.J.Henrickson KJ ," Parainfluenza viruses". Clin Microbiol Rev 16: 242–264.2003.

[11] K.E.Templeton, S.A. Scheltinga, M.F. Beersma, A.C.Kroes , E.C. Claas,". Rapid and sensitivemethod using multiplex real-time PCRfor influenza a and influenza B viruses, diagnosis of infections by respiratory syncytial viru, and parainfluenzaviruses 1, 2, 3, and 4". J. Clin. Microbiol. 42, 1564–1569.2004.

[12]J.O. Wishaupt, A.Russcher, L.C.Smeets, F.G.Versteegh,N.G. Hartwig ," Clinicalimpact of RT-PCR for pediatric acute respiratory infections: a controlled clinical trial". Pediatrics 2011.