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Molecular Detection of *Parainfluenza virus* in Infants and Young Children in AL-Muthanna Governorate

Thesis

Submitted to the Department of Biology , College of Science, Muthanna University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology / Microbiology

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بسم الله الرحمن الرحيم

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الأقـــــرار

نحن نشهد كأعضاء لجنة المناقشة قد اطلعنا على هذه الأطروحة الموسومة ب **(التحري الجزيئي لفيروس *parainfluenza* في الرضع و الأطفال في محافظة المثنى)**, وقد ناقشنا الطالبة (**ابرار عبدالله كاظم**) في تاريخ 2/6/2016. و وجدنا بأن الأطروحة تفي بمستوى الحصول على درجة الماجستير في كلية علوم الحياة / علم الأحياء المجهرية.

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Dedication

First to my beloved mother and father

And my family the rock of my life

To my brothers with love

To my dearest friend Fatima abdullkadem

To my colleagues and teachers

To everyone who has encouraged and helped me in every way to make this work possible .

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First of all, I would like to thank Allah, the most merciful for helping me during my study and my live ,blessings and peace for the prophet Mohammed bin Abdullah and his house hold for blessing me in my live and in completing this work .

*I would like to thanks my family whom the sours of my pride and appreciation in life, they light up the way and take care of me (**Mother , Father, and my brothers**).*

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To all thank you very much

And sorry to those I forgot…….

Abrar

Abstract

Human Parainfluenza virus (HPIV) is an important pathogen that causes upper and lower respiratory infection in infant , every person at least had been infected one time in his live . The virus considered the second most infective agent after *respiratory syncytial virus (RSV)* that causes respiratory infection . *Human parainfluenza virus* are generally believed spread through contact and large airborne droplet transmission .

In response to clinical importance of the virus this study was designed to detect the virus in acute respiratory tract infection patients and to estimate infection rate in addition to investigate the predominant genotypes . Molecular characterization and phylogenetic analysis of local strains have been undertaken as compared with globally published strains .

Three hundred nasal swaps were collected from infants and preschool children whom were hospitalized for acute respiratory infections in AL-Muthanna governorate at the period from January 2015 to March 2015 , written constants were taken . Most frequent clinical signs have been estimated which were fever , breathing difficulty , wheezing , cough and runny nose more than one sign were recorded at the same case .

The results of the clinical study showed that the acute respiratory infection was the higher infection rate in Rumaythah with 38% and the lowest infection rate in Khider with 1.33% . the total infection rate of both male and female were 54.33% for male and 45.66% for female .

Revers transcription real time polymerase chine reaction technique was applied for detection of nucleoprotein gene (*NP* gene).of *human parainfluenza virus* by using specific primers and TagMan probe .

The results of *HPIV* infection rate by using RT-RT-PCR was 45.38% . The results of infection rate according to the gender showed that the percentage rate of female was 61.11% which was higher than male 36.66% . with significant differences at ($p \leq 0.01$) between male and female. The results of infection rate by using RT-qPCR according to regions of study were (58.3% , 47.5% , 50.1% , 38.5% , , 33.3 %, 25% , , 0% and 0%)

in Samawa, , Hilal , Rumaythah, Warka, Sweer, Najme, Maged, and Khider respectively .Samawa showed the highest infection rate 58.3% , while Najme was the lowest 25% with significant differences (P≤0.01) .Maged and Khider were not recorded any positive results. And there was no significant differences between Rumaythah and Hilal and significant differences (p≤0.01) with other regions . There was significant differences between Warka and other regions (p≤0.01). There was significant differences between Sweer and other regions (p≤0.01). There were significant differences between Najme and other region in (p≤0.01) .And there was no significant differences between Maged and Khider and significant differences (p≤0.01) with other regions

Ten positive RT-PCR samples were subjected to endpoint convential PCR by using specific primers for identification of *NP* gene (523bp) . The positivity of all ten samples were confirmative .

Ten isolate (convential PCR products) were sequenced and submitted in NCBI – GenBank and took their accession number , seven of them were *HPIV-1* **(KT763053 , KT763054 ,KT763055 , KT763056 , KT763057 , KT763058,KT763060)** , and the other three isolate were HPIV-3 **(KT763052 , KT763059, and KT763061)** , while there were no HPIV-2 and HPIV-4 recorded .

Phylogenetic tree construction of the isolate was achieved . The results of phylogenetic analysis were Seven of our ten isolate was match to HPIV-1 . The seven isolate are **(KT763053 , KT763054 ,KT763055 , KT763056 , KT763057 , KT763058,KT763060)** The isolate **(KT763053 , KT763054)** showed high homology with **(EU346886.1,)** which isolated from Lithuania and with**(D01070.1, JQ901971.1)** which isolated from USA. (**KT763055 , KT763056 , KT763057 , KT763058,KT763060)** showed high homology with **(M62850.1)** which isolated from USA . And the isolate showed similarity with other isolation in different percentage, (**AF457102.1 , JQ902004.1 , KF530212.1 , KF687311.1 , KF530203.1**) that isolated from USA, **(S38060.1)** that isolated from Japan and**(KM190940.1)** that isolated in Thailand

Three of the ten isolate **(KT763052 , KT763059, and KT763061)** was match to HPIV-3. **(KT763061)** was match to **(M11849.1, M14552.1)** that isolated from Chile and

(X04612.1) that isolated from India.**(KT763052 , KT763059**) was match to **(EU346887.1)** that isolated from Lithuania . And showed similarity in different presenting with other isolation , **(U51116.1 , Z11575.1 , KJ672605.1 , KF530245.1)** that isolated from USA , **(AB736166.1)** that isolated from Japan , **(KM190938.1)** that isolated from Thailand, and **(FJ455842.2)** that isolated from China

In conclusion *HPIV* was considered one of the most important causative agent of acute respiratory infection in infants and young children in al Muthanna and *HPIV 1* and *HPIV 2* were the predominant genotype recorded in different area of study . Phylogenetic analysis permitted the arrangement of Iraqi strains of the current study with some other some world lineages as USA, Lithuania , Chile and other .

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List of Abbreviation

Abbreviation	Terms
Acces. NO.	Accession number.
ARI	Acute respiratory infections.
ARTIs	Acute respiratory tract infections.
A	Adenine.
AAP	American Academy of Pediatrics.
bp	Base pair.
BLAST	Basic Local Alignment Search Tool.
cDNA	Complimentary DNA.
CP	Crossing point .
CP	Crossing Point.
CT	Cycles of threshold.
C	Cytosine.
^{o}C	Degree Celsius.
dNTPs	Deoxynucleotide Triphosphate.
DEPC	Diethyl pyrocarbonate.
ddH_2O	Double-distilled water
ELISA	Enzyme-linked immunosorbent assay.

F	Fusion protein
G	Guanine.
HAdI	Hemadsorption inhibition.
HI	Hemagglutinin inhibition.
HN	Hemagluttinin -neuraminidase.
HPIV	*Human parainfluenza virus* .
HCL	Hydrochloric acid.
IF	Immuno fluorescence.
Ig	Immunoglobulin.
IL	Interleukin.
ICTV	International Committee on Taxonomy of Viruses.
KB	Kilobase.
L	large polymerase protein.
LCA	Last Common Ancestor.
LRTI	Lower respiratory tract infection.
$MgCl_2$	Magnesium Chloride.
M	Matrix protein
MRC	Medical Research Center .
MS_2	MegaSquirt2
M	Membranes protein
mRNA	Messenger ribonucleic Acid.
µl	Microliter.
ml	milliliter
MEGA 6	Molecular evolution Genetic Analysis version 6.
nm	Nanometer.
NCPI	National Center for Biotechnology Information.
NGS	Next generation Sequencing.
NS	Nonstructural protein.
NP	Nucleocapsides protein.
OTUs	Operational taxonomic units.
PIV	*parainfluenza virus*.
P	Phosphoprotein.
PCR	Polymerase chain reaction.
KCL	Potassium Chloride
qPCR	Quantitative polymerase chain reaction.
RSV	Respiratory syncytial virus.
RELPs	Restriction fragment length polymorphisms.
RT	Reverse transcription .
RT-qPCR	Reverse transcription quantitative Polymerase chain reaction.
rpm	Revolution per minute.
RNase	Ribonuclease
RNA	Ribonucleic Acid.
RNA-Seq	RNA Sequencing.

SNP	Single Nucleotide Polymorphism .
TCID 50	Tissue culture infective doses 50.
HL	Transformed HeLa.
UV	Ultra Violate .
URTI	Upper respiratory tract infection.
vRNA	Viral RNA.
WHO	World Health Organization .

Appendixes

Chapter One

Introduction

1.1. Introduction .

Respiratory viral infections (RVIs) are a leading cause of morbidity, hospitalization and mortality, throughout the world **(Abed and Boivin 2006),(Armstrong *et a1*., 1999).** Again acute lower respiratory tract infections (ALRI) is a leading cause of paediatric morbidity and mortality One of the most common viral agents associated with respiratory tract infections are parainfluenza viruses (*PIV*) **(van den Hoogen *et al.*, 2001).**

Human parainfluenza viruses (HPIVs) are important human pathogens that cause upper and lower respiratory tract infections, especially in infants and young children. They are typical members of the family *Paramyxoviridae* and There are four recognized serotypes, *HPIV* types 1 to 4 (*HPIV-1* to -4) . *HPIV-1* and *HPIV-3* belong to the genus *Respirovirus*, whereas *HPIV-2* and *HPIV-4* belong to the genus *Rubulavirus*. *HPIV-4* is further divided into two subtypes, 4A and 4B, on the basis of antigenic differences **(Lamb and Parks, 2007).**

HPIV are demonstrated to be pleomorphic enveloped viruses .Their envelope is derived from the host cell that they last infected. This family of viruses are medium sized, between 150 and 250 nm, but much larger virions or virus aggregates have been reported. Virus particles usually contain single-stranded RNA with negative polarity (complementary to mRNA). **(Henrickson, 2003).**

Parainfluenza virus are major causes of upper and lower respiratory tract diseases in infants and young children, causing croup, bronchiolitis, and pneumonia **(Hall, 2001).** Additionally, these viruses have all been identified as important causes of severe lower respiratory tract disease, with significant morbidity and mortality, in elderly and immnunocompromised patients **(Greenberg, 2002) , (Ljungman *et al*., 2001)**

Human parainfluenza virus (*HPIV*) causes serious lower respiratory tract diseases in young children. It is one of the most common causes of hospitalization for fever and or acute respiratory illness in children under 5 years old , and in particular ,in infant aged 0-5 months **(Murphy, *et al*.,1988) (Weinberg, *et al*., 2009)**.

Virus transmission occur through contact with infectious fluids, either directly or indirectly through contaminated fomites, or through inhalation of airborne particles in the

form of large droplets or small droplet nuclei **(Goldmann , 2000)** . Respiratory paramyxoviruses such as *HPIV* are generally believed spread through contact and large airborne droplet transmission **(Hall , 2001)**.

A rapid, sensitive, and specific diagnostic tool is important for management of patients presenting with a respiratory infection **(Adcock *et al*., 1997).** Electron microscopy can easily demonstrate *HPIV* , many paramyxoviruses appear the same **(Henrickson,2003).** Cell culture is still used in laboratory detection of respiratory viruses. However, cell culture is slow and has a low sensitivity and viral culture can often result in delays of several weeks before test results are available making such results clinically irrelevant **(Martin *et al*. 2008).** Immunofluorescence (IF) techniques used for the detection of viral antigens, IF provides rapid results, but it often lacks sensitivity in detecting some viruses and further confirmation by viral culture may sometimes be required **(Liolios , 2001).**

The availability of *HPIV*-specific detection assays is important because other respiratory pathogens cause similar illnesses. In recent years, molecular methods have resulted in sensitive, specific, and rapid detection of respiratory viruses

Polymerase chain reaction (PCR) assays have been shown to be more sensitive than culture or antigen testing for the detection of some respiratory viruses in specimens from various populations, **(Kuypers *et al*., 2006).(Weinberg *et al*.,2004)**. Molecular techniques as both Real-Time PCR and conventional PCR were rapid , specificity and highly sensitive technique to detect of *NP* gene of the *HPIV* . In addition to enhanced sensitivity, the benefits of real-time PCR assays over conventional endpoint detection methods include their large dynamic range, a reduced risk of cross contamination, an ability to be scaled up for high throughput applications and the potential for accurate target quantification . The combined properties of high sensitivity and specificity, low contamination risk, and speed has made real-time PCR technology a highly attractive alternative to tissue culture- or immunoassay-based methods for diagnosing many infectious diseases **(Espy *et al*., 2006).**

DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species

(Pettersson *et al* ., 2009). RNA-Seq is one possible method for transcriptomics that shows a high potential to improve the understanding of development and diseases .

virus sequences in the GenBank will throw light on the molecular epidemiology and emergence of any new variant *HPIV* in the field. So the sequence study is considered to be useful in understanding *HPIV* scenario in the endemic areas. **(Hosamani, *et al*., 2007).** Molecular phylogenetics uses the structure and function of molecules and how they change over time to infer these evolutionary relationships **(Hall , 2004).**

1.2. Aims of study .

1- To evaluate the clinical and epidemiological features of *HPIV* in young children in AL-Muthanna province .

2-To investigate the role of HPIV in acute respiratory tract infection and the predominant genotypes of *Parainfluenza virus* in AL-Muthanna province .

3-Molecular characterization and phylogenetic analysis of local strain of *HPIV* by sequencing and alignment with globally published strains in the GenBank .

Chapter Two

Literature Review

2.1. History

Acute respiratory infections (ARI) are one of the major causes of morbidity and mortality in young children throughout the world especially in developing countries **(Denny and Loda ,1986)**. Data from WHO estimated the burden of ARI at 94037000 disability-adjusted life years (DALYs) and 3.9 million deaths in 2001 **(WHO,2001)**. These viruses were first discovered in the late 1950s, when three different viruses recovered from children with lower respiratory disease proved to be unique and easily separated from the myxoviruses (*influenza virus*) they closely resembled. This new family of respiratory viruses grew poorly in embryonated eggs and shared few antigenic sites with influenza virus. In 1959 **(Canchola *et al.*,1964**). a fourth virus was found that also met these criteria, and a new taxonomic group was created called parainfluenza viruses. **(Morris *et al*., 1956).**

Human parainfluenza virus (*HPIV*) types 1,2, 3, and 4 have been known primarily as respiratory pathogens in young children. They are now recognized as important pathogens in adults as well as young children. Adults infected with these viruses tend to have more variable and less distinctive clinical findings than children, and the viral cause of the infection is often unsuspected. The consistency of the annual outbreaks of these agents and the frequency of reinfection suggest that they impose a considerable, but ill-defined, disease burden throughout life **(Chanock, *et al*., 1957).**

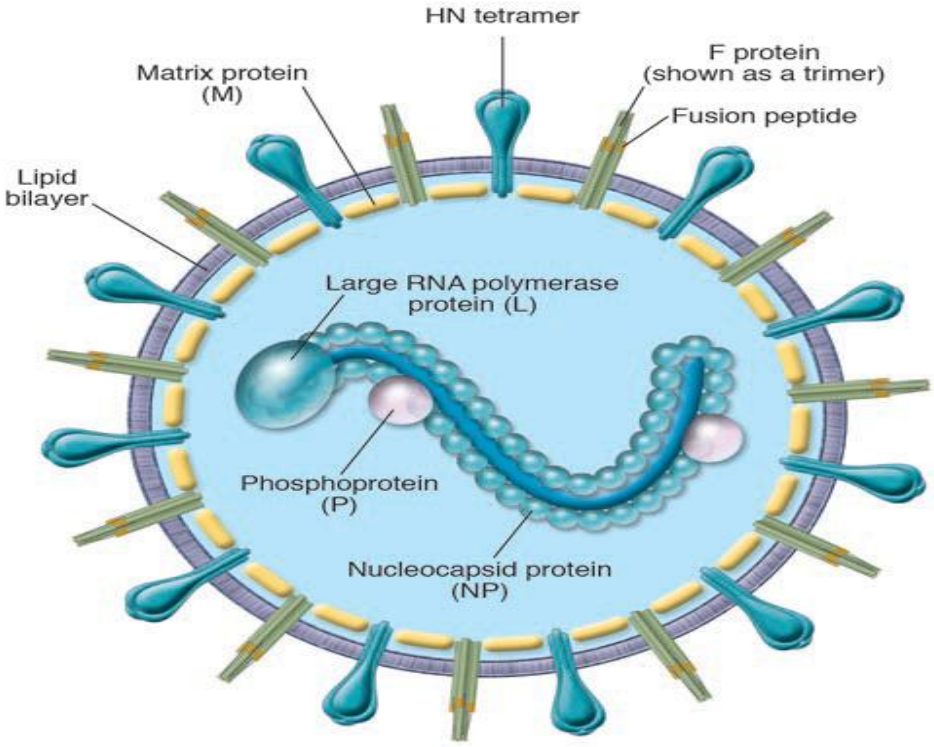
The parainfluenza viruses, is one the chief cause of hospitalization for respiratory tract illness in young children. In 1991, it was estimated that infection of children with *parainfluenza virus* types 1 and 2 accounted for 250000 visits to emergency rooms, 70000 hospitalizations **(Henrickson *et al*.,1994)**.Parainfluenza viruses are also leading causes of hospitalization in adults with community-acquire respiratory disease
(Marx *et al*., 1999 ; Falsey *et al*., 1995).

A further systematic analysis also estimated 1.575 million (uncertainty range: 1.046 million - 1.874 million) deaths of children worldwide in 2008 as due to ARI **(Black *et al*., 2008)** majority of acute lower respiratory tract infections.

Despite four decades of efforts, there are no effective means to control *Parainfluenza virus* infections. The development of vaccines has been confounded by the lack of durable immunity, even after natural infection, and the diversity and ubiquity of populations at risk for infection **(Hall, 2001).**

2.2. Etiology

Human Parainfluenza viruses (HPIVs) are important human pathogens that cause upper and lower respiratory tract infections, especially in infants and small children. They are typical members of the family *Paramyxoviridae* **(Vainionpaa and Hyypia , 1994)** , There are four recognized serotypes, *HPIV* types 1 to 4 (*HPIV*-1 to -4) **(Collins *et al.*, 1996)**. *HPIV*-1 and *HPIV*-3 belong to the genus *Respirovirus*, whereas *HPIV-2* and *HPIV-4* belong to the genus *Rubulavirus*. *HPIV*-4 is further divided into two subtypes, 4A and 4B, on the basis of antigenic differences **(Henrickson, 2003)**. These viruses are pleomorphic enveloped particles that are 150 to 300nm in diameter. The virion consists of a filamentous, herringbone-like nucleocapsid core surrounded by a lipid envelope with virus-specific glycoprotein spikes. The nucleocapsid is composed of the RNA genome, which is linear, non-segmented, negative-sense and tightly coated with the nucleocapsid protein. The viral envelope contains two virus-specific proteins, the hemagglutinin-neuraminidase (HN) and the fusion (F) proteins, both largely integral to immunity and pathogenesis **(Hall , 2001 ; Vainionpaa and Hyypia, 1994)(Figure2-1)** .



(Figure2-1):A schematic diagram of the *parainfluenza* virion. L, large RNA polymerase protein; M, matrix protein; *NP*, nucleocapsid protein; P, phosphoprotein **(Hall,. 2001).**

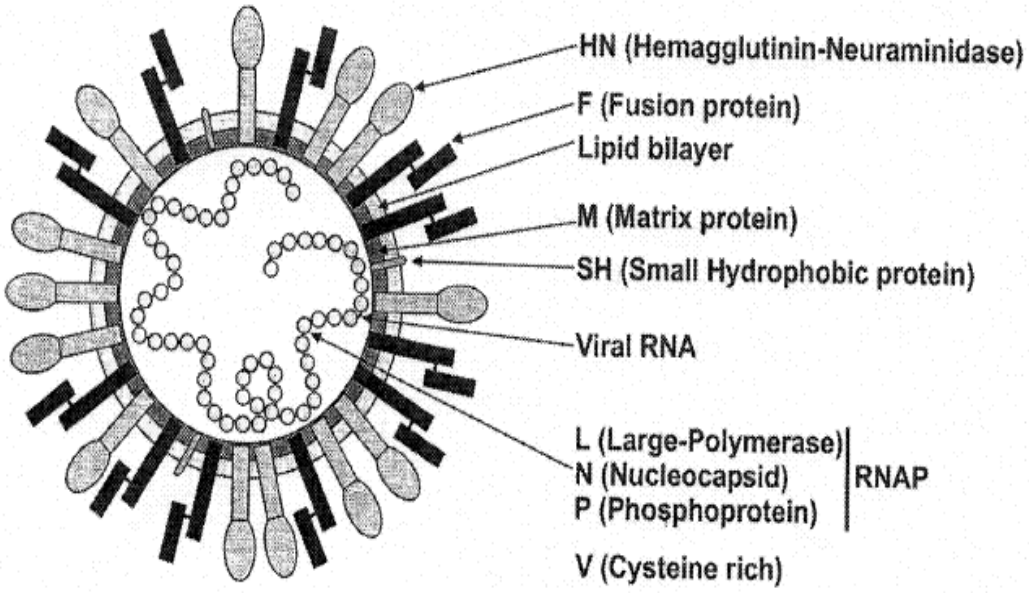
2.2.1. Morphology

By electron microscopy, *HPIV* are demonstrated to be pleomorphic enveloped viruses**.** Their envelope is derived from the host cell that they last infected. This family of viruses are medium sized, between 150 and 250 nm, but much larger virions or virus aggregates have been reported Virus particles usually contain single-stranded RNA with negative polarity **(Lamb and Parks, 2007 ; Kingsbury, 1985) ,**

The *HPIV* genome contains approximately 15000 nucleotides. These are organized to encode at least six common structural proteins (3'-N-P -M-F-HN-L-5') **(Wechsler *et al*,. 1985 ; Storey, *et al*,. 1984).**

Electrophoresis demonstrates great similarity in protein size between the four major *HPIV* types except for the phosphoprotein two surface glycoproteins are found in all HPIV the hemagglutinin-neuraminidase (HN) and the fusion protein (F_o) **(Komada, *et al*,. 1989 ; Cowley and Barry. 1983)**, the membrane protein (M) is strongly associated with and found just beneath the viral membrane. and have an envelope composed of host cell lipids and viral glycoproteins

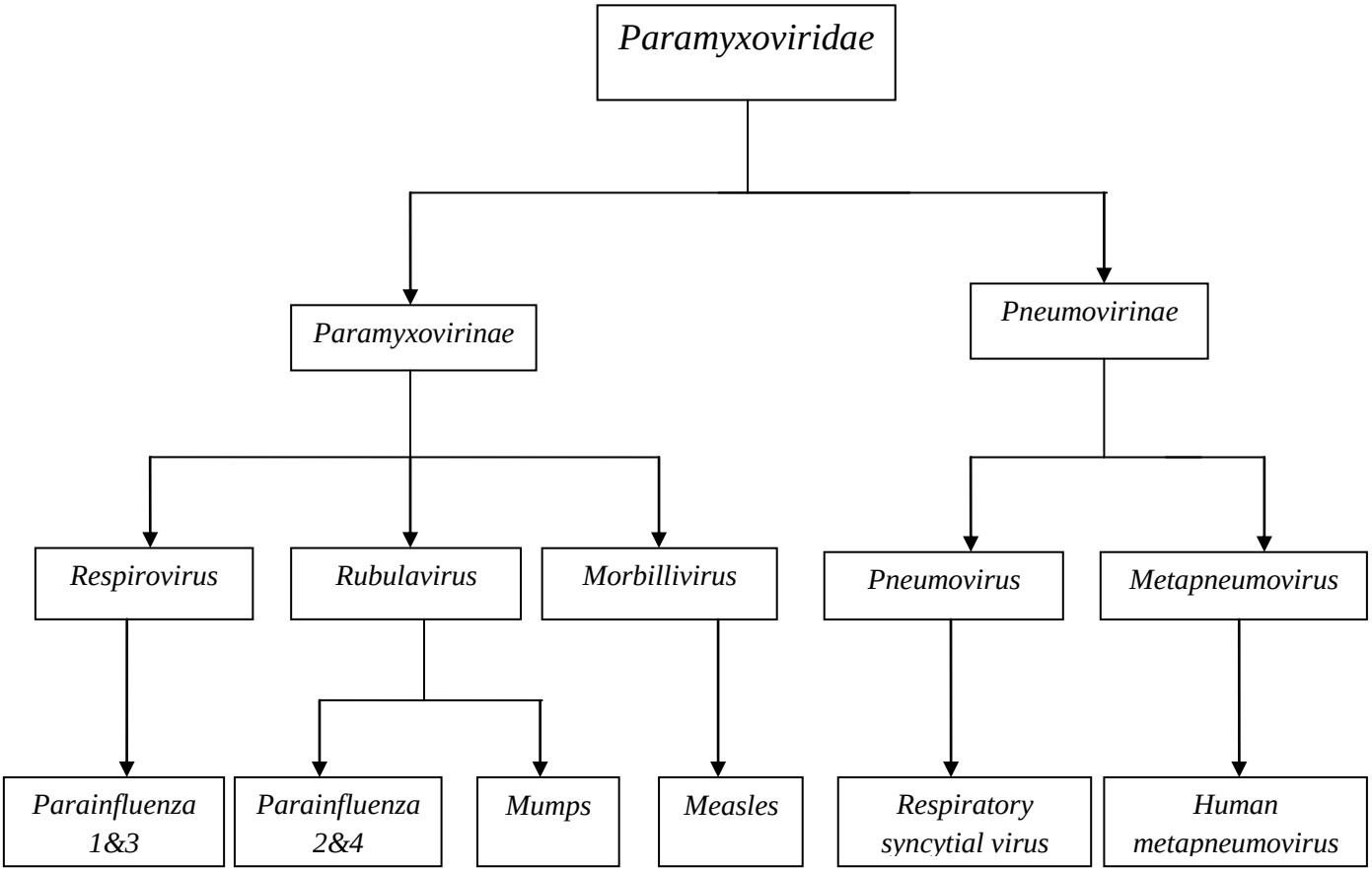
derived from the plasma membrane of the host cell during viral budding. The *HPIV* genome is single-stranded, negative-sense RNA that must be transcribed into message-sense RNA before it can be translated into protein. Like all negative-stranded RNA viruses, the HPIVs encode and package an RNA-dependent RNA polymerase in the virion particles .The RNA genome is approximately 15,500 nucleotides in length and is encapsidated by the viral nucleocapsid protein, forming helical nucleocapsids **(Figure2-2) (Moscona, 2005).**



(Figure 2-2) : Schematic diagram of the member of *paramyxoviridae* **(Farideh ,2008).**

2.2.2. Classification

The family of *Paramyxoviridae* has been reclassified in 2000 by international committee on the taxonomy of viruses into two subfamilies , the *paramyxovirinae* and the *pnemovirinae*. *Paramyxovirinae* contains three genera : *Respirovirus* ,*Rubulavirus* and *Morbillivirus*. *pneumovirinae* contains the genera : *Pneumovirus* and *metapneumovirus* **(ICTV , 2000)** .



(Figure 2-3): Classification of **Parainfluenza virus (Farideh ,2008).**

The new classification is based on morphologic criteria, the organization of genome, the biologic activation of proteins , and the sequence relationship of encoded protein. The pnemoviruses can be distinguished from *paramyxovirinae* morphologically as they contain nucleocapsides with smaller diameter .In addition , the number of encoded proteins differs and the pnemoviruses also contain an attachment protein that is very different from that of paramyxoviruses **(Lamb & Kolakofsky, 2001)** . Paramyxoviruses contain nonsegmented, single strand RNA genomes of negative polarity, and they replicate entirely in the cytoplasm. Their genomes are 15 to 19 KB in length, and the genomes contain six to ten linked genes **(figure 2-3).**

2.2.3. Serotypes

HPIV has four predominant serotypes. Serologic and antigenic analysis of all of the species within the *Paramyxovirinae* subfamily demonstrates four basic genera *HPIV*-1, *HPIV*-3, *HPIV*-2, *HPIV*-4, **(Henrickson and Savatski , 1996 ; Tsurudome *et al*., 1989).**

The *HPIV* all induce variable levels of heterotypic antibody during infection and have common antigens. This often makes it impossible to determine whether serologic positivity represents specific amnestic responses or cross-reactions to similar antigens on different HPIVs. However, specific hyper immune animal serum (e.g., hamster or guinea pig) can usually differentiate between these viruses in standard hemagglutinin inhibition (HI), hemadsorption inhibition (HAdI), complement fixation, neutralization tests or enzyme-linked immunosorbent assay (ELISA) **(Henrickson , 2003 ;Sarkkinen *et al*., 1981**

Two major subtypes of *HPIV*-4 (A and B) were discovered shortly after this virus was first identified 40 years ago. HAdI and neutralization assays could easily distinguish these subtypes, but complement fixation (CF) could not (**Canchola *et al*., 1964).**

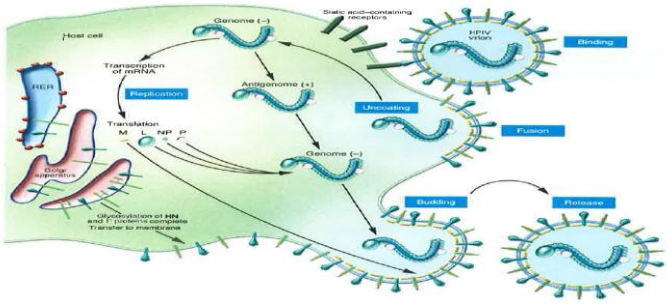
During the same decade, several *HPIV-2* strains were isolated that could be differentiated serologically from the type strain **(Numazaki *et al*., 1968)**, and then about 10 years ago studies demonstrated significant antigenic variation between different *HPIV*-2 clinical isolates **(Ray *et al*., 1992)**. At about the same time, strains of *HPIV*-1 were reported that could be separated from the type strain by ELISA, HI, and neutralization assays **(Henrickson, 1999).** Molecular analyses of all four types have demonstrated more antigenic and genetic heterogeneity than was initially appreciated **(Henrickson and Savatski, 1996)**,. In fact, the data suggest that all four major *HPIV* serogroups (*HPIV*-1 to *HPIV*-4) have subgroups or populations that have unique antigenic and genetic characteristics. Even HPIV-4 subtypes A and B demonstrate this variability **(Komada *et al*.,1990).** The variability and changes seen in *HPIV* suggest an evolutionary pattern similar to that of influenza B virus. Polyclonal serologic testing can detect most *HPIV* strains using common "type" antigens, but subgroups of *HPIV*-1 (A, C, and D) and *HPIV*-3 have been reported with progressive antigenic change **(Henrickson, 1999)**. In addition, *HPIV*-1 strains isolated over the last 12 years demonstrate persistent antigenic and genetic differences compared to the 1957 type strain, including differences between genotypes

within the same epidemic and same geographic location **(Henrickson, 1999; Henrickson and Savatski, 1996 ; Komada *et al*., 1992),** This progressive antigenic change (although slow) will cause standard reference sera raised to HPIV isolates from the 1950s, or antigen prepared from these same "type" strains, to not universally react in routine serologic assays in the future. Examples of this have already occurred, leading to failure of commercial diagnostic products. Investigators now often use more recent strains of HPIV as sources of antigen or genomic material**.(Scaparrotte *et al*.,2013)**.

2.2.4. Replication

The first step in viral replication and reproduction is the fusion of the virus and host cell lipid membranes **(Kingsbury,1985).** The virus attaches to sialic acid receptors on the host cell and fuses with the cell membrane, make it possible to the nucleocapsides to enter the cytoplasm **(figure 2-4**). Once it is became inside the cell, transcription takes place using the polymerase L protein (RNA dependent RNA polymerase) and P protein. Viruses dock to the cell using a glycoprotein or glycolipid cell receptor and its own G, H, or HN spike protein The virion enters the cell by mediating fusion of its lipid envelope with the external plasma membrane of the cell and this vital event of catalysed "fusion from without" is mediated by the F protein. The genome is immediately released into the cytoplasme **(Collier and Oxford,2000)**.

The virus uses host ribosomes to help translate the viral messenger RNA (mRNA) into viral proteins, which direct replication of the virus genome, firstly to a positive sense strand and then into a negative sense strand. The genome of negative-sense RNA viruses cannot function directly as mRNA but must first be transcribed to virus-specific mRNA species by RNA-dependent RNA polymerase , thin it is encapsulated with NP and can be released from the cell by budding **(Moscona , 2005 ;Henrickson, 2003; Chanock ,2001)**.



(Figure 2-4) : Schematic representation of the life cycle of *parainfluenza virus.* **(Moscana, 2005).**

2.3. Epidemiology

Infants and young children are clearly infected by this virus, but it is rarely isolated. Serologic surveys have demonstrated that most children between 6 and 10 years of age have evidence of past infection, suggesting mild or asymptomatic primary infections **(Henrickson, 2003).**

Respiratory infections cause 3 to 18% of all admissions to pediatric hospitals, and *HPIV* can be detected in 9 to 30% of these patients depending on the time of year **(Carballal *et al*,. .2001) ,(Kim *et al*., 2000).**

The majority of infections and deaths are observed among young infants, immnunocompromised, and elderly individuals. Malnutrition, overcrowding ,Environmental conditions such as temperature, humidity, pH, and the composition of the storage fluid easily affect *HPIV*. Viral survival markely decreases at temperature above 37°C, until at 50°C almost all virus is inactivated within 15 min **(Mahony, 2008)** , *HPIV*-1 and occur in both hemispheres. HPIVs generally cause upper and lower respiratory infections it has been estimated that 12% of the 500000 to 800000 lower respiratory infection (LRI) cases reported annually in USA are caused by HPIV 1-3. It has also been estimated that, worldwide, 10% of the total LRIs in preschool children are caused by HPIVs and 25 to 30% of these result in death. Nosocomial infections are also common, especially among young infants; with HPIV3 being the most frequently transmitted among the four HPIVs **(Marx *et al*,. 1997).**

Human parainfluenza 3 also infects children early in life: 60% and 80% will have been infected before the ages of 2 and 4 years, respectively infection with *HPIV*-1 and *HPIV*-2 occurs when children are slightly older, but, by 5 years of age, most children have been infected with these viruses at least once **(Durbin and Karron, 2003)**.

Human parainfluenza 1 causes biennial epidemics which peak during the fall season. During these epidemics the majority of infections (50%) occur in children aged 7 to 36 months and peaking during the second and third year of life. HPIV-2 also causes biennial infections, either with *HPIV*-1 or during alternate years from HPIV-1, or annual epidemics, which peak during fall to early winter. The majority of infections (60%) caused by *HPIV*-2 occur in children younger than 5 years of age and peak between the first two years of life. Outbreaks caused by *HPIV*-3 tend to occur yearly and peak during early spring to summer (for North America and Europe). The majority of these infections (40%) occur in children during the first year of life**(Koivisto, 2004).** Little is known about the epidemiology of *HPIV*-4 due to the small number of studies conducted. Generally, it has been noted that the rate of infection is relatively the same in age groups from young infants to adults. An outbreak of *HPIV*-4 within a developmental disabilities unit involving 38 institutionalized children and 3 staff members has also been described **(Lau *et al*,. 2005).**

The four human parainfluenza viruses (HPIVs) are important upper and lower respiratory tract pathogens in infants and young children. Each of the HPIVs has distinct clinical and epidemiologic features, but each can cause a full spectrum of acute respiratory tract illnesses. *HPIV*-1 is the most common pathogen associated with croup or laryngotracheobronchitis**(Denny and Clyde, 1986)** Although its epidemiology is less well-defined, *HPIV*-2 infection is also associated with croup hospitalizations. **(Henderson, 1987)** It has been reported that HPIV-3 ranks behind only respiratory syncytial virus (*RSV*) as a cause of bronchiolitis and pneumonia among infants and young children**.(Glezen and Denny, 1997)**. *Human parainfluenza 4* is infrequently detected , and consequently less is known about its clinical and epidemiologic characteristics. Infection with all four *HPIV* serotypes is common, and most children have serologic evidence of infection by 5 years of age. **(Collins *et al*., 1996)** . The age at which first infection occurs varies by serotype. First infections with *HPIV*-1 and -2 are most common during the second year of life. *HPIV*-3 infections occur frequently during the first year of life, and it is the serotype most often associated with HPIV infection during the first 6 months of life. **(Knott *et al*., 1994)**. Because *HPIV* infections do not confer complete protective immunity, reinfection occur

throughout life **(Marx *et al*.,1999)**. Most *HPIV* infections are detected during seasonal epidemics. In the United States *HPIV*-1 infections have been epidemic in the fall of odd-numbered years since 1973. *HPIV*-2 infections are epidemic in the fall every year nationally, but community epidemics can occur annually or biennially in the fall.15 *HPIV*-3 infections are usually widespread in the United States during late spring and summer **(Glezen &Denny, 1997; Collins, 1996).**

2.3.1. Transmission

Parainfluenza viruses are common human pathogens, and they are second only to the respiratory syncytial virus (*RSV*) in causing lower-respiratory-tract infections in young children In spite of the relative importance of parainfluenza viruses as human pathogens, our understanding of the modes and vehicles for their spread is still very limited **(Chanock and McIntosh. 1990)**. These viruses have been found to survive for at least a few hours in air and on environmental surfaces **(Brady *et al*,. 1990 ;Parkinson *et al*,. 1983)**.

Respiratory virus transmission can occur through contact with infectious fluids, either directly or indirectly through contaminated fomites, or through inhalation of airborne particles in the form of large droplets or small droplet nuclei**(Goldmann , 2000)**.

Respiratory paramyxoviruses such as *HPIV* are generally believed spread through contact and large airborne droplet transmission **(Hall , 2001)**. Few studies have specifically investigated *HPIV* transmission. Infectious *HPIV*-1 virus was recovered from air samples taken 60 cm away from only 1 of 30 *HPIV*-1 infected children, making transmission by small droplet nuclei unlikely. The HPIVs can be recovered from experimentally contaminated non-porous surfaces for up to 10 hours **(Brady *et al*,. 1990)** however, *HPIV*-3 quickly lost infectivity when placed on the hands (**Ansari *et al*,.1991)** In general terms, the potential of a vehicle to spread a given infectious agent is directly related to the capacity of the agent to survive in or on that vehicle. Hands have long been implicated in the spread of infectious diseases and are often suggested to be the most important vehicle **(Adler, 1988 ; Hendley and Gwaltney, 1988).** Beyond these studies, surprisingly little is known about *HPIV* transmission in humans. The study of respiratory

virus transmission in humans is difficult due to ethical, safety, environmental, and budgetary considerations. For these reasons, the use of small animal models to study transmission of respiratory viruses has been widely utilized. The HPIVs poorly infect mice, and *HPIV* infection in cotton rats, hamsters, guinea pigs, and ferrets is usually asymptomatic with minimal or undetectable pathology **(Karron and Collins ,2007)**

Human parainfluenza viruses are reported to cause 40% of acute respiratory tract illnesses in children and 20% of respiratory illnesses in hospitalized children **(Reed *et al*., 1997)**. Of the four recognized HPIVs, *HPIV*-1, -2 and -3 have been regarded as major causes of lower respiratory tract illness in infants and young children **(Collins *et al*., 1996) .**In particular, *HPIV*-1 and *HPIV*-3 were the important causes of outbreaks of respiratory tract infection, especially in institutional settings **(Cortez *et al*., 2001 ;Fiore *et al*., 1998 ;Karron *et al*., 1993).** Beside these, medical studies showed that significant mortality and morbidity caused by HPIVs occur frequently in immnunocompromised individuals **(Apalsch *et al*., 1995 ; Arola *et al*., 1995) .** While the impact of the *HPIV*-1, -2 and -3 have been well appreciated, the clinical importance of *HPIV*-4 is far less well defined **(Lau *et al*., 2005)**. More than 40 years after the discovery of *HPIV*-4, only few individual cases or case series of *HPIV*-4 infection have been described **(Lindquist *et al*., 1997 ;Rubin *et al*., 1993).** The scarcity of reported cases may be attributed to the difficulties in isolating *HPIV*-4. *HPIV*-4 is the most difficult HPIV to grow in cell culture, with late hemadsorption and cytopathic effect, and therefore is rarely detected by conventional virological methods **(Aguilar *et al*., 2000).** The another reason for the lack of information on *HPIV*-4 is that the virus was traditionally associated with mild respiratory disease (**Aguilar *et al*., 2000 ;Collins, *et al*., 1996.).** Over the past decades, it was regarded as less clinically important; and therefore was usually not included in the routine panels of respiratory virus detection in most clinical virology laboratories (**Lam Siu Yan , 2007).** However, a growing body of studies suggest that the prevalence of *HPIV*-4 has been underestimated. Several recent studies have found that *HPIV*-4 is associated with more severe respiratory illness in children, and is even more frequently detected than HPIV-2 **(Lindquist *et al*., 1997 ;Rubin *et al*., 1993)**. *HPIV*-4 infection may therefore be more prevalent and severe than was previously thought. Particularly, an outbreak of *HPIV*-4

infection reported in Hong Kong provided important evidence. This was the first local outbreak of *HPIV*-4 infection in a regional hospital, involving 38 institutionalized children and three staff members during a 3-week period in autumn 2004. A total of 7% children were infected with lower respiratory tract infections. The outbreak initially aroused the general awareness of *HPIV*-4 infection that *HPIV*-4 may be an important cause of more severe respiratory illness in children. Therefore, it is now a need to further investigate the role of *HPIV*-4 as causes of respiratory disease among children in our locality **(Lau *et al*., 2005)**.

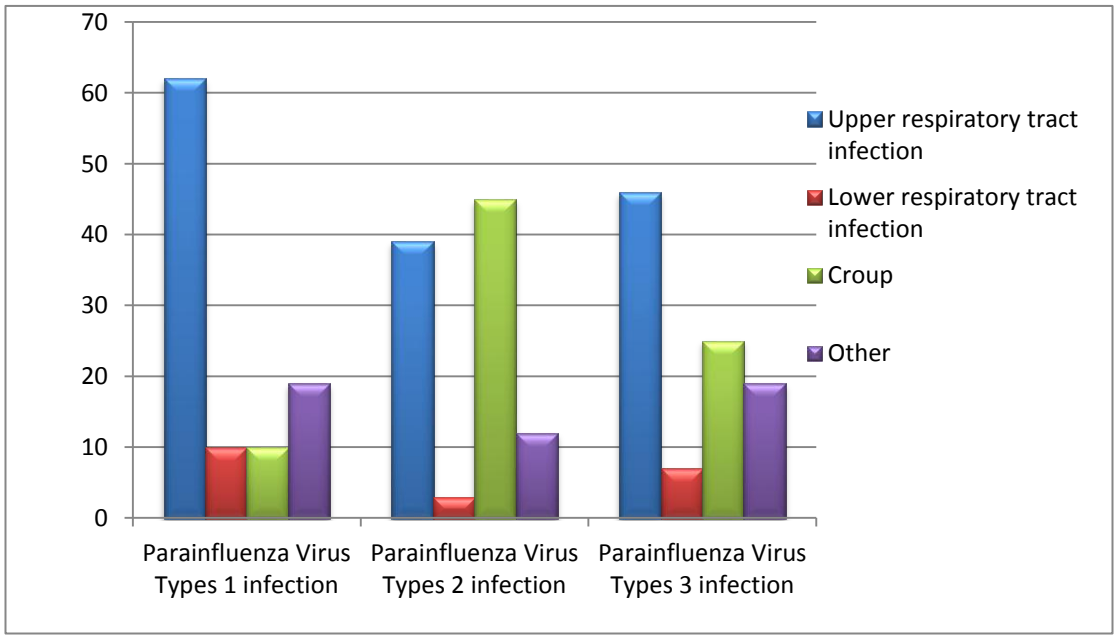
2.4. Symptoms and clinical signs .

These viruses have been primarily known as children's viruses, with good reason the parainfluenza viruses cause a spectrum of respiratory illnesses similar to those caused by *RSV* **(Figure 2-5),** but result in fewer hospitalizations **(Hall ,2001 ; Reed *et al*.,1997 ; Knott *et al*.,1994).** Most are upper respiratory tract infections, of which 30 to 50 percent are complicated by otitis media**.(Knott *et al*.,1994 ; Henderson *et al*.,1982)**

About 15 percent of *parainfluenza virus* infections involve the lower respiratory tract, and 2.8 of every 1000 children with such infections require hospitalization ,Most children are infected by *parainfluenza virus* type 3 by the age of two years and by *parainfluenza virus* types 1 and 2 by the age of five years. Pneumonia and bronchiolitis from *parainfluenza* virus type 3 infection occur primarily in the first six months of life, as is the case for RSV infection, but with a lower frequency **(Reed *et al*.,1997)**

Croup is the signature clinical manifestation of infection with parainfluenza virus, especially type 1, and is the chief cause of hospitalization from *parainfluenza* infections in children two to six years of age **(Marx *et al*.,1997 ;Knott *et al*.,1994**) .In those with chronic pulmonary disorders, *RSV* and *parainfluenza virus* infections lead to complications that are indistinguishable from those resulting from other infectious or noninfectious causes **(Arnold *et al*.,1999)**.

Parainfluenza virus infections are often unsuspected in immune compromised hosts, since they may mimic other opportunistic infections more commonly associated with an immune compromised state. Furthermore, upper respiratory tract signs, if present, may appear inconsequential **(Hill, 2001)** .



(Figure 2-5): Clinical Syndromes Caused by the Parainfluenza Virus Types 1, 2, and 3 in Pediatric Outpatients **(Knott *et al*,.1994).**

2.5. Disease caused by parainfluenza

Human Parainfluenza 1 is the major cause of acute croup in infants and young children but also causes mild URTI, pharyngitis, and tracheobronchitis in all age groups **(Leland , 1996).** Outbreaks in temperate climates tend to occur mostly in the autumn months. *Human Parainfluenza* 2 is generally associated with lower infection rates than *HPIV*1 or *HPIV*3 and has been associated with mild URTI, croup in children, and, occasionally, LRTI. Infections occur predominantly in fall months. *HPIV*3 is a major cause of severe LRTI in infants and young children, often causing croup, bronchitis, and pneumonia in children 1 year of age **(Wright *et al*,. 2005)**. In older children and adults, it can cause URTI ortracheobronchitis **(He *et al*,. 2007).** Infections with *HPIV*3 can occur in any season but are most prevalent in winter and spring months in temperate climates **(AAP,2003).** PIV4 is the least common of this group and is generally associated with mild URTI. As a group, HPIVs cause 15 to 30% of nonbacterial respiratory disease in children requiring hospitalization **(Griffin *et al*., 2004).** The onset of illness can either be abrupt as an acute spasmodic cough or begin as a mild URTI evolving over 1 to 3 days to involve

the lower tract. The duration of acute illness can vary from 1 to 3 weeks but generally lasts 7 to 10 days **(AAP, 2003)**. *Human parainfluenza* also causes LRTI in the elderly and immune compromised patients including bone marrow recipients **(Madden *et al*.,2004 ; Raboni *et al*., 2003)**

2.6. Pathogenesis

Human parainfluenza viruses cause several serious respiratory diseases in children for which there is no effective prevention or therapy. Parainfluenza viruses initiate infection by binding to cell surface receptors and then, via coordinated action of the 2 viral surface glycoproteins, fuse directly with the cell membrane to release the viral replication machinery into the host cell's cytoplasm. During this process, the receptor-binding molecule must trigger the viral fusion protein to mediate fusion and entry of the virus into a cell. There are several steps during the process of binding, triggering, and fusion that are now understood at the molecular level, and each of these steps represents potential targets for interrupting infection **(Moscona, 2005).**

Viral antigen has been localized to the apical portion of epithelial cells in infected cotton rats **(Porter *et al*., 1991).** Actin and the cytoskeleton have been reported to play roles in transcription, maturation, and the movement of viral glycoproteins to the surface of infected cells. **(Bose *et al*., 2001).**

Human parainfluenza virus replicates in the respiratory epithelium of the upper respiratory tract and spread from there to the lower respiratory tract , Epithelial cells of the small airways become infected. Resulting in inflammation of the airways or bronchiolitis. This inflammation is accompanied by The relationship among the tissue damage caused by the virus, the immune responses that help to clear the virus, and the inflammatory responses that contribute to disease is still quite enigmatic. However, the damage to epithelial cells appears to result from inflammation, rather than the virus itself, but this concept remains unclear. In most cases, the virus is non-cytopathic and can lead to persistent infections. Tissue damage leads to necrosis of cells and increased mucus secretions, obstructing airflow, resulting in wheezing or coughing. Recovery from

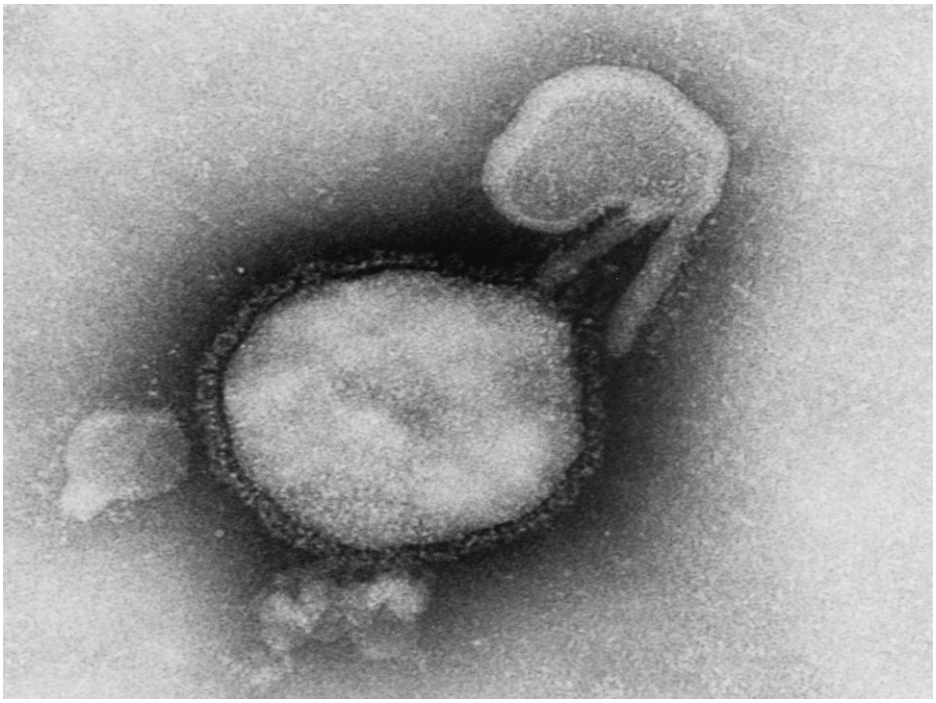
infection usually occurs within a few days of symptoms **,(Henrickson , 2003 ; Hall , 2001).**

2.7. Diagnosis

Acute respiratory tract infections are the most widespread types of infections in adults and children and are responsible for considerable morbidity and mortality worldwide (**Lopez *et al*., 2006).** Unfortunately, the etiology remains undetermined in more than 50% of cases **(File, 2003).** For these reasons, a rapid, sensitive, and specific diagnostic tool is important for management of patients presenting with a respiratory infection **(Adcock *et al*., 1997 ;Woo, *et al*., 1997).** Throat swabs, nasopharyngeal swabs, nasal washes, and nasal aspiration have all been used successfully to recover *HPIV* **(Frayha *et al* . 1989).**

2.7.1. Electron microscopy .

Electron microscopy can easily demonstrate *HPIV* **(Figure 2-6).** However, many paramyxoviruses appear the same (e.g., mumps virus). No large study looking at the diagnostic utility of electron microscopy for HPIV infection has been published **(Henrickson,2003)** , and less expensive methods of diagnosis have been developed.



(Figure 2-6) : Electron micrograph of HPIV, Magnification, ×275,000. , **(Henrickson , 2003).**

2.7.2. cell lines

Cell culture is still the “gold standard” for the laboratory detection of respiratory viruses. However, cell culture is slow and has a low sensitivity. Therefore, its implementation for routine virus detection is suboptimal and the viral culture can often result in delays of several weeks before test results are available making such results clinically irrelevant**(Downham *et al*., 1974).** Cultured for respiratory viruses by conventional tube culture. A number of primary and secondary cell lines support the growth of HPIV. On rhesus monkey kidney, African green monkey kidney, transformed HeLa (HL), and human foreskin fibro blast (HDF) cell lines**(Nichols *et al*., 2001).** *HPIV* can also replicate in organ cultures from mouse, guinea pig, ferret, and human fetal respiratory epithelium **(Henrickson, 2003).** Paramyxoviruses are known to induce apoptosis in tissue culture cells , in a study found that *HPIV 1* induced a potent apoptotic response. Both phenotypes appeared to contribute to attenuation in African green monkeys (AGMs) and in cultures of ciliated human airway epithelium (HAE) **(Emmalena et al., 2008)**

2.7.3. immunofluorescence

The development of sensitive and specific immunofluorescence (IF) techniques for the detection of viral antigens in the cells of the nasopharyngeal secretions, collected at the acute stage of the disease, has greatly improved the rapid laboratory diagnosis of viral respiratory diseases .The problem of specimen transportation, however, has caused some limitations to the use of the technique since cell preparations from the nasopharyngeal secretions are destroyed rapidly during transportation by the proteolytic enzymes present in the specimens. The method also requires a highly skilled microscopist to evaluate the test and is not easily automated**.(Sarkkinen *et al*,. 1981).** It provides rapid results, but it often lacks sensitivity in detecting some viruses and further confirmation by viral culture may sometimes be required **(Liolios , 2001)** .In addition, some *HPIV* strains may be missed entirely by IF assays with specific monoclonal antibodies **(Swierkosz *et al*., 1995).**Although the combination of both of these techniques can provide an increase in the proportion of positive results, it has been reported that a significant number of specimens still remain negative, despite clinical and epidemiological suspicions of viral infection **(Ellis *et al*., 1997),** (**Freymuth *et al*., 1995**),

2.7.4. Molecular Technique .

Virus isolation may not always be successful as a compared to molecular detection methods (**Elnifro *et al*., 2000).** PCR is nowadays one of the most powerful and applied methods in virus diagnostics **(Mackay *et al*., 2002).**

A. Convential Polymerase chain reaction (PCR)

To overcome the limitations in the diagnosis , there has been a keen interest in the development of new nucleic acid-based assays. Reverse transcription-PCR (RT-PCR) assays have been shown to be rapid, sensitive, and specific for the detection of respiratory viruses **(Liolios , 2001).**

Nucleic acid detection methods have become available for the diagnosis of virus infection **(Watzinger *et al.*, 2006).** The classical polymerase chain reaction (PCR), which amplifies DNA in a specific manner, has improved the sensitivity of the direct diagnosis of viruses dramatically **(Belák and Ballagi-Pordány, 1993)**. Reverse transcriptase PCR technique has been frequently used for detection of the *human parainfluenza virus* (**Vaucher *et al*., 2008).**

Nucleic acid detection by means of end point PCR involves three steps extraction of nucleic acid from a sample, nucleic acid amplification, and detection of amplified products. Originally, detection of PCR amplicons relied on gel electrophoresis in the presence of ethidium bromide that allows subsequent visualization of the amplicons during UV irradiation. As an alternative, PCR amplicons may be captured onto a solid phase and detection by enzyme immunoassay. These methods are characterized by time consuming and necessitates multiple PCR product handling steps that increase the risk of carry over contamination and false positive results in subsequent assay**(Lassauniere, 2010).**

B-Real-Time PCR

Real-time PCR-based molecular virology testing requires assays that are sensitive, specific, and that can distinguish between virus types (**van Raak *et al.*, 2010**). Comparative studies have shown that for the detection of respiratory viruses real time RT-PCR is significantly more sensitive than conventional detection methods. **(Gharabaghi *et al*, 2008).** The majority of real-time RT-PCR detection assay for respiratory viruses are qualitative in nature. Even though qualitative real-time PCR in the diagnostic setting has

many advantages in compare with traditional detection methods. Quantitative real-time PCR provides qualitative as well as quantitative information. Advantages of quantitative real-time PCR is that it permits the assessment of viral load at a given time point, facilitates the monitoring of response to treatment, and offers the possibility to determine the dynamics of virus proliferation **(Watzinger *et al.*, 2006).** The Real-Time PCR assay can provide a high sensitivity and requires therefore only one round of amplification, which further reduces working time and decreases the risk of contamination of samples. Real time PCR generates a threshold (CT) or crossing point (CP) cycle for each sample. This is the point where product (fluorescence) crosses a predetermined threshold. The higher the amount of starting target, the lower the CT. The CT for an unknown sample is analyzed against a standard curve to yield a target DNA or RNA copy number (**Tang and Stratton, 2006).**

There are two primary ways that real-time RT-PCR can be carried out.

One method involves including the RT step into the same tube as the PCR reaction (one-step). The other method involves creating cDNA first by means of a separate reverse transcription reaction and then adding the

cDNA to the PCR reaction (two-step). There are advantages and disadvantages to both systems. The advantages to one-step real-time RT- PCR is that it is quicker to set up, less expensive to use, and involves less handling of samples, thereby reducing pipetting errors, contamination, and other sources of error. With the one-step method, gene-specific primers are used and both the reveres transcripts and PCR occur in one reaction tube; therefore, other genes of interest cannot be amplified for later analysis **(Gallina *et al.*, 2006).** The RNA from the original sample must be initially aliquoted for archival storage and future testing. The main advantage to two-step RT-PCR is that typically random hexamer or oligo primers are used in an RT reaction in a separate tube. This allows for the ability to convert all the messages in a RNA sample into cDNA, which would allow for archiving of samples and future testing of other genes **(Wacker and Michael, 2005).**

2.7.5 Sequencing .

DNA sequencing is the process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of

the four bases—adenine, guanine, cytosine, and thymine—in a strand of DNA. The advent of rapid DNA sequencing methods has greatly accelerated biological and medical research and discovery. DNA sequencing technology has been instrumental in the sequencing of complete DNA sequences, or genomes of numerous types and species of life, including the human genome and other complete DNA sequences of many animal, plant, and microbial species **(Pettersson *et al* ., 2009).**

Several decades passed before fragments of DNA could be reliably analyzed for their sequence in the laboratory. RNA sequencing was one of the earliest forms of nucleotide sequencing. The major landmark of RNA sequencing is the sequence of the first complete gene and the complete genome of Bacteriophage MS2, identified and published by Walter Fiers and his coworkers at the University of Ghent (Ghent, Belgium), in 1972 **(Min Jou *et al* ., 1972) .**

Several notable advancements in DNA sequencing were made during the 1970s. **Frederick Sanger** developed rapid DNA sequencing methods at the MRC Centre, Cambridge, UK and published a method for "DNA sequencing with chain-terminating inhibitors" in 1977 **(Sanger *et al* ., 1977).**

RNA-Sequencer (RNA-Seq) is a whole transcriptome shotgun sequencing method that uses next generation sequencing (NGS) methods, like either Illumina sequencing by synthesis, 454 -Pyrosequencing or Solid sequencing by ligation. The NGS step follows several preparation steps, as for example RNA isolation and preparation.

First, RNA has to be isolated Second, the isolated RNA has to be prepared for cDNA library construction. The RNA preparation steps depend on the following NGS method. Typically rRNA is depleted , reverse transcribed, indexed (adaptor attachment to one or both ends) and pooled before library construction and final sequencing steps. Third, cDNA library construction is followed by NGS **(Roth, 2009).**

RNA-Seq has several benefits compared to other techniques in transcriptomic , which are , RNA-Seq does not rely upon knowledge about the genome sequence **.** In comparison, DNA microarray, which is the most frequently used transcriptomic technique, requires genome information to enable oligonucleotide synthesis for microarray chip 3 production. Consequently, RNA-Seq is attractive for non-model organisms too. Furthermore, RNA -

Seq shows a low background signal due to the possibility to map the cDNA sequences to unique regions in the genome **(Wang *et al.*, 2009).** Likewise, RNA-Seq enables the identification of alternative spliced RNA is forms, antisense transctipts and fusion genes by mapping the transcripts to the genome sequence **(Ozsolak and Milos, 2011)** . As a result, RNA-Seq does not rely upon genome sequence information, but, nevertheless, genomic information are useful for these purposes. Moreover RNA-Seq has no upper quantification limit and a large dynamic detection range **.** In contrast, DNA microarray shows a detection limitation due to the number of fixed oligonucleotides on a microarray chip. Finally, RNA-Seq shows a high reproducibility both for technical and biological replicates **(Wang *et al.*, 2009).**In spite of all the benefits, some challenges still remain for RNA-Seq. Although there are just a few steps in RNA-Seq, there are still some manipulation stages, such as PCR amplification, RNA fragmentation and reverse transcription**(Ozsolak and Milos, 2011)**.

An ideal method for transcriptomics should be able to directly identify and quantify all RNAs. The direct RNA sequencing**(Ozsolak *et al.*, 2009)** method avoids the reverse transcription and PCR amplification, but the problem with fragmentation of large RNA molecules (e.g. to identify splicing patterns in eukaryotic transcriptomes) still remains.

Another point which should be mentioned is that an increase in sequencing depth, which could be reached by a longer read length, would lead to a greater coverage and more significant data. Finally, RNA-Seq also faces bioinformatical challenges **(Wang *et al.*, 2009).** High-throughput sequencing approaches generate a high amount of data that have to be processed. Therefore, two challenges are to reduce Errors in image analysis and to remove low-quality reads .In the end, RNA-Seq is one possible method for transcriptomics that shows a high potential to improve the understanding of development and diseases. Nevertheless, it is assumed that RNA-Seq does not replace techniques like DNA microarrays, because it takes (at the moment) a much longer time to perform a RNA-Seq experiment.

2.7.6 Molecular Phylogenetics .

Phylogenetics is the area of research concerned with finding the genetic relationships between species. The basic idea is to compare specific characters (features) of the species,

under the natural assumption that similar species (i.e., species with similar characters) are genetically close.

The similarity of biological functions and molecular mechanisms in living organisms strongly suggests that species descended from a common ancestor. Molecular phylogenetics uses the structure and function of molecules and how they change over time to infer these evolutionary relationships. This branch of study emerged in the early 20th century but didn't begin in the 1960s, with the advent of protein sequencing, PCR, electrophoresis, and other molecular biology techniques **(Hall , 2004).**

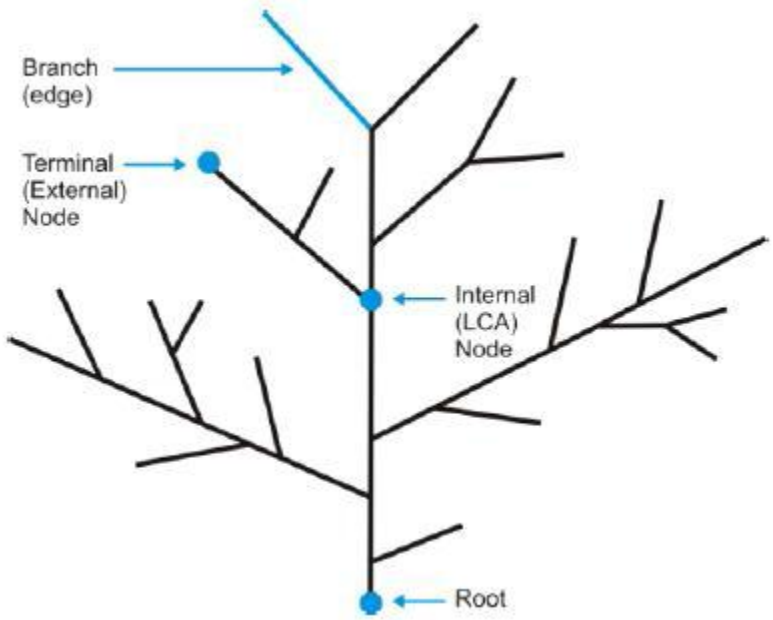
Over the past 30 years, as computers have become more powerful and more generally accessible, and computer algorithms more sophisticated, researchers have been able to tackle the immensely complicated stochastic and probabilistic problems that define evolution at the molecular level more effectively. Within past decade, this field has been further reenergized and redefined as whole genome sequencing for complex organisms has become faster and less expensive. As mounds of genomic data becomes publically available, molecular phylogenetics is continuing to grow and find new applications. **(Patthy , 1999 ; Liò and Goldman , 1998 ; Li , 1997)**.

The objective of molecular phylogenetic studies is to recover the order of evolutionary events and represent them in evolutionary trees that graphically depict relationships among species or genes over time. This is an extremely complex process, further complicated by the fact that there is no one right way to approach all phylogenetic problems. Phylogenetic data sets can consist of hundreds of different species, each of which may have varying mutation rates and patterns that influence evolutionary change. Consequently, there are numerous different evolutionary models and stochastic methods available. The optimal methods for a phylogenetic analysis depend on the nature of the study and data used. **(Ewens and Grant , 2005; Linder and Warnow , 2005).**

Evolution is a process in which the traits of a population change from one generation to another, by Means of Natural Selection, Darwin proposed that, given overwhelming evidence from his extensive comparative analysis of living specimens and fossils, all living organisms descended from a common ancestor**.(Hartwell *et al*,. 2008 ; Warnow ,2004)**.

Phylogenetics infers trees from observations about existing organisms using morphological , physiological , and molecular characteristics. The “tree of life” represents a phylogeny of all organisms, living and extinct. Other, more specialized species and molecular phylogenies are used to support comparative studies, test biogeographic hypotheses, evaluate mode and timing of speciation, infer amino acid sequence of extinct proteins, track the evolution of diseases, and even provide evidence in criminal cases **(Linder and Warnow , 2005)**.

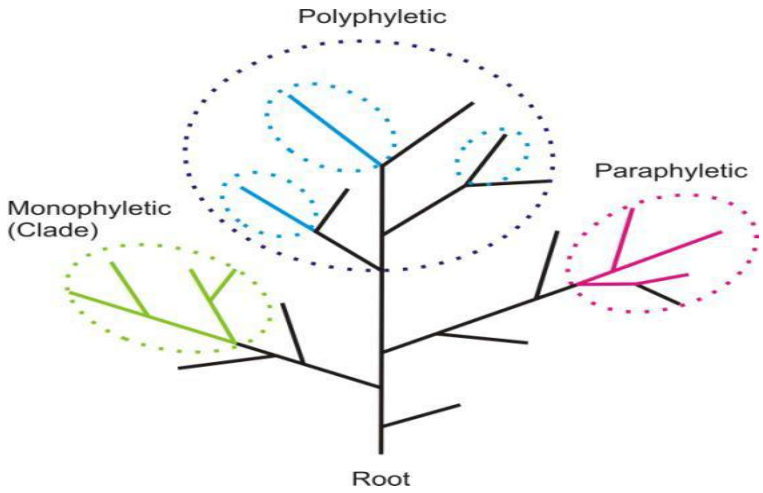
Phylogenetic trees are composed of branches, also known as edges, that connect and terminate at nodes. Branches and nodes can be internal or external (terminal), **(Figure 2-7)** . The terminal nodes at the tips of trees represent operational taxonomic units (OTUs). OTUs correspond to the molecular sequences or taxa (species) from which the tree was inferred. Internal nodes represent the last common ancestor (LCA) to all nodes that arise from that point. Trees can be made of a single gene from many taxa (a species tree) or multi-gene families (gene trees) . **(Baldauf , 2003) .**



(Figure 2-7): Basic elements of a phylogenetic tree **(Baldauf , 2003) .**

A tree is considered to be “rooted” if there is a particular node or outgroup (an external point of reference) from which all OTUs in the tree arises. The root is the oldest point in the tree and the common ancestor of all taxa in the analysis. In the absence of a known out

group, the root can be placed in the middle of the tree or a rootless tree may be generated. Branches of a tree can be grouped together in different ways**(Hall , 2004)** (**Figure 2-7).**

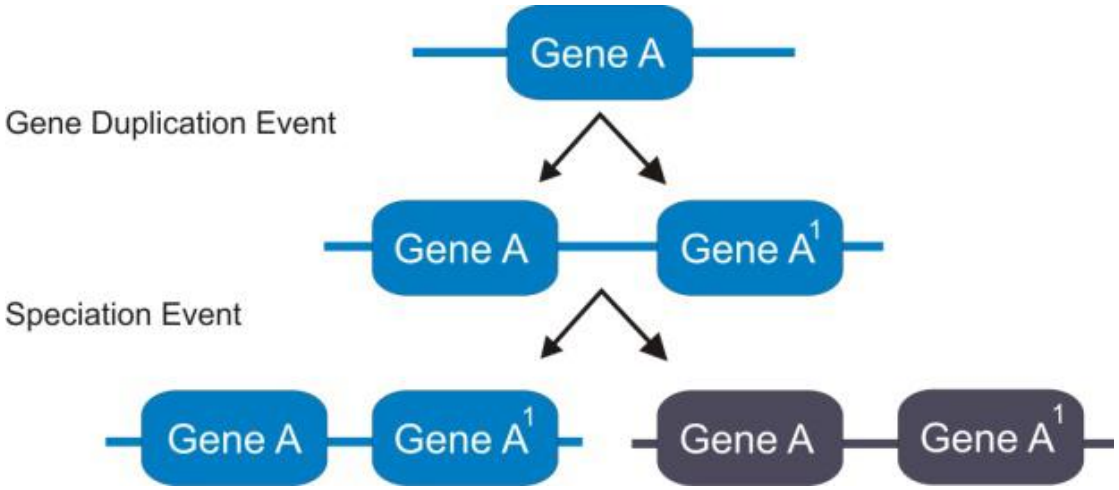


(Figure 2-8): Groups and associations of taxonomical units in trees **(Hall , 2004).**

A monophyletic group consists of an internal node and allOTUs arising from it. All members within the group are derived from a common ancestor and have inherited a set of unique common traits. A paraphyletic group excludes some of its descendents (for examples all mammals, except the marsupialiataxa). And a polyphyletic group can be a collection of distantly related OTUs that are associated by asimilar characteristic or phenotype, but are not directly descended from a common ancestor **(Li , 1997).** Evolution is shaped by homology, which refers to any similarity due to common ancestry. Similarly, phylogenetic trees are defined by homologous relationships. Paralogs are homologous sequences separated by a gene duplication event. Orthologs are homologous sequences separated by a speciation event (when one species diverges into two). Homologs can be either paralogs or orthologs **(Hartwell *et al* ., 2008).**

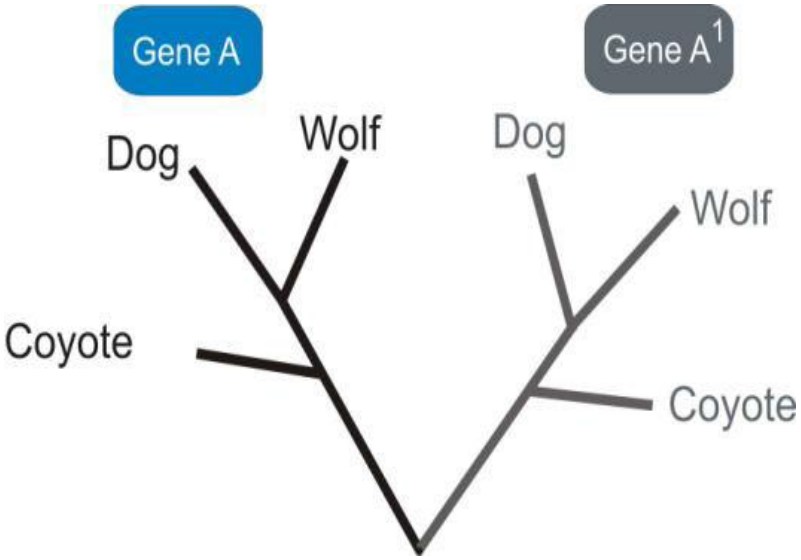
Molecular phylogenetic trees are drawn so that branch length corresponds to amount of evolution (the percent difference in molecular sequences) between nodes **(Linder and Warnow , 2005)**.

Paralogs are created by gene duplication events (**Figure 2-9**) . Once a gene has been duplicated, all subsequent species in the phylogeny will inherit both copies of the gene, creating orthologs. Interestingly, evolutionary divergence of different species may result in many variations of a protein, all with similar structures and functions, but with very different amino acid sequences. Phylogenetic studies can trace the origin of such proteins to an ancestral protein family or gene **(Patthy , 1999).**



(Figure 2-9) : Understanding paralogs and orthologs, **(Patthy , 1999).**

One way to ensure that paralogs and orthologs are appropriately referenced in a phylogenetic tree, and guard against misrepresentation due to missing or incomplete taxonomic information is to generate mirror phylogenies (**Figure 2-10**) in which paralogs serve as each other's outgroup **(Linder and Warnow , 2005).**



(Figure 2-10) : Mirror Phylogenies. Gene A and Gene A1 are paralogs, whereas all instances of Gene A are orthologs of each other in different Canid species **(Linder and Warnow , 2005).**

Molecular phylogenetic trees are generated from character datasets that provides evolutionary content and context. Character data may consist of biomolecular sequence alignments of DNA, RNA, or amino acids, molecular markers, such as single nucleotide

polymorphisms (SNPs) or restriction fragment length polymorphisms (RFLPs), morphology data, or information on gene order and content. **(Patthy ,1999)**.

2.8. Control and prevention

There are no licensed vaccines available for these non-segmented, negative strand RNA viruses **(Abed and Boivin , 2006)**. Hand washing is an effective way of preventing the transmission of respiratory viruses. Transmission appears to be related to contact with infectious droplets**.(Hill, 2001)**.

There is no licensed *HPIV* vaccine. However, the live attenuated *HPIV3* vaccine is showing considerable promise, as it can induce good humoral immune responses in seronegative children **(Belshe *et a1*., 2004).** Also, no antiviral drugs have been approved yet to treat *HPIV* infections. However, ribavirin, which is a drug that can inhabit viral polymerase activity, and the neuraminidase inhibitor, zanamivir, exhibited *in vitro* antiviral activity against *HPIV* (**Abed and Boivin, 2006 ; Henrickson,2003).** Thus, progress in this area is evident and will hopefully provide new therapies to treat *HPIV* infections in future years.

2.9. Immunity to Human parainfluenza viruses

The mechanisms of viral clearance and immune responses to *HPIV* are unclear. However, antibodies against *HPIV* are produced, mainly in response to the two major viral envelope proteins, HN and F. The majority of serum antibodies produced against these glycoproteins, are IgG antibodies and IgA antibodies are found in the mucosa. These antibodies protect against upper and lower respiratory tract infections **(Henrickson, 2003 ; Chanock, 2001).**

Also, cytotoxic T cells, that can kill virus infected cells, appear to be important in the clearance of virus from the lower respiratory tract, especially during *HPIV3* infections **(Henderson, 1981).** This cell mediated immunity is also important for recovery from viral infection, as *HPIV3* infected infants, with a severe T cell deficiency, can lead to a fatal case of pneumonia **(Chanock, 2001).** Therefore, this lack of cellular immunity appears to exacerbate the disease, emphasising the important role of T cells in the immune response

to viral infections. However, although adequate protection appears to be mounted against *HPIV*, no long lasting immunity to these viruses ever develops **(Henrickson, 2003).** Thus, reinfection with *HPIV* can occur throughout life, suggesting a lack or deficiency in immunological memory

HPIV can modulate certain aspects of the immune system, thereby enhancing its own survival. *HPIV3* can infect dendritic cells, leading to limited T cell proliferation (**Plotnicky-Gilquin *et a1*., 2001),** which is important for viral clearance and inducing memory T cells. Moreover, this virus has also been shown to induce IL-10 secretion from virus infected peripheral blood mononuclear cells, which is a potent immunoregulatory cytokine that can Inhibit T cell proliferation **(Sieg *et a1*., 1996)**. Also, *HPIV* can interfere with signalling components of the interferon pathway **(Young *et a1*., 2000),** which may affect interferon production and antiviral immunity. Thus, *HPIV* modulation of host immune responses, may account for the frequent infection rate and lack of lifelong immunity, associated with *HPIV*.

Chapter Three

Materials and Methods

3.1 Materials.

3.1.1. Instruments and Equipments.

Table (3-1): Instruments and equipments that used in this study with their companies and countries of origin .

No.	Equipment & instrument	Company
1	High Speed Coold centrifuge	Eppendorf /Germany
2	Incubator	Mammert/Germany
3	Sensitive Balance	Sartorius/Germany
4	Water Bath	Mammert
5	Vortex	CYAN/ Belgium
6	Micropipettes 5-50, 0.5-10, 100-1000µl	CYAN
7	Refrigerator	Concord /Lebanon
8	Thermocycler PCR	MyGene/USA
9	Exispin centrifuge	Bioneer/ Korea
10	Eppendorf tubes	Bioneer
11	Disposable syringe 10 ml, 5ml and 3ml	Sterile EO. / China
12	Sterile test tube	Superestar/ India
13	UV Transilluminator	ATTA/ Korea
14	Gel electrophoresis	Shandod Scientific/ UK
15	Digital camera	Samsung/ china
16	Real Time PCR thermocycler	Bio-Rad/ USA
17	Latex gloves	China
18	Cooled box	China

19	Sample containers	Jordan
20	Nanodrop	Bioneer

3.1.2. Chemicals.

Table (3-2): The chemicals with their companies and countries of origin used in this study.

No.	Chemical	Company and Origin
1	Ethanol	BDH (England)
2	Isopropanol	BDH
3	DEPC water	Bioneer/ Korea
4	Free nuclease water	Bioneer
5	DNA ladder (100bp)	Bioneer
6	Agarose gel	BioBasic/ Canada
7	Ehidium Bromide	BioBasic
8	TBE buffer	BioBasic

3.1.3. Kits .

Table (3-3): The kits used in this study with their companies and countries of origin.

No.	Kit	Company and Origin
1	**Total RNA Extraction Kit AccuZol™**	**Bioneer \ Korea**
	Trizol reagent 100ml	
2	**AccuPower® RocketScript™ RT-qPCR PreMix (RT-qPCR)**	**Bioneer\ Korea**
	RocketScript RT enzyme	
	RT buffer	
	Taq DNA polymerase	
	DNTPs	
	10X PCR buffer	
	RNase inhibitor	
3	**AccuPower® RT-PCR PreMix (convential PCR)**	**Bioneer\ Korea**
	RT enzyme	
	RT buffer	
	Taq DNA polymerase	
	DNTPs	
	10X PCR buffer	
	RNase inhibitor	
	Loading dye	

3.1.4. Primers and Probe

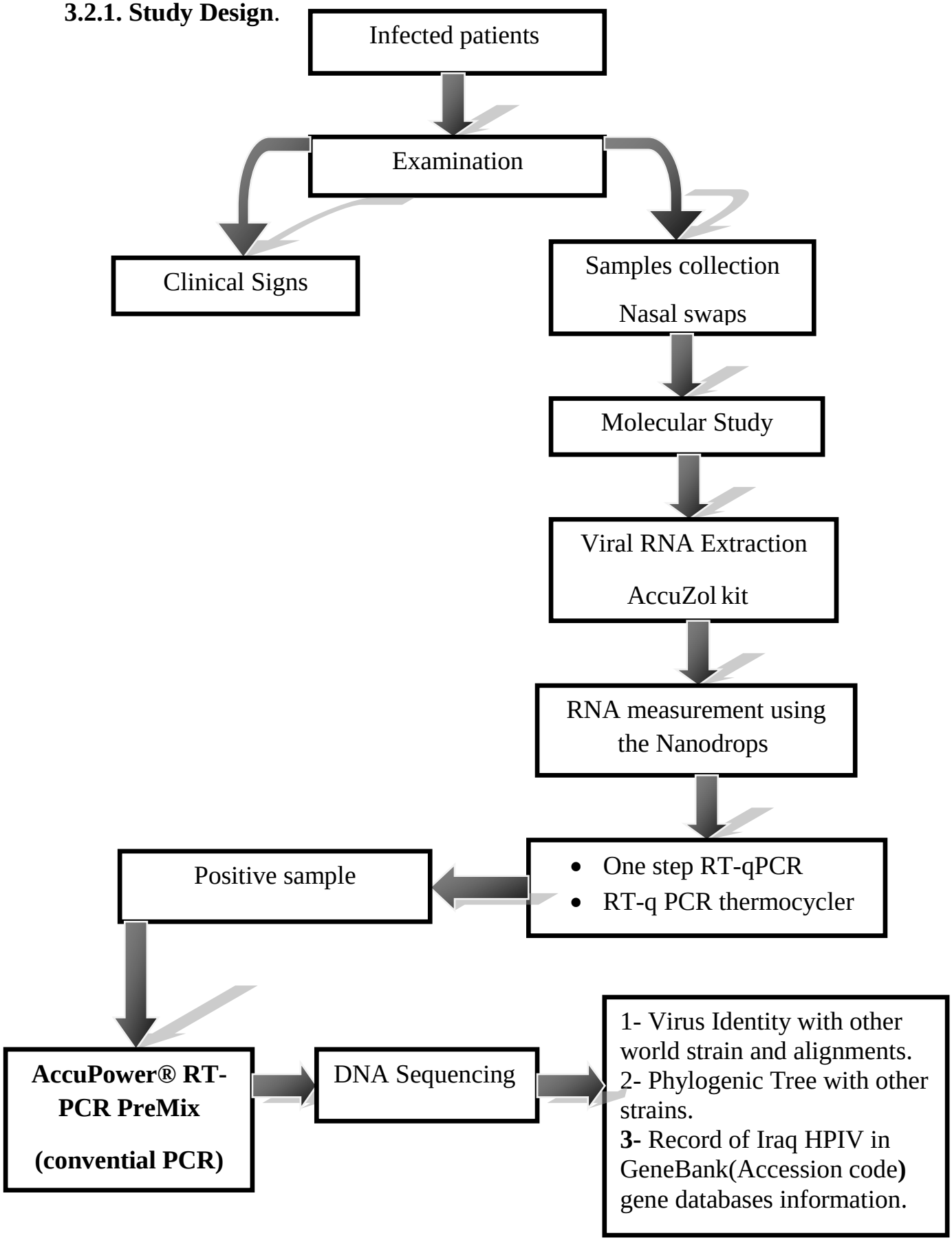
The primers and probe were designed in this study by using the complete sequence of Human parainfluenza virus nucleoprotein (*NP)gene* (GenBank: EU346886.1) from NCBI-Genbank and Primer3 plus design. These primers and probe were used in Real-Time PCR assay for rapid detection of Human parainfluenza virus. Also another PCR primers for nucleoprotein (*NP) gene* were used in end point PCR amplification that used in DNA sequence method for genotyping study based phylogenetic tree analysis table (3-4).

Table (3-4) : Primers and probe used for molecular diagnosis .

Primer	Sequence		Amplicon
NP-qPCR primers For RT-qPCR	F	5- ACTGGAAGCACGGAAAGAAG-3	85bp
	R	5- TTGTTGGTGAGCTTGTTGCC-3	
NP-qPCR probe	5-FAM-TGAGCTGGAGACATCCACAGCCA-BHQ1-3		
NP- PCR Primers For end point PCR	F	5-GCCCGAGTGTGACAGATGAT-3	523bp
	R	5-GTGTCTCCCGTGAAGACCAG-3	

3.2. Methods.

3.2.1. Study Design.



3.2.2. Clinical examination.

Clinical examination was conducted by special physician , singe as fever , cough , runny nose, chest pain, sore throat, shortness of breath, wheezing, general breathing difficulty were considered(**Ellis** , 2015) (**López** , 2009). Data patient recorded in applicant form designed for this purpose include, age, gender, address, data and clinical signs. Written information consents was obtained from parents of patients for publication of this study.

3.2.3. Samples collection.

Three hundred nasopharyngeal specimens were collected from January 2015 to march 2015 from Rumaitha General Hospital and maternity and children Hospital in AL-Muthanna from preschool children and infant whom were hospitalized with acute respiratory infections .

Fresh nasopharyngeal specimens were collected from patients by using sterile cotton swap and kept in transport medium and transferred as soon as possible to the laboratory by cooled box .

3.2.4. Viral RNA extraction

Viral RNA was extracted from nasopharyngeal fluid swab for 96 samples by using AccuZol^{TM} Total RNA extraction kit and done according to company instructions as following steps:

1. A 0.5 ml nasopharyngeal fluid sample was transferred by sterile pipette into sterile and clean 1.5ml eppendorf tube, then 1ml of Accuzol reagent an mixed by vortex.
2. A volume of two hundred µl chloroform were added to each eppendorf tube and mixed vigorously for 30 seconds.
3. The mixture was incubated on ice for 5 minutes. After that, the mixture was centrifuged at 12000 rpm, 4C°, for 15 minutes.
4. Supernatant was then transferred to a new Eppendorf tube, and a volume 500µl isopropanol was added.

5. The mixture mixed by inverting the tube 4-5 times and incubated at 4C° for 10 minutes.
6. The mixture was centrifuged at 12,000 rpm, at 4C°, for 10 minutes.
7. The supernatant was discarded.
8. Eighty percentage Ethanol was added into each tube and mixed by vortex, then centrifuged at 12,000 rpm, 4C° for 5 minutes.
9. The supernatant was discarded and the RNA pellet left to dry at room temperature for 5 minutes.
10. After that, DEPC water (50µl) was added to RNA pellet tubes and mixed by vortex to dissolved the RNA pellet.

12.The extracted RNA sample was kept at -20c freezers

3.2.5. RNA extraction profile.

The extracted RNA was checked by using Nanodrop spectrophotometer that check DNA concentration and estimation of DNA purity through reading the absorbance in at (260 /280 nm) as following steps **(Turner et al., 2005)** .

1. After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, RNA).
2. A dry Chem-wipe was taken and cleaned the measurement pedestals several times. Then carefully pipette 2µl of ddH_2O onto the surface of the lower measurement pedestal.
3. The sampling arm was lowered and clicking OK to blank the Nanodrop, then cleaning off the pedestals.
4. After that, the pedestals are cleaned and pipette 1µl of RNA sample for measurement.

3.2.6. Reverse Transcription Real-Time PCR

It was performed for detection of human parainfluenza virus by using the primers and TaqMan probe specific for nucleoprotein (*NP*)*gene* and this technique was carried out according to method described by Lassaunière**(2010**).

3.2.6.1. RT-Real-Time PCR master mix preparation

RT-Real-Time PCR master mix was prepared by one step Reverse Transcription and Real-Time PCR detection kit (AccuPower RocketScript RT-qPCR PreMix), and done according to company instructions as following table:

Table (3-5): Component of RT-qPCR master mix .

RT-qPCR master mix	Volume
Total RNA	10µL (5-50 ng\20ml)
Forward NP gene primer (20pmol)	2µL
Reverse NP gene primer (20pmol)	2µL
TaqMan NP gene probe (25pmol)	2µL
DEPC water	34 µL
Total	50µL

The RT-qPCR master mix reaction components that mentioned in table (3-5) were added into standard qPCR tube containing (8 wells strips tubes which were contained RocketScript reverse transcriptase and TaqMan probe premix). Then all strips tubes vortex for mixed the components and centrifuge at 3000rpm for 3 minutes in Exispin centrifuge, after that transferred into Real-Time PCR thermocycler.

3.2.6.2. Real-Time PCR Thermocycler conditions

Real-Time PCR thermocycler conditions was set according to primer annealing temperature and RT-qPCR TaqMan kit instructions table (3-6).

Table (3-6): Real-Time PCR Thermocycler conditions .

Step	Condition	Cycle
Reverse transcription	50 °C 15 min	1
Pre-Denaturation	95 °C 5 min	1
Denaturation	95 °C 20 sec	50
Annealing/Extension	60 °C 30 sec	
Detection (Scan)		

3.2.6.3. Real-Time PCR Data analysis.

RT-qPCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification of Human parainfluenza virus *NP gene*. **(Yuan ,*et al*,. 2006).**

3.2.7. Convential PCR method

RT-PCR method was performed for amplification of human parainfluenza virus nucleoprotein *(NP) gene* in positive samples of real-time PCR, these PCR products were used in RNA sequence method as following steps:

3.2.7.1. Convential PCR master mix preparation

PCR master mix was prepared by using (AccuPower® RT-PCR PreMix Kit) and this master mix done according to company instructions table (3-7) :

Table (3-7): Component of PCR master mix preparation .

PCR Master mix	Volume
RNA template	5µL (5-50 ng\20ml)
NP gene Forward primer (10pmol)	0.0015 ml
NP gene Reveres primer (10pmol)	0.0015 ml
PCR water	0.012 ml
Total volume	0.02 ml

The PCR master mix components that mentioned above placed in standard AccuPower PCR PreMix Kit which was contained all other components those were for RT-PCR reaction such as (RT enzyme, RT buffer, Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$,stabilizer, and tracking dye) .

Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (MyGene).

3.2.7.2. Convential PCR Thermocycler Conditions

PCR thermocycler conditions for detection of *NPgene* were done by using convential PCR thermocycler system table (3-8):

Table (3-8) : PCR Thermocycler Conditions.

PCR step	Temp.	Time	Repeat
cDNA synthesis	42	1hours	1
Denaturation	95 ^{o}C	5min	1

Denaturation	95 ^{o}C	30sec.	30 cycle
Annealing	60 ^{o}C	30sec	
Extension	72 ^{o}C	1min	
Final extension	72 ^{o}C	5min	1
Hold	4 ^{o}C	Forever	-

3.2.7.3. End point PCR product analysis.

The PCR products of *NP gene* was analyzed by agarose gel electrophoresis following steps **(Lee , *et al*,. 2012)**

1- one and half percent Agarose was prepared by using 1X TBE and dissolving in water bath at 100 °C for 15 minutes, after that, left to cool 50°C.

2- Then 3µ of ethidium bromide stain were added to agarose gel solution.

3- Agarose solution was poured in tray after fixed the comb in proper position after that, left to solidified for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added into each comb well and 5 µl of (100bp Ladder) in one well.

4- The gel tray was fixed in electrophoresis chamber and filled by 1X TBE buffer. Then electric current was performed at 100 volt for 1hour.

5- PCR products (523bp) as specific for *NP gene* were visualized by using UV Transilluminator.

3.2.8. DNA sequencing method.

Ten positive PCR products were chosen for DNA sequencing method *NP gene* of local *HPIV* 523 bp by DNA sequencing system , by Bioneer company in Korea.

The sequencing of the PCR product (cDNA) of NP gene was performed by using Dye-terminator sequencing method.

3.2.9. Nucleic sequence and geneBank submitting

Ten sequenced isolate of local *HPIV* of this study were submitted to NCBI- GeneBank for recording and published global and for taking specific accession number to prepare this sequenced clone in phylogenetic analysis and phylogenetic tree construction .

3.2.10. Genomic characterization of HPIV 1, 3

The evolutionary history was inferred using the Neighbor-Joining method **(Saitou and Nei , 1987).** The optimal tree with the sum of branch length = 0.09636524 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates **(Felsenstein , 1985).** The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree . The evolutionary distances were computed using the Tamura 3-parameter method **(Tamura , 1992)** and are in the units of the number of base substitutions per site. The analysis involved 18 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 472 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 **(Tamura *et al*., 2013)** .

Phylogenetic tree was built by using neighbor joining method by application of MEGA-6 according to (Tamura , 2007) . Phylogenetic tree were inferred with distance parsimony and maximum likelihood methods, the reliability of the tree was determined by sets bootstrap resembling method

A sequence similarity search often provides the first information about a new DNA or protein sequence. A search allows scientists to infer the function of a sequence from similar sequences. There are many ways of performing a sequence similarity search, but probably the most popular method is the "Basic Local Alignment Search Tool" (BLAST) **(Madden ,2013)**.

3.3. Statistics analysis

Data were statically analyzed ANOVA and Chi-square (x^2), and to find out whether there was significant differences by using Duncan multiple test by using statistical program SPSS (2008).

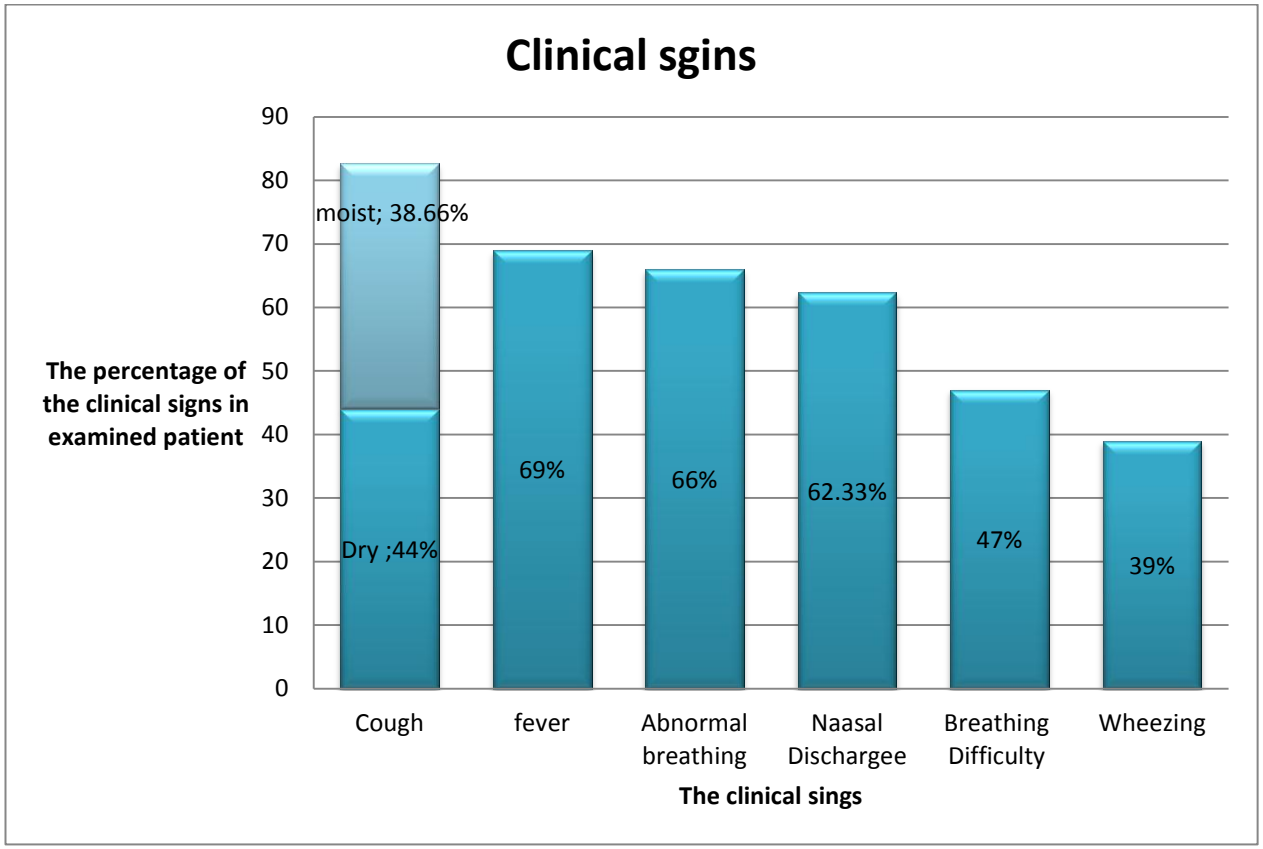
Chapter Four

Results

4. Results

4.1. Clinical study

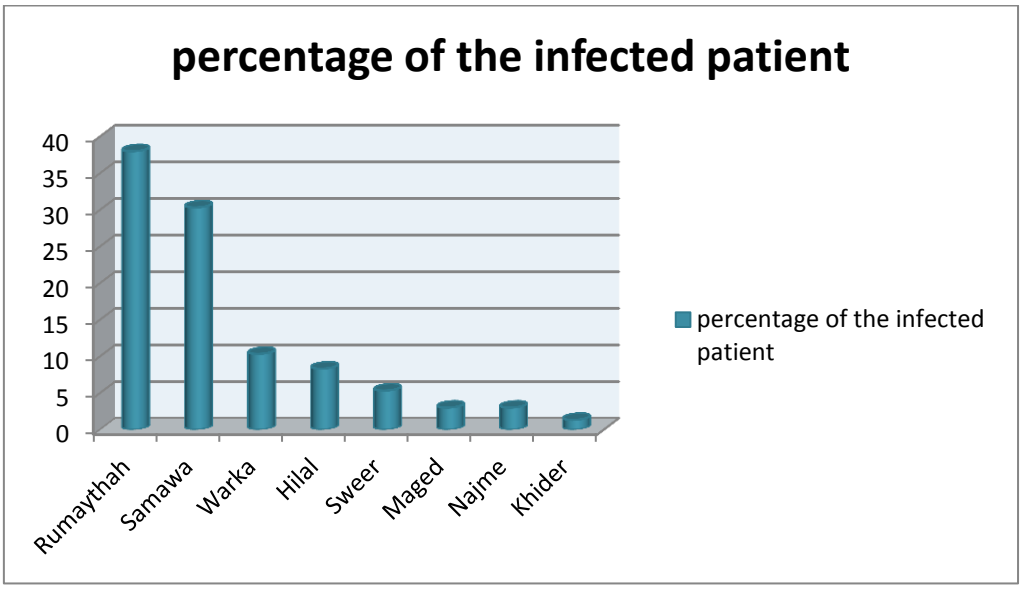
The clinical features of a cut respiratory associated hospitalized pediatric patients were studied to analyze the clinical presentation of infection . The results showed that respiratory distress , runny nose , moderate fever , with moist or dry cough. In severe cases there were sore through and wheezing and the patients were needed an oxygen supply. Some patients were also associate with bronchiolitis ,pneumonia and asthma exacerbation More than one clinical sign occurred at the same time in many cases. , figure (4-1).



(**Figure 4-1):** The percent of clinical signs in examined patient .

4.2. ARI distribution according to the regions

The resultrs of acut respiratory infection rate distribution acoording to different study area in AL-Muthanna showed that high infection rate in Rumaythah with 38% and the loset infection rate in Khider with 1.33 % ,figure (4-2).



(Figure 4-2): Results of ARI distribution according to the study area

The total infection rate of both male and female were 54.33% for male and 45.66% for female Figure (4-3).

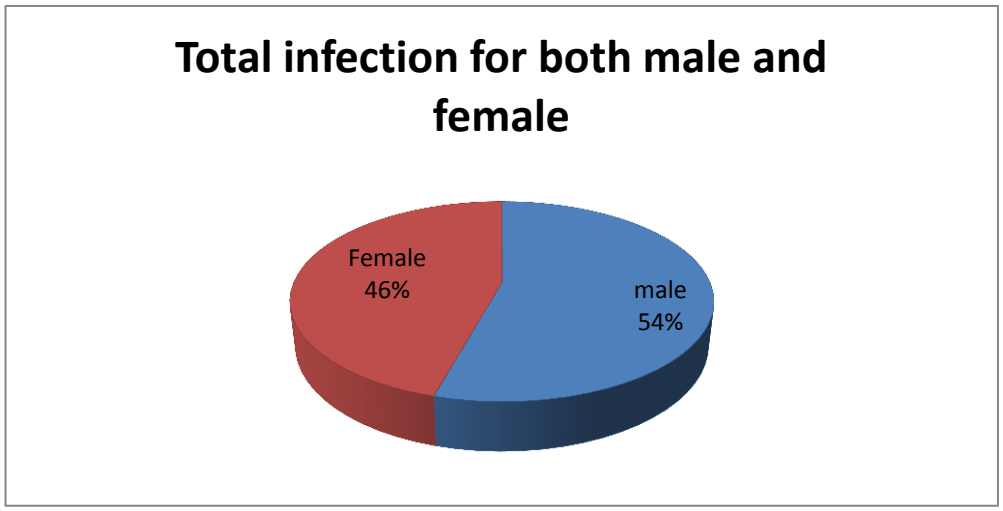


Figure (4-3): Results of the total infection rate of both male and female .

4.3. Molecular detection using (RT-qPCR)

The results of *HPIV* detection by using RT-qPCR technique showed that the total percentage of infection rate was 45.38% from the 96 sample that was examined by molecular technique . Figure (4-4), Figure (4-5) .

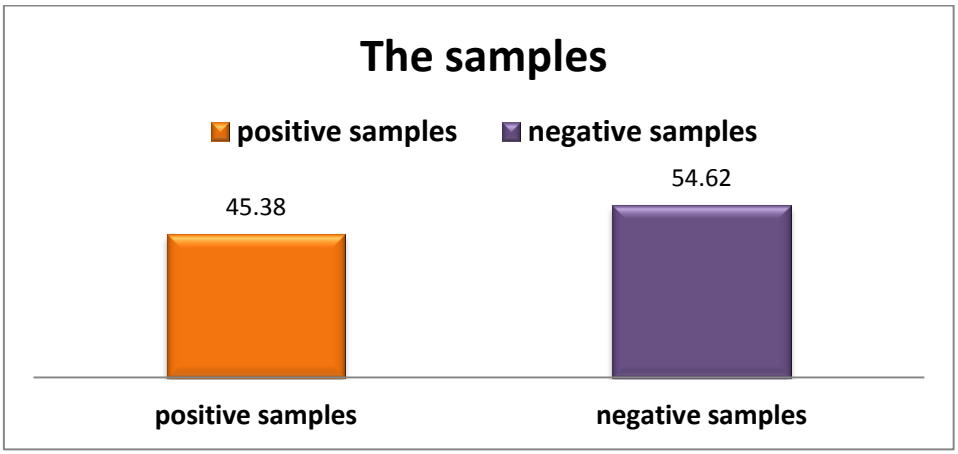


Figure (4-4): Results of molecular test using RT-qPCR **.**

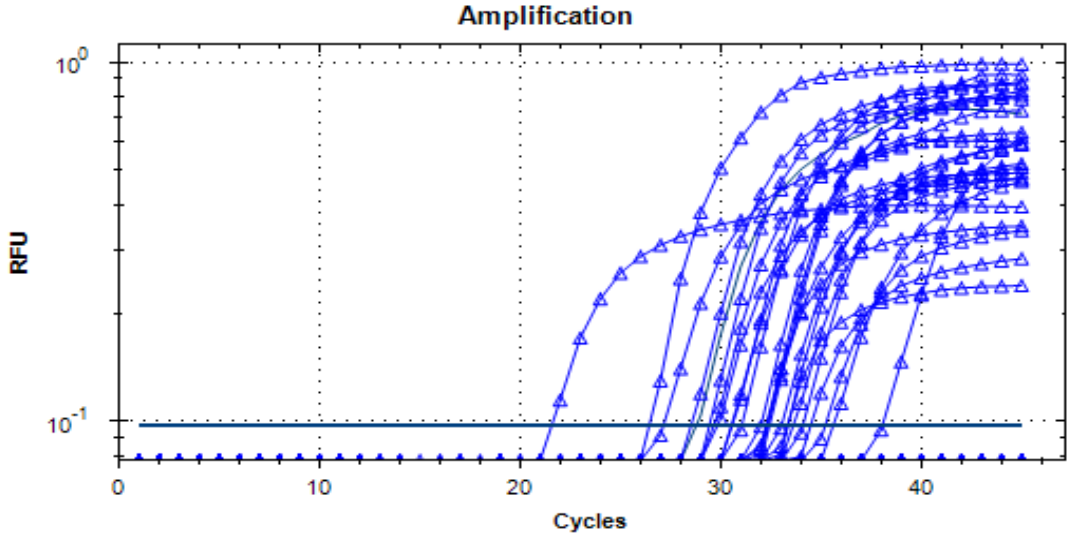


Figure (4-5): Real-Time PCR amplification log plot that showed result of *HPIV* for nucleoprotein *(NP) gene* of *HPIV* , that showed cycles of positive results ranged from CT:21.59 to CT:38.05.

4.3.1. Infection rate according to the gender using RT-qPCR

The results of infection rate according to the gender showed that the percentage rate of female was 61.11% which was higher than male 36.66% . with significant differences ($p \leq 0.01$) between male and female. Figure (4-6).

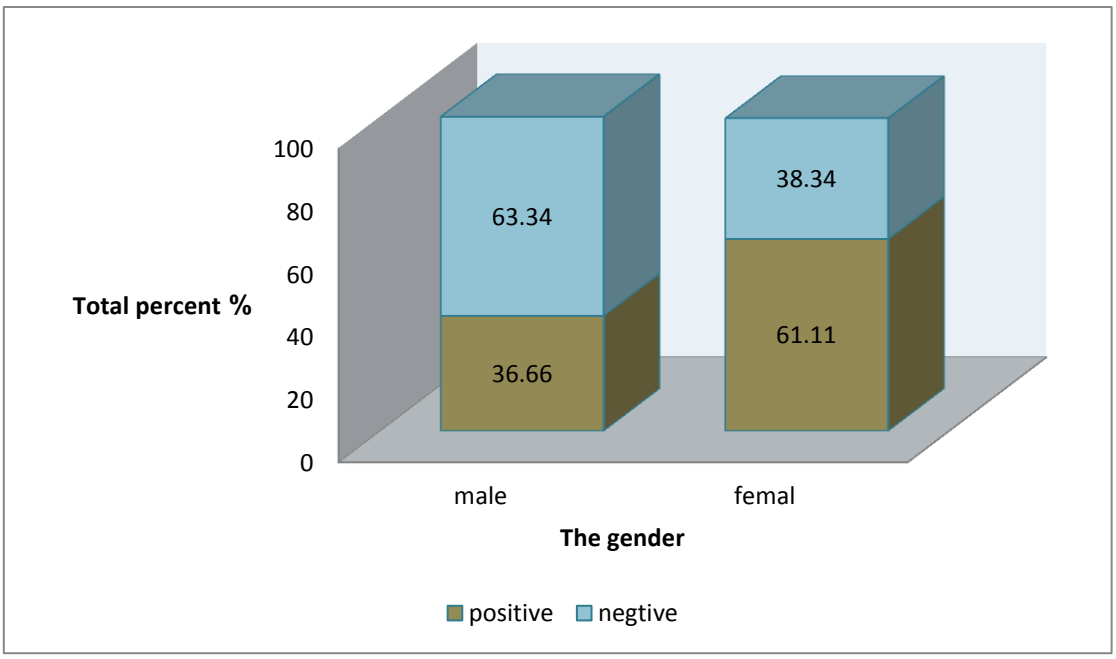


Figure (4-6): Results of infection rate according to the gender.

4.3.2. Infection rate according to the study regions by using RT-qPCR .

The results of infection rate by using RT-qPCR according to regions of study (58.3% , 50.1% , 47.5% , 38.5% , 33.3% , 25% , 0% , 0%) in Samawa, Hilal , Rumaythah , Warka , Sweer, Najme, Maged, and Khider respectively .Samawa showed the highest infection rate 58.3% , while Najme was the lowest 25% with significant differences ($P \leq 0.01$) .Maged and Khider were not recorded any positive results.

There significant differences between Samawa and other regions at ($p \leq 0.01$) . There was no significant differences between Rumaythah and Hilal at ($p \leq 0.01$) . There was significant differences between Warka ,Sweer and Najme .

And there was no significant differences between Maged and Khider and significant differences at ($p \leq 0.01$) with other regions . figure (4-7).

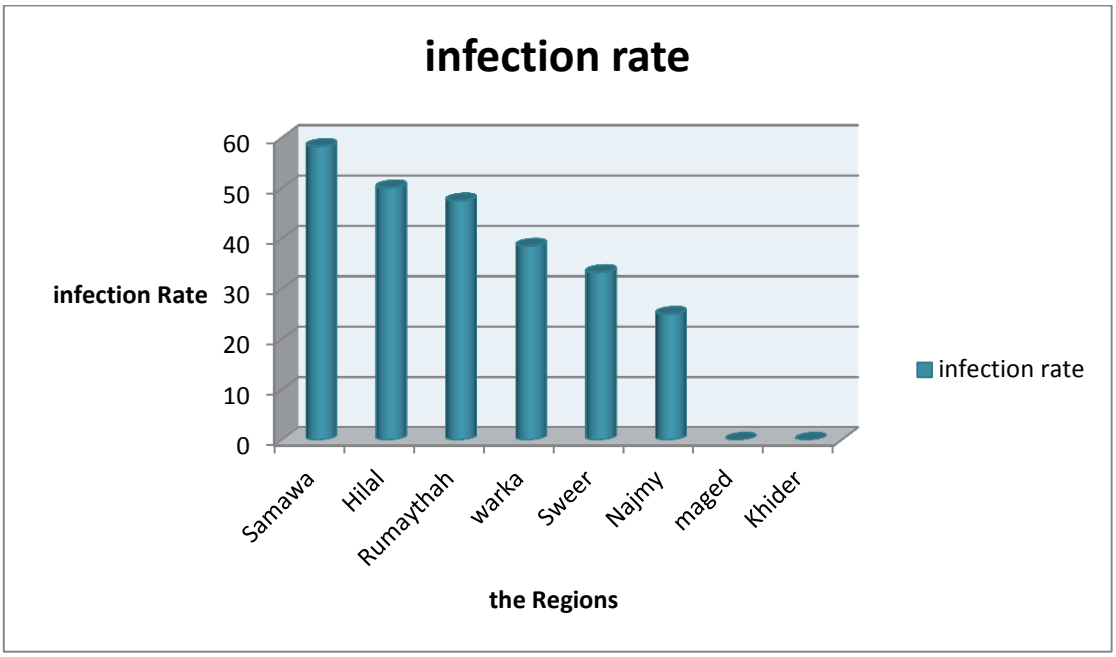


Figure (4-7): The Results of infection rate according to the study regions by using RT-qPCR.

4.3.3. Infection rate according to the age groups by using RT-qPCR .

The results of infection rate according to the age groups in (less than 1 year, More than 1-2 years , More than 2-3 years, More than 3-4 years ,and More than 4-6 years) were (49% , 38% , 36.4% , 66.6% ,and 40.1%) respectively.

The results showed that the highest rate of infection 66.6% were in the age group more than 3-4 years old. While the lowest infection rate 36.4% was in the age group more than 2-3 years old .

there was significant differences between age group (More than 3- years old)and other age group at ($p \leq 0.01$) . There was significant differences between age group (less than 1 years old) and other age group at ($p \leq 0.01$) . there were no significant differences between age group (more than 1-2 years old) and (more than 2-3 years old) and (more than 4-6 years old) at ($p \leq 0.01$) . figure (4-8)

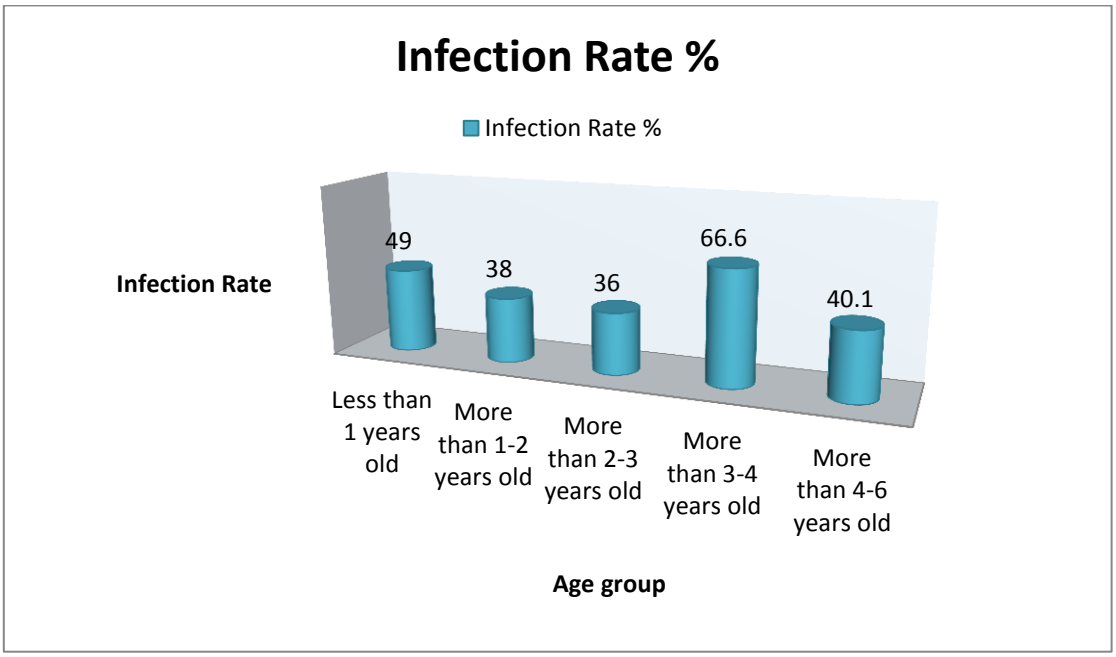
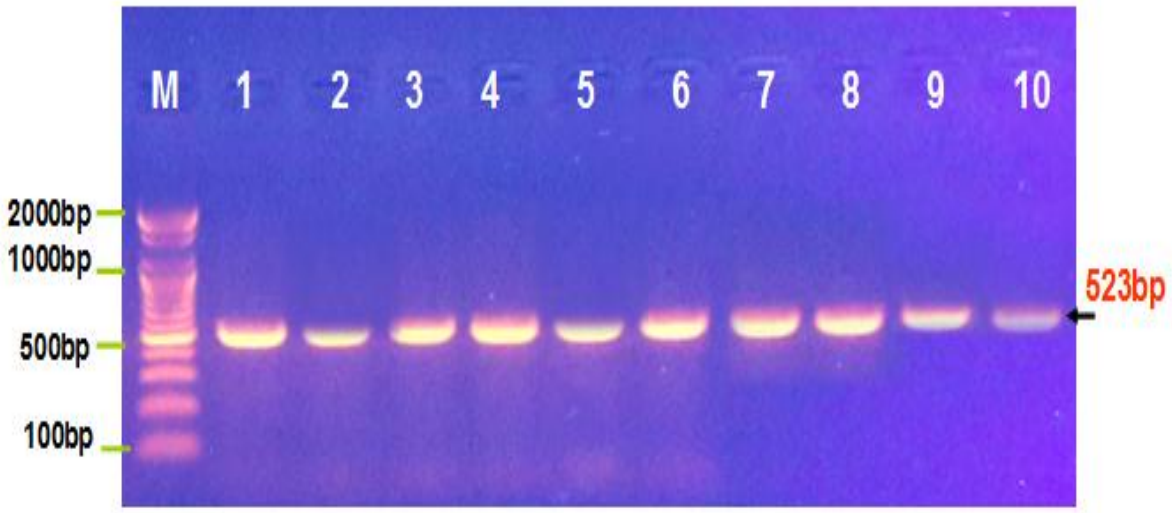


Figure (4-8): The results of infection rate according to the age group by using RT-qPCR.

4.4. Conventional PCR

The results of end point PCR for detection of *HPIV* using specific primers for *(NP) gene* (523bp) ,showed that 10 isolation from the 44 positive RT-qPCR ,gave positive results, figure (4-9).



(Figure 4-9): Agarose gel electrophoresis image that show the RT-PCR product analysis of nucleoprotein *(NP) gene* in *Human parainfluenza virus* clones . Where M: DNA marker (100-

2000bp), lane (1-10) positive *parainfluenza virus* clones for(523bp) of reveres transcript-PCR product.

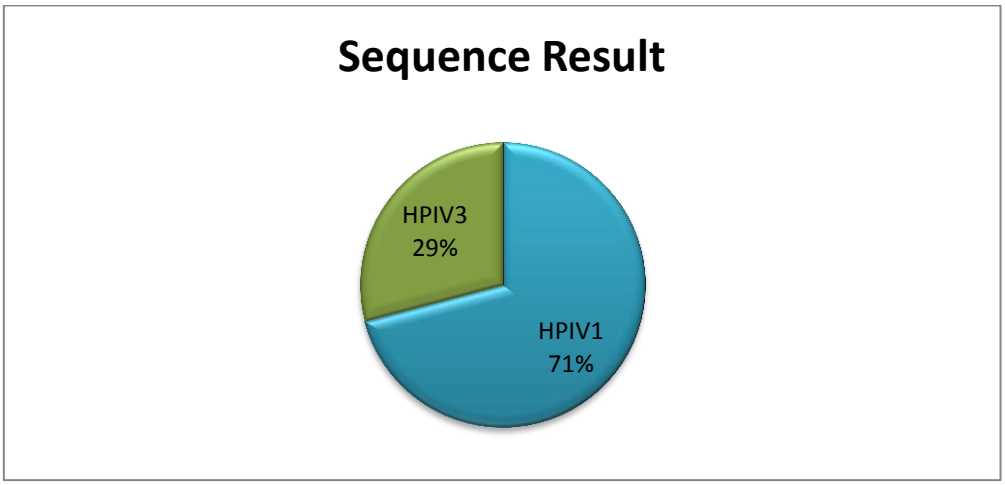
4.5. Sequencing and phylogenetic results

Ten isolate of the this study were sequenced and took the symbol that represent number of clone(seq 1- 10) host and sample source (human , nasal swab) region of sample collation (Samawa), country and organism *HPIV* which were submitted in the GenBank .**Appendix (2).**

4.5.1. GenBank submission .

The results of submission showed that the ten clones of this study were took accession number as **(seq1 BankIt1856417 KT763052), (seq2 BankIt1856417 KT763053), (seq3 BankIt1856417 KT763054) , (seq4 BankIt1856417 KT763055), (seq5 BankIt1856417 KT763056), (seq6 BankIt1856417 KT763057), (seq7 BankIt1856417 KT763058), (seq8 BankIt1856417 KT763059), (seq9 BankIt1856417 KT763060), (seq10 BankIt1856417 KT763061)** . **Appendix (3,4)**.

The sequence results showed the two type *HPIV-1* and *HPIV-3* are the dominant types in the 10 sample that was sequenced , and the result were among ART infection patients of *HPIV* the major positive results 32.17% were caused by *HPIV-1* while the percentage of infect rate of *HPIV-3* was 13.21% and there were no *HPIV-2* and *HPIV-4* have been detected. Figure(4-10).



(Figure 4-10): Result of the sequence that show the dormant type.

4.5.2. multiple sequence alignment .

The Results of multiple sequence alignment of the ten clones with the GenBank published strain showed that high identity and homology with strain isolated from different area .

Human Parainfluenza virus 1 showed high homology with strain isolated from Lithuania USA , Japan and Thailand. While *HPIV-3* showed high homology with strain isolated from Lithuania, Chile , India , Thailand , Japan , and USA .**Appendix (,5 ,6 ,7 ,8 ,9.10.11,12).** That show the non-homologous regions . Figure (4-11), Figure (4-12).

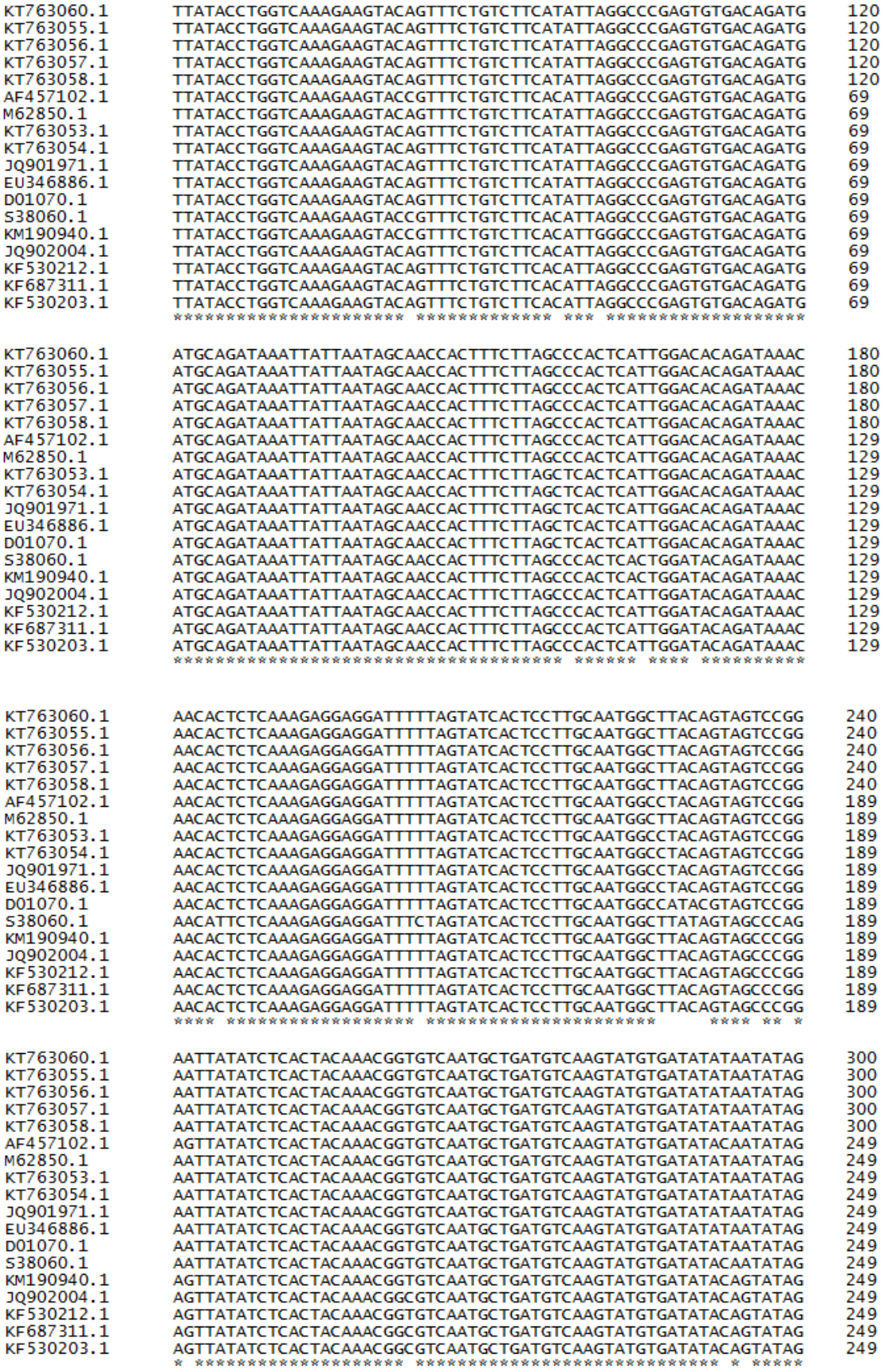


Figure (4-11): Multiple sequence alignment of HPIV-1.

```
KT763061.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    120
M11849.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACGA    64
KT763059.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
KT763052.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
EU346887.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
M14552.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
X04612.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
FJ455842.2      TCCTGGACAGAAAAATACTGTCTCTATATTTGCTCTTGGACCGACAATAACTGATGATAA    64
KM190938.1      CCCTGGACAGAAAAATACTGTCTCTATATTCGCTCTTGGACCAACAATAACCGATGATAA    76
U51116.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA    71
Z11575.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA    64
AB736166.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
KJ672605.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
KF530245.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
                 *********************** ***** ** ******** *******  *****  *

KT763061.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    180
M11849.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
KT763059.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
KT763052.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
EU346887.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
M14552.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
X04612.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
FJ455842.2      TGAGAAAATGACATTAGCTCTTCTTTTTTTATCTCATTCACTGGATAATGAGAAACAACA    124
KM190938.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    136
U51116.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    131
Z11575.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
AB736166.1      TGAGAAAATGACATTGGCCCTTTTGTTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
KJ672605.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
KF530245.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
                *************** ** *** * *** *********** * *****************

KT763061.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    240
M11849.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KT763059.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KT763052.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
EU346887.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
M14552.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
X04612.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
FJ455842.2      TGCACAAAGGGCAGGGTTCCTGGTGTCCTTATTATCAATGGCTTATGCCAATCCAGAGCT    184
KM190938.1      TGCACAAAGAGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAACT    196
U51116.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    191
Z11575.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
AB736166.1      TGCACAAAGAGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KJ672605.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KF530245.1      TGCACAAAGGGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
                ********* ********* ******* ***** *********************** **

KT763061.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    300
M11849.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTAGGAA    244
KT763059.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
KT763052.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
EU346887.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
M14552.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    244
X04612.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    244
FJ455842.2      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA    244
KM190938.1      CTACTTGACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    256
U51116.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    251
Z11575.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
AB736166.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
KJ672605.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA    244
KF530245.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
                 *** * ************************** ** *********** *****   ***
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Figure (4-12): Multiple sequence alignment of HPIV-3

4.5.3. The Sequence and Phylogenetic analysis .

The result of phylogenetic tree analysis showed that our ten clones , **(KT763052 ,KT763053 , KT763054, KT763055 , KT763056 , KT763057 , KT763058 , KT763059 ,KT763060 , KT763061)** cluster with **(AF457102.1, M62850.1, JQ901971.1, EU346886.1, D01070.1, S38060.1, KM190940.1, JQ902004.1, KF530212.1, KF687311.1, KF530203.1)** for HPIV-1 table (4-1).

(M11849.1, EU346887.1, M14552.1, X04612.1, FJ455842.2, KM190938.1, U51116.1, Z11575.1, AB736166.1, KJ672605.1, KF530245.1) . for HPIV-3 table (4-2).

Table (4-1): Results of Sequence and phylogenetic analysis for HPIV-1.

NO.	Strain	Genotype	Origin	Reference	Identical percent %
1	KT763053	HPIV 1	Iraq	This Study	
2	KT763054	HPIV 1	Iraq	This Study	
3	KT763055	HPIV 1	Iraq	This Study	
4	KT763056	HPIV 1	Iraq	This Study	
5	KT763057	HPIV 1	Iraq	This Study	
6	KT763058	HPIV 1	Iraq	This Study	
7	KT763060	HPIV 1	Iraq	This Study	
8	JQ901971.1	HPIV 1	USA	(Beck *et al.*, 2012)	100%
9	EU346886.1	HPIV 1	Lithuania	(Juozapaitis *et al*., 2008)	100%
10	M62850.1	HPIV 1	USA	(Matsuoka, and Ray, 1991)	99%
11	D01070.1	HPIV 1	USA	(Lyn *et al*,. 1991)	99%
12	AF457102.1	HPIV 1	USA	(Newman *et al*., 2002)	98%
13	S38060.1	HPIV 1	Japan	(Miyahara *et al*., 1992)	96%
14	JQ902004.1	HPIV 1	USA	(Beck *et al*., 2012)	96%
15	KF530212.1	HPIV 1	USA	(Lorenzi *et al*., 2013)	96%

16	KF687311.1	HPIV 1	USA	(Lorenzi *et al*., 2013)	96%
17	KF530203.1	HPIV 1	USA	(Lorenzi *et al*., 2013)	96%
18	KM190940.1	HPIV 1	Thailand	(Rutvisuttinunt *et al.*,2015)	95%

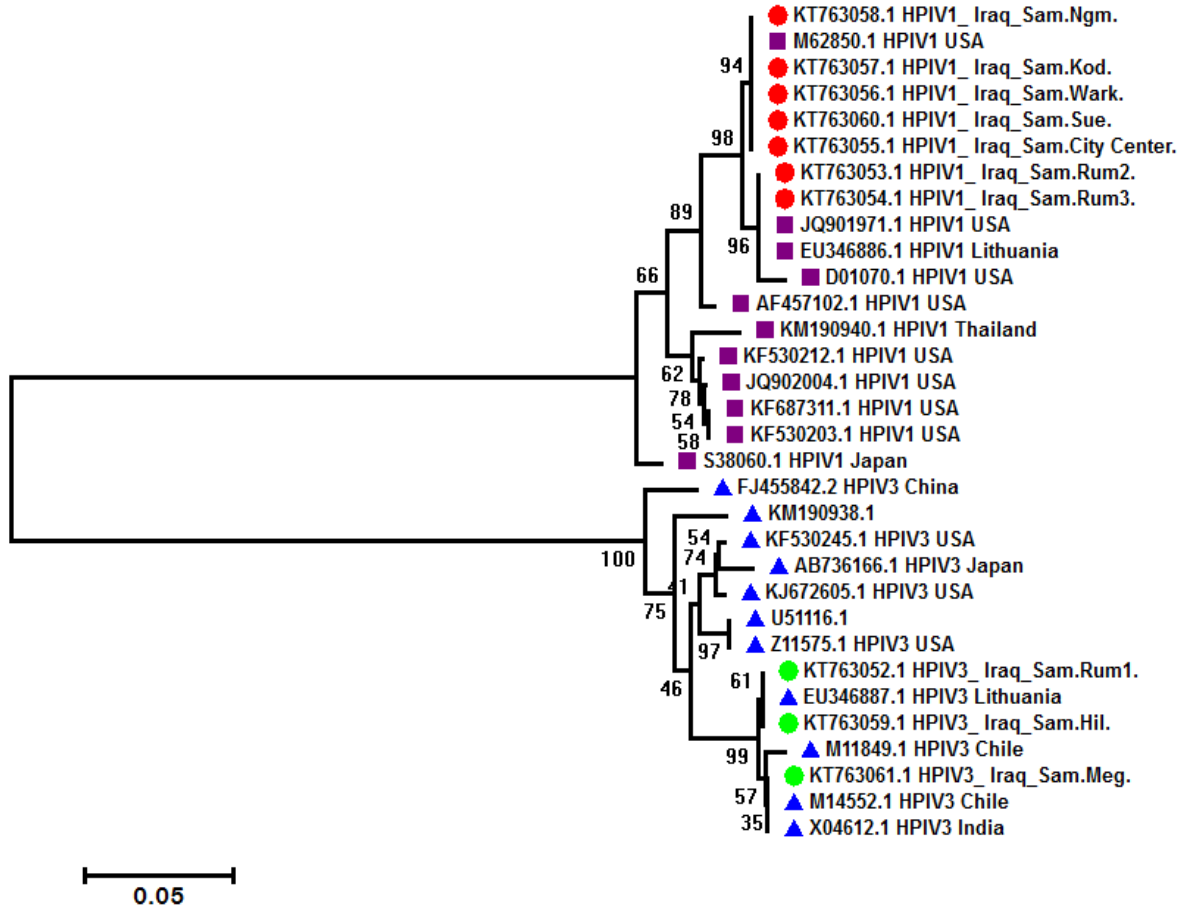
Table (4-2): Results of Sequence and phylogenetic analysis for HPIV-3.

NO.	Strain	Genotype	Origin	Reference	Identical percent %
1	KT763052	HPIV 3	Iraq	This Study	
2	KT763059	HPIV 3	Iraq	This Study	
3	KT763061	HPIV 3	Iraq	This Study	
4	EU346887.1	HPIV 3	Lithuania	(Juozapaitis *et al*., 2008)	100%
5	M11849.1	HPIV 3	Chile	(Galinski et al., 1986)	99%
6	M14552.1	HPIV 3	Chile	(Sanchez *et al*., 1986)	99%
7	X04612.1	HPIV 3	India	(Jambou *et al*., 1986)	99%
8	U51116.1	HPIV 3	USA	(Stokes *et al*., 1993)	96%
9	Z11575.1	HPIV 3	USA	(Stokes *et al.*, 1992)	96%
10	KJ672605.1	HPIV 3	USA	(Wentworth *et al*.,2014)	96%
11	KF530245.1	HPIV 3	USA	(Lorenzi *et al.*, 2013)	96%
12	FJ455842.2	HPIV 3	China	(Yang *et al*.,2011)	95%
13	KM190938.1	HPIV 3	Thailand	(Rutvisuttinunt *et al*., 2015)	95%
14	AB736166.1	HPIV 3	Japan	(Sasaki *et al.*, 2013)	95%

4.5.3.1. Phylogenetic tree analysis.

Our ten clones **(KT763052 ,KT763053 , KT763054, KT763055 , KT763056 , KT763057 , KT763058 , KT763059 ,KT763060 , KT763061)** showed match with different isolate from the world **(AF457102.1, M62850.1, JQ901971.1, D01070.1, JQ902004.1, KF530212.1, KF687311.1, KF530203.1, U51116.1, Z11575.1, KJ672605.1, KF530245.1)** that isolated from USA and **(EU346886.1, EU346887.1)** that isolated from Lithuania . and **(S38060.1, AB736166.1)** that isolated from Japan and **(KM190940.1, KM190938.1)** that isolated from Thailand and **(M11849.1, M14552.1)** that isolated from Chile and **(FJ455842.2)** that isolated from china and **(X04612.1)** that isolated from India .Figure (4-13) .

.



(Figure 4-13): Traditional phylogeny tree using a neighbor – joining method constructed based on *NP gene* of HPIV , general HPIV-1 and HPIV-3.

Chapter Five

Discussion

Parainfluenza viruses cause up to one third of all respiratory infections in infants and preschool children and most often associated with Laryngeotrachitis (Croup) ,type three usually causes lower respiratory tract infection as bronchiolitis and pneumonia . **(Henrickson, 2003**)

Human parainfluenza virus is very common virus infection which responsible for many cases of bronchiolitis and pneumonia in young children , *HPIV* are important etiologic agent of acute respiratory infection in childhood and rank second among most common diseases in this age group. **(Teo , 2010 ; Karron, and Collins, 2007; Henrickson, 2003)**.

5.1 Clinical and epidemiological features

The results of the clinical examination in different study area showed typical signs of acute respiratory diseases which were (Fever , Dry Cough ,Moist cough, nasal discharge , Abnormal breathing , wheezing, breathing difficulty .(69% , 44%, 38.66%, 62.33%, 66%, 39% , 47%) respectively . The result showed that the most common clinical sign in the respiratory tract infection is the cough with 82,66% divided no dry cough with 44% and moist cough with 38.66% ,in agreement with **(Knutson *et al*.,2002)** who referred that the most commonly observed symptom of respiratory infection beginning within 2 days of infection in 85% of patients.

Marika *et al*.,2004 found that in respiratory infection associated viruses the most common clinical sign was fever as 65% for influenza virus and 38% for other viruses including *HPIV* and *RSV*.

Other signs and symptoms that include dyspnea, wheezing, sputum production, chest pain, fever, is a sign of respiratory infection **(Chesnutt and Prendergast , 2002 ; Mufson ,2000).**

Other study respiratory viruses showed that the most common clinical sign in the respiratory viruses is fever with 65% for *influenza* and 38% for other viruses including *HPIV* and *RSV*, **(Marika *et al*.,2004) .**

All patients showed typical symptoms of respiratory tract infection and more than one clinical signs usually occur in the same case. A study done on *Parainfluenza virus* as a cause of acute respiratory infection in hospitalized children found the following result Cough, Fever ,Shortness of breath , Wheezing , Dyspnea , Cyanosis (8.9% ,82.2%,48.9% , 62.2% , 66.7%, 60% , 8.9%) respectively, which is almost with the same average of our result and showed the cough and fever are the most frequents symptoms (**Rogério *et al*., 2004)** . Fever and cough and other symptoms could be as result of the virus mechanism of infection **(Moscona ,2005)**.

The total infection rate of the acute respiratory tract infection for the pedreatic pateint of both male and female were 54.33% for male and 45.66% for female .The difference of infection rate of ARI according to the gender may be due to variety of etiological agent as *Influenza* , *parainfluenza* , *Respiratory Syncytial Virus* , *Adenovirus* , *Rhinovirus* and other respiratory virus . or could be from co infection of more than on virus or as could be a result from bacteria that causes the respiratory infection and all this etiological agent causes a high percentages of children that infected with acute respiratory tract infection every year and usually frequents infection in one year . It has been reported that boys are more prone to recurrent infections at least during the first years of life **(Kim et al. 2000; Monto et al. 1974) .**

Acute respiratory tract infections are the most common illnesses in childhood, comprising as many as 50% of all illnesses in children less than 5 years old **(Daniel *et al*,.1999) .**

acoording to different study area in AL-Muthanna showed that high infection rate in Rumaythah with 38% and the loset infection rate in Khider with 1.33 % High infection rate in the ARI could be as result of Immunity to HPIVs is incomplete immunity and occurrence of infection throughout life **(Henrickson , 2003).** This differences in the number could be as result of the number of sample that collected from each area or as result of differences in the geographic distribution of the patient . Small cities the countryside with high infection rate of respiratory tract infection could due to the less of health care , poverty of sanitary , shortage of vaccination , the distance between this cities and hospitals ,animal spread and a few health awareness.

We saw that children in the 5 or 6 first years of live are highly effected with respiratory viruses either were female or male The recurrent respiratory infections in infants and children are among the most common

causes of counselling and admission to the hospital. They are responsible for significant morbidity. Many factors can play an important role in the genesis of respiratory tract infection that can act alone or together. In some children, it is possible to detect also transient or permanent immune system deficiencies **(Bellanti, 1997).** It should be pointed, that a true immunodeficiency is rare and the first cause of recurrent respiratory infections is the childhood itself **(Wheeler, 1996),** because both humoral and phagocytic immunity reach their best efficacy during the first fifth or sixth years of age **(Wheeler and Steiner, 1992 ; Yang and Hill, 1991).** Typically, children with recurrent respiratory infections are usually not affected by severe alterations of the immune system functions. The majority of these children do not have recognized immunodeficiency, but some may have low levels of some laboratory parameters, usually of immunoglobulin isotypes or rarely other immunological parameters such as phagocytosis. Some of the observed immunological alterations are of questionable significance and not convincingly related to an increased susceptibility to respiratory infections **(Litzman *et al*., 1999).** Most children with recurrent respiratory infections do not have an immunodeficiency. If they do, this is often due to an antibody deficiency. **(Finocchi *et al*. 2002)** evaluated humoral immune defects in apparently 67 non-atopic patients with recurrent infections and in 55% a humoral defect was diagnosed.

5.2 Molecular study

Revers transcription (RT) followed by convential polymerase chine reaction (PCR) is the technique of choice to detect viral genome extracted from various samples **.(Morrison *et al*., 1998).**

In this study we use Real Time PCR for the diagnosis of *HPIV* Real-time reverse transcription polymerase chain reaction (RT-PCR) which is a commonly used technique. There are types of Real Time PCR , one step and two steps . in our study we use one step to avoid contamination and for less handling and reducing pipetting errors.. **(Wacker and Michael 2005).**

The results of the present study was used Real Time-PCR for the detection of *NP* gene of *HPIV*. RT-PCR indicated rapidity .(523bp) HPIVs amplification plot showed different positive reaction cycle of threshold (CT) and this started at (CT 21.59 to CT 38.05) in the extracted RNA that collected from the patients , and that showed a high specificity for the detection of *NP* gene . End point PCR that done after RT-PCR is considered confirmatory test to the RT-PCR. The PCR products of *NP gene* was analyzed by agarose gel electrophoresis .Ten positive PCR products were chosen for DNA sequencing method for local *HPIV NP gene* 523 bp by DNA sequencing system **.(Bouda et al.,2000)**

The results of *HPIV* detection by using RT-qPCR technique showed that the total percentage of infection rate in *HPIV* was 45.38%.

In a study done to rapid detection for respiratory viruses in the USA found that *RSV* followed by *HPIVS* and followed by *Influenza*, **(Jiang *et al*.,1997).**

The results showed differences in the infection rate between male and female . The infection rate of female was 61.11% is more than male which was 36.66% with significant differences between male and female, female is more sensitive than male for in infection in *HPIV* . In another study that have been done and showed that females are infected with 44% with HPIV **(Rogério *et al*., 2015).** that could be due to biological reason, the number of administrated patients in the hospitals , and female are tend to be more infected with diseases than male.

A review of mortality differentials for males and females aged 1–5 years from 82 developing countries found evidence of higher childhood mortality in females in south-central Asia, northern Africa and western Asia, China, Guatemala and Niger, and to a lesser extent in Burundi, Namibia and Togo. Research has found some differences between the immune systems of boys and girls that seem to confer an immunological advantage to girls, but neither the biological mechanisms involved nor the relative importance of these differences in determining morbidity and mortality have been fully identified **((WHO,2007 ; Population Division, 1998).**

The results of HPIV rates in different regions were (58.3% , 50.1% , 47.5% , 38.5% , , 33.3 %, 25% , and 50.1% , 0%) in (Samawa , Hilal , Rumaythah, Warka , Sweer , Najme , Maged and Khider) respectively

The highest rates were in Samawa 58.3% while the lowest one were in Maged and Khider 0% statically , there was significant differences in ($p>0.01$) between Samawa and other regions . the highest number were in Samawa and Rumaythah that could be due to that both of these cities are the one with the biggest area and with the biggest population and more crowded than the other region and the *HPIV* transmission done through the large droplet and direct contact with the infected people and surfaces and through hands , and the *HPIV* cannot survive for a long time in the air that could be the reasons why the biggest city are with the highest infection rate .

The result of infection rate according to the age group in (less than 1 year, More than 1-2 years , More than 2-3 years, More than 2-3 years , More than 3-4 years , More than 4-6 years) were (49% , 38% , 36.4% , 66.6% , 40.1%) respectively . The results showed that the highest rate of infection 66.6% were in the age group more than 3-4 years old. there was significant differences among the following age group less than 1 years - More than 1-2 years - More than 3-4 years - More than 4-6 years, and no significant differences between the following age group More than 1-2 years- More than 2-3 years - More than 4-6 years. In one study, 10% of children aged less than 5 years had at least one *parainfluenza virus* 3 infection **(Reed et al. 1997)** .And the other study that agreed with our study and showed that the higher infection in the same age group (3-5) is **(Holly , 2013 ;Henrickson . 2003; Komada *et al.*, 1990)**. In a study done in Hong Kong and agreed with our study found that children aged from 1to 3 years old were infected with *HPIV-1* were 52.9% , and found that 64% of the patients that were positive for *HPIV-3* were aged one year old and less , and found that that majority of hospitalized children (85.5%) that were infected with *HPIV* less than 5 years of age. **)(Lam Siu Yan, 2007)**
This difference may be attributable to the numbers of our patients who were hospitalized or a difference in the predisposition of children with underlying medical conditions to acquiring *HPIV* at an older age, as a higher proportion of our patients had underlying medical conditions compared with previous reports, or may reflect strain variation.

5.3 Sequencing and genomic study

The sequencing study is considered to be a useful molecular approach in understanding the infectious/contagious virus scenario worldwide. Sequence analysis of virulent of *HPIV*

and its comparison with other *HPIV* sequences in GenBank gave an idea on the molecular epidemiology and emergence of any new variant *HPIV* in the field , on the other hand it is very important in control measurement of the disease in the country.

The results of molecular characterization showed that 10 Iraqi *HPIV* clones of *NP* were sequenced and nucleotide sets were determined.

This study among ARI patients of *HPIV* the major positive results 32.17% were caused by *HPIV-1* while the percentage of infection rate of *HPIV-3* was 13.21% and there were no *HPIV-2* and *HPIV-4* have been detected. And that match to other study that find the *HPIV-1* and *HPIV-3* are with the higher rate of infection as expected, Although all four type causes disease , most significant infection are caused by *HPIV-1* and *HPIV-3* . *HPIV-1* is the major cause of the syndrome referred to as croup **(Mao *et al*., 2012 ;Jose , 2000 ; Tellez , 1990)**

In comparing with other study we found that the *HPIV-1* is the more frequents in discrepancy to other study that showed that the *HPIV-3* is the most etiological agent **(Calvo *et al* ., 2011)**. It is known that *HPIV-3* is the *HPIV* type most frequently detected in hospitalized patients, particularly among young infants, and associated with the bronchiolitis and pneumonia **(Sato , 2006).** In another study done in Iraq in AL-Najaf governorate found that *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 **(Mahdi , 2014).**

Possible explanations for these phenomena include nonspecific viral interference **(Glezen and Denny , 1973)** or heterotypic cross-protection of antibodies. It is possible that prior infection might infer some protection against infection from the other serotypes during that season. Several studies also suggested that seasonal increases in *HPIV* activity may be associated with increased risk of nosocomial transmission **(Apalsch, *et a.,l* 1995 ; Heidemann , 1992).**

In the results of multiple sequence alignment among Iraqi isolate of this study showed high identity , and the identity result were the major positive results caused by *HPIV-1* and *HPIV-3* ,other study that also showed that the predominate type is *HPIV-1* and *HPIV-3* is **(Denny and, Clyde , 1986)** and there were no *HPIV-2* and *HPIV-4* have been

detected. The low level of *HPIV-2* seen in our study population is consistent with global epidemiologic data **(Hsieh *et al*., 2010; Counihan *et al*., 2001) .**

These results of sequencing were important because these information was used in the submission of all data of every sample of this study to GeneBank databases information for recording and publishing isolates of this study.

GenBank have provided accession numbers for the ten clones of this study**(KT763052 ,KT763053 ,KT763054 ,KT763055 ,KT763056 ,KT763057 ,KT763058, KT763059 ,KT763060 ,KT763061).** Results of GeneBank submissions and recording strains of this study helped in the phylogenic analysis by using phylogenic analyses steps . First step of analyses by alignment of all isolates of this study with other world selected reference *HPIV* strains by using online (Clustal W2) program . This program demonstrated had accurate degree of the identity with all world strains including the isolates of this study. The results of phylogeny tree and sequences analysis of our isolates under accession numbers **(KT763053 ,KT763054 ,KT763055 ,KT763056 ,KT763057 ,KT763058,KT763060)** which were matched to *HPIV-1* .

(KT763055 ,KT763056 ,KT763057 ,KT763058,KT763060) showed the highest homology with world strains as **(M62850.1)** *HPIV* strain isolated from USA in the same branch with (99%), while (**KT763053 ,KT763054**)were showed highest homology with (**JQ901971.1 , and D01070.1)** isolated from (USA) with (100% and 99%) respectively, and **(EU346886.1)** that isolated from (Lithuania) in the same branch with (100%). However , some of our isolates had high relationship with other world strains in the same phylogeny tree and located in the second tree branch as (**S38060.1)** which isolated from Japan with (96%) **, (KM190940.1)** which isolated from Thailand with (95%) and **(JQ902004.1, KF530212.1, KF687311.1, KF530203.1)** with (96%), **AF457102.1)** with (98%) which isolated from USA .

While our three clones **(KT763052, KT763059 , KT763061)** were matched with *HPIV-3.* .

(KT763061) showed the highest homology with other world strains as **(M11849.1, M14552.1)** that isolated from Chile with(99%), **(X04612.1**) that isolated from India with

(99%).(**KT763052, KT763059)** showed the highest homology with other world strains as **(EU346887.1)** that isolated from(Lithuania) with (100%).

some of our isolates had high relationship with other world strains in the same phylogeny tree and located in the second tree branch as **(FJ455842.2)** that isolated from China with(95%) **, (KM190938.1)** that isolated from Thailand with (95%) **,(AB736166.1)** that isolated from Japan with (95%),and **(U51116.1, Z11575.1, KF530245.1**,**KJ672605.1**) , that isolated from USA with (96%) .

Chapter Six

Conclusions and Recommendations

6.1. Conclusions

1- Human Parainfluenza was considered as one of the most important causative agent of a cut respiratory infection and young children in Muthanna province.

2- Human parainfluenza infection showed characteristic clinical and epidemiological features , ranging from mild to severe form in different age groups.

3- Nucleotides sets alignment for *NP* gene of Iraqi HPIVs strains of this study showed a high degree of homology and identity with close relationship among other world reference published strains.

4- Human Parainfluenza 1 followed by *HPIV-3* were the predominant genotypes recorded in different areas of the study .

5- Phylogenetic analysis permitted the arrangement of Iraqi strains of the current study with some other world lineages as USA , Lithuania ,India and Chile.

6.2. Recommendations

1- HPIVs infection must be included in the treatment and application of prevention and control measures of acute respiratory infection in young children and infection and infants in Iraqi as widely distributed and high infection rate.

2- The predominate genotypes which are *HPIV-1* and *HPIV-2* must be regarded in immunization and vaccination programs .

3- Further phylogenetic analysis of Iraqi strains is essential to determine the ancestral origin and evolutionary pathway of circulating strains to confirm the existence of distinct Iraqi lineage .

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Appendixes

Appendix (1) : Examination card for The general databases of the *Parainfluenza virus* in this study .

<table>
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<tr><td></td><td colspan="2"></td><td></td><td></td><td></td><td colspan="2"></td></tr>
<tr><th colspan="8">Physical Exam</th></tr>
<tr><th rowspan="2">Temp</th><th colspan="2">Cough</th><th rowspan="2">Nasal Discharge</th><th rowspan="2">Wheezing</th><th rowspan="2">Abnormal breathing</th><th rowspan="2" colspan="2">Breathing Difficulty</th></tr>
<tr><th>Dry</th><th>Moist</th></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td><td colspan="2"></td></tr>
<tr><th colspan="2">Notes</th><td colspan="6"></td></tr>
</table>

Appendix (2) : Gene submission sequence.

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  ORGANISM  Human para-influenza virus
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  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

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            Assembly Name         :: hh
            Sequencing Technology :: Illumina
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      361 tgcagaacgg cagaaacaat tcaacgattg aagatcttgt tcacacattt gggtatccat
      421 catgtttagg agctcttata atacagatct ggatagtttt ggtcaaagcc atcactagca
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```

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SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
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REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

            ##Assembly-Data-START##
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            Assembly Name         :: hh
            Sequencing Technology :: Illumina
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                     /isolation_source="Nasal Swap"
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      121 aacatgcaca aagggcaggg ttcttggtgt ctttattgtc aatggcttat gccaatccag
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      241 agaaagatct aaaacggcaa aagtatggag gatttgtggt taagacgaga gagatgatat
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      361 tgcagaacgg cagaaacaat tcaacgattg aagatcttgt tcacacattt gggtatccat
      421 catgtttagg agctcttata atacagatct ggatagtttt ggtcaaagcc atcactagca
      481 tctcagggtt aagaaaaggc tttttcactc gattagaggc ttt
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KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 525)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 525)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

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            Assembly Name         :: hh
            Sequencing Technology :: Illumina
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
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       61 tgacagatga tgcagataaa ttattaatag caaccacttt cttagctcac tcattggaca
      121 cagataaaca acactctcaa agaggaggat ttttagtatc actccttgca atggcctaca
      181 gtagtccgga attatatctc actacaaacg gtgtcaatgc tgatgtcaag tatgtgatat
      241 ataatataga gagagatcct aaaagaacaa aaacagatgg gttcattgtc aaaacgagag
      301 acatggagta tgaaagaacc acagagtggt tgtttggacc tatgatcaat aagaatccat
      361 tgttccaagg gcaaagagag aatgcggatc tagaggcatt gcttcagaca tatggatatc
      421 ctgcatgtct cggagctata atagttcaag tttggatagt gttggtcaaa gccataacaa
      481 gtagtgctgg tctaagaaaa ggattcttca atagattaga agcat
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LOCUS       seq3                      525 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq3
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 525)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 525)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
            Assembly Name         :: hh
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
BASE COUNT      181 a     88 c    116 g    140 t
ORIGIN
        1 gaggagcaat tatacctggt caaagaagta cagtttctgt cttcatatta ggcccgagtg
       61 tgacagatga tgcagataaa ttattaatag caaccacttt cttagctcac tcattggaca
      121 cagataaaca acactctcaa agaggaggat ttttagtatc actccttgca atggcctaca
      181 gtagtccgga attatatctc actacaaacg gtgtcaatgc tgatgtcaag tatgtgatat
      241 ataatataga gagagatcct aaaagaacaa aaacagatgg gttcattgtc aaaacgagag
      301 acatggagta tgaaagaacc acagagtggt tgtttggacc tatgatcaat aagaatccat
      361 tgttccaagg gcaaagagag aatgcggatc tagaggcatt gcttcagaca tatggatatc
      421 ctgcatgtct cggagctata atagttcaag tttggatagt gttggtcaaa gccataacaa
      481 gtagtgctgg tctaagaaaa ggattcttca atagattaga agcat
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LOCUS       seq4                      523 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq4
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
            Assembly Name         :: hh
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
BASE COUNT      175 a     91 c    118 g    139 t
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      121 atgcagataa attattaata gcaaccactt tcttagccca ctcattggac acagataaac
      181 aacactctca aagaggagga tttttagtat cactccttgc aatggcttac agtagtccgg
      241 aattatatct cactacaaac ggtgtcaatg ctgatgtcaa gtatgtgata tataatatag
      301 agagagatcc taaaagaaca aaaacagatg ggttcattgt caaaacgaga gacatggagt
      361 atgaaagaac cacagagtgg ttgtttggac ctatgattaa caagaatcca ttgttccaag
      421 ggcaaagaga gaatgcggat ctagaggcat tgcttcagac atatggatat cctgcatgtc
      481 tcggagctat aatagttcaa gtttggatag tgttggtcaa agc
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LOCUS       seq5                    525 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq5
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 525)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 525)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
            Bankit Comment: TOTAL # OF SETS:10.

            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
            Assembly Name         :: hh
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
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      121 atgcagataa attattaata gcaaccactt tcttagccca ctcattggac acagataaac
      181 aacactctca aagaggagga tttttagtat cactccttgc aatggcttac agtagtccgg
      241 aattatatct cactacaaac ggtgtcaatg ctgatgtcaa gtatgtgata tataatatag
      301 agagagatcc taaaagaaca aaaacagatg ggttcattgt caaaacgaga gacatggagt
      361 atgaaagaac cacagagtgg ttgtttggac ctatgattaa caagaatcca ttgttccaag
      421 ggcaaagaga gaatgcggat ctagaggcat tgcttcagac atatggatat cctgcatgtc
      481 tcggagctat aatagttcaa gtttggatag tgttggtcaa agcca
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LOCUS       seq6                      523 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq6
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
            provided by the submitter.
            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
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            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
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            Sequencing Technology :: Illumina
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
BASE COUNT      175 a     91 c    118 g    139 t
ORIGIN
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      121 atgcagataa attattaata gcaaccactt tcttagccca ctcattggac acagataaac
      181 aacactctca aagaggagga tttttagtat cactccttgc aatggcttac agtagtccgg
      241 aattatatct cactacaaac ggtgtcaatg ctgatgtcaa gtatgtgata tataatatag
      301 agagagatcc taaaagaaca aaaacagatg ggttcattgt caaaacgaga gacatggagt
      361 atgaaagaac cacagagtgg ttgtttggac ctatgattaa caagaatcca ttgttccaag
      421 ggcaaagaga gaatgcggat ctagaggcat tgcttcagac atatggatat cctgcatgtc
      481 tcggagctat aatagttcaa gtttggatag tgttggtcaa agc
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LOCUS       seq7                      523 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq7
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
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            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
            Bankit Comment: TOTAL # OF SEQS:10.
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            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
            Assembly Name         :: hh
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FEATURES             Location/Qualifiers
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                     /mol_type="other RNA"
                     /isolate="Almuthana"
                     /isolation_source="Nasal Swap"
                     /host="human"
                     /country="Iraq"
BASE COUNT      175 a     91 c    118 g    139 t
ORIGIN
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      121 atgcagataa attattaata gcaaccactt tcttagccca ctcattggac acagataaac
      181 aacactctca aagaggagga tttttagtat cactccttgc aatggcttac agtagtccgg
      241 aattatatct cactacaaac ggtgtcaatg ctgatgtcaa gtatgtgata tataatatag
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      361 atgaaagaac cacagagtgg ttgtttggac ctatgattaa caagaatcca ttgttccaag
      421 ggcaaagaga gaatgcggat ctagaggcat tgcttcagac atatggatat cctgcatgtc
      481 tcggagctat aatagttcaa gtttggatag tgttggtcaa agc
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LOCUS       seq8                      523 bp    RNA     linear       14-SEP-2015
DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq8
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
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            Bankit Comment: TOTAL # OF SEQS:10.
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            ##Assembly-Data-START##
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            Sequencing Technology :: Illumina
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      361 tgcagaacgg cagaaacaat tcaacgattg aagatcttgt tcacacattt gggtatccat
      421 catgtttagg agctcttata atacagatct ggatagtttt ggtcaaagcc atcactagca
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DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq9
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 523)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 523)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
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            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
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            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
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            Sequencing Technology :: Illumina
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                     /host="human"
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BASE COUNT      175 a     91 c    118 g    139 t
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      181 aacactctca aagaggagga tttttagtat cactccttgc aatggcttac agtagtccgg
      241 aattatatct cactacaaac ggtgtcaatg ctgatgtcaa gtatgtgata tataatatag
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      361 atgaaagaac cacagagtgg ttgtttggac ctatgattaa caagaatcca ttgttccaag
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DEFINITION  UNVERIFIED: [Nucleoprotine gene] partial CDS.
ACCESSION   seq10
VERSION
KEYWORDS    UNVERIFIED.
SOURCE      Human para-influenza virus
  ORGANISM  Human para-influenza virus
            Unclassified.
REFERENCE   1  (bases 1 to 527)
  AUTHORS   abdullah,A.A.
  TITLE     Molecular detection of human parainfluenza virus in infants
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 527)
  AUTHORS   Alrodhan,M.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2015) Zoonotic diseases Unit, University of
            Al-Qadissya, University street, Diwanya, Qadissyah 00964, Iraq
COMMENT     GenBank staff is unable to verify sequence and/or annotation
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            Bankit Comment: ALT EMAIL:hmz.rodhan@gmail.com.
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            ##Assembly-Data-START##
            Assembly Method       :: mega v. 6.0
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            Sequencing Technology :: Illumina
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      121 tgagaaaatg acattagctc ttctatttct atctcattca ctagataatg agaaacaaca
      181 tgcacaaagg gcagggttct tggtgtcttt attgtcaatg gcttatgcca atccagagct
      241 ttacctgaca acaaatggaa gtaatgcaga tgttaaatat gtcatatata tgattgagaa
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      361 aaagacaact gagtggatat ttggaagtga cctggattat gaccaggaaa ctatgctgca
      421 gaacggcaga aacaattcaa cgattgaaga tcttgttcac acatttgggt atccatcatg
      481 tttaggagct cttataatac agatctggat agttttggtc aaagcca
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Appendix (3): Ten clones of this study .

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CAGATGTCAAATATGTCATATATATGATTGAGAAAGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGT
TAAGACGAGAGAGATGATATATGAAAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGACCAG
GAAACTATGCTGCAGAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCAT
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>KT763053.1 HPIV1_ Iraq_Sam.Rum2.
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AGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGGAATTATATCTCACTACAAACG
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>KT763055.1 HPIV1_ Iraq_Sam.City Center.
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GCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAACAACACTCTCAAAGAGGAGGATTTTTAGTAT
CACTCCTTGCAATGGCTTACAGTAGTCCGGAATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAA
GTATGTGATATATAATATAGAGAGAGATCCTAAAAGAACAAAAACAGATGGGTTCATTGTCAAAACGAGA
GACATGGAGTATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATTAACAAGAATCCATTGTTCCAAG
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AATAGTTCAAGTTTGGATAGTGTTGGTCAAAGC

>KT763056.1 HPIV1_ Iraq_Sam.Wark.
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GCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAACAACACTCTCAAAGAGGAGGATTTTTAGTAT
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>KT763058.1 HPIV1_ Iraq_Sam.Ngm.
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>KT763059.1 HPIV3_ Iraq_Sam.Hil.
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>KT763060.1 HPIV1_ Iraq_Sam.Sue.
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>KT763061.1 HPIV3_ Iraq_Sam.Meg.
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CTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCA

Appendix (4) : GeneBank Submission Massage

Dear GenBank Submitter:
Thank you for your direct submission of sequence data to GenBank. We
have provided GenBank accession numbers for your nucleotide sequences:

BankIt1856417 seq1	KT763052
BankIt1856417 seq2	KT763053
BankIt1856417 seq3	KT763054
BankIt1856417 seq4	KT763055
BankIt1856417 seq5	KT763056
BankIt1856417 seq6	KT763057
BankIt1856417 seq7	KT763058
BankIt1856417 seq8	KT763059
BankIt1856417 seq9	KT763060
BankIt1856417 seq10	KT763061

The GenBank accession numbers should appear in any publication that
reports or discusses these data, as it gives the community a unique
label with which they may retrieve your data from our on-line servers.
You may prepare and submit your manuscript before your accessions are
released in GenBank.

Submissions are not automatically deposited into GenBank after being
accessioned. Each sequence record is individually examined and processed
by the GenBank annotation staff to ensure that it is free of errors or problems.

You have not requested a specific release date for your sequence data.
Therefore, your record(s) will be released to the public database once
they are processed. If this is not what you intended, please contact
us as soon as possible with the correct release date.

Since the flatfile record is a display format only and is not an editable
format of the data, do not make changes directly to a flatfile. For
complete information about different methods to update a sequence record,
see: http://www.ncbi.nlm.nih.gov/Genbank/update.html

Any inquiries about your submission should be sent to gb-admin@ncbi.nlm.nih.gov
For more information about the submission process or the available
submission tools, please contact GenBank User Support at
info@ncbi.nlm.nih.gov.

Please reply using the original subject line.
This will allow for faster processing of your correspondence.
Sincerely,
Anjanette Johnston, PhD

The GenBank Direct Submission Staff
Bethesda, Maryland USA
****
gb-admin@ncbi.nlm.nih.gov (for updates/replies to GenBank entries)
info@ncbi.nlm.nih.gov (for general questions regarding GenBank)
www.ncbi.nlm.nih.gov/books/NBK51157/ GenBank Submissions Handbook
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Appendix (5) : Multiple sequence alignment of HPIV-1

CLUSTAL O(1.2.1) multiple sequence alignment .

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KT763060.1      CTTTTGACACATTTAGTTCCAGGAGAAGTGAGAGCATCAACAAGTCTGGCGGAGGAGCAA    60
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KT763056.1      CTTTTGACACATTTAGTTCCAGGAGAAGTGAGAGCATCAACAAGTCTGGCGGAGGAGCAA    60
KT763057.1      CTTTTGACACATTTAGTTCCAGGAGAAGTGAGAGCATCAACAAGTCTGGCGGAGGAGCAA    60
KT763058.1      CTTTTGACACATTTAGTTCCAGGAGAAGTGAGAGCATCAACAAGTCTGGCGGAGGAGCAA    60
AF457102.1      ---------------------------------------------------GAGGAGCAA     9
M62850.1        ---------------------------------------------------GAGGAGCAA     9
KT763053.1      ---------------------------------------------------GAGGAGCAA     9
KT763054.1      ---------------------------------------------------GAGGAGCAA     9
JQ901971.1      ---------------------------------------------------GAGGAGCAA     9
EU346886.1      ---------------------------------------------------GAGGAGCAA     9
D01070.1        ---------------------------------------------------GAGGAGCAA     9
S38060.1        ---------------------------------------------------GAGGAGCAA     9
KM190940.1      ---------------------------------------------------GAGGAGCAA     9
JQ902004.1      ---------------------------------------------------GAGGAGCAA     9
KF530212.1      ---------------------------------------------------GAGGAGCAA     9
KF687311.1      ---------------------------------------------------GAGGAGCAA     9
KF530203.1      ---------------------------------------------------GAGGAGCAA     9
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KT763057.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG    120
KT763058.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG    120
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M62850.1        TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
KT763053.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
KT763054.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
JQ901971.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
EU346886.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
D01070.1        TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG     69
S38060.1        TTATACCTGGTCAAAGAAGTACCGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG     69
KM190940.1      TTATACCTGGTCAAAGAAGTACCGTTTCTGTCTTCACATTGGGCCCGAGTGTGACAGATG     69
JQ902004.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG     69
KF530212.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG     69
KF687311.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG     69
KF530203.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG     69
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KT763058.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC    180
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KF530212.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
KF687311.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
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KT763058.1      AGAGAGATCCTAAAAGAACAAAAACAGATGGGTTCATTGTCAAAACGAGAGACATGGAGT    360
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KT763054.1      AGAGAGATCCTAAAAGAACAAAAACAGATGGGTTCATTGTCAAAACGAGAGACATGGAGT    309
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EU346886.1      AGAGAGATCCTAAAAGAACAAAAACAGATGGGTTCATTGTCAAAACGAGAGACATGGAGT    309
D01070.1        AGAGAGATCCTAAAAGAACAAAAACAGATGGGTTCATTGTCAAAACGAGAGACATGGAGT    309
S38060.1        AGAGAGATCCCAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
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KF687311.1      AGAGAGATCCTAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
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S38060.1        AGAGAGATCCCAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
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JQ902004.1      AGAGAGATCCTAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
KF530212.1      AGAGAGATCCTAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
KF687311.1      AGAGAGATCCTAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
KF530203.1      AGAGAGATCCTAAAAGGACAAAAACAGATGGGTTCATTGTCAAAACAAGAGACATGGAGT    309
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KT763060.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATTAACAAGAATCCATTGTTCCAAG    420
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KT763056.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATTAACAAGAATCCATTGTTCCAAG    420
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KT763058.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATTAACAAGAATCCATTGTTCCAAG    420
AF457102.1      ATGAAAGAACAACAGAGTGGTTGTTTGGACCTATGATCAACAAGAACCCATTGTTCCAAG    369
M62850.1        ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATTAACAAGAATCCATTGTTCCAAG    369
KT763053.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATCAATAAGAATCCATTGTTCCAAG    369
KT763054.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATCAATAAGAATCCATTGTTCCAAG    369
JQ901971.1      ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATCAATAAGAATCCATTGTTCCAAG    369
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D01070.1        ATGAAAGAACCACAGAGTGGTTGTTTGGACCTATGATCAATAAGAATCCATTGTTCCAAG    369
S38060.1        ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGATCAACAAGAACCCATTGTTCCAAG    369
KM190940.1      ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGGTCAACAAGAACCCACTGTTCCAGG    369
JQ902004.1      ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGGTCAACAAGAATCCATTGTTCCAAG    369
KF530212.1      ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGGTCAACAAGAACCCATTGTTCCAAG    369
KF687311.1      ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGGTCAACAAGAACCCATTGTTCCAAG    369
KF530203.1      ATGAAAGAACAACAGAGTGGTTGTTCGGACCTATGGTCAACAAGAACCCATTGTTCCAAG    369
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KT763060.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    480
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KT763056.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    480
KT763057.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    480
KT763058.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    480
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M62850.1        GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
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KT763054.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
JQ901971.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
EU346886.1      GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
D01070.1        GGCAAAGAGAGAATGCGGATCTAGAGGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
S38060.1        GGCAAAGAGAAAATGCAGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
KM190940.1      GGCAGAGAGAGAATGCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
JQ902004.1      GGCAAAGAGAGAATGCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
KF530212.1      GGCAAAGAGAGAATGCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
KF687311.1      GGCAAAGAGAGAATGCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
KF530203.1      GGCAAAGAGAGAATGCGGATCTAGAAGCATTGCTTCAGACATATGGATATCCTGCATGTC    429
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KT763055.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGC----------------  523
KT763056.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCA--------------  525
KT763057.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGC----------------  523
KT763058.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGC----------------  523
AF457102.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTTAAAGCCATAACAAGTAGTGCTG  489
M62850.1        TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCATAACAAGTAGTTCTG  489
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KT763054.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCATAACAAGTAGTGCTG  489
JQ901971.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCATAACAAGTAGTGCTG  489
EU346886.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCATAACAAGTAGTGCTG  489
D01070.1        TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTCAAAGCCATAACAAGTAGTGCTG  489
S38060.1        TCGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTTAAAGCCATAACAAGTAGTGCTG  489
KM190940.1      TTGGAGCTATAATAGTTCAAGTTTGGATAGTGTTGGTTAAGGCCATAACAAGTAGTGCTG  489
JQ902004.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGCTGGTTAAGGCCATAACAAGTAGTGCTG  489
KF530212.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGCTGGTTAAGGCCATAACAAGTAGTGCTG  489
KF687311.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGCTGGTTAAGGCCATAACAAGTAGTGCTG  489
KF530203.1      TCGGAGCTATAATAGTTCAAGTTTGGATAGTGCTGGTTAAGGCCATAACAAGTAGTGCTG  489
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KT763060.1      ------------------------------------  523
KT763055.1      ------------------------------------  523
KT763056.1      ------------------------------------  525
KT763057.1      ------------------------------------  523
KT763058.1      ------------------------------------  523
AF457102.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
M62850.1        GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KT763053.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KT763054.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
JQ901971.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
EU346886.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
D01070.1        GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
S38060.1        GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KM190940.1      GTCTAAGGAAAGGATTCTTCAATAGATTAGAAGCAT  525
JQ902004.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KF530212.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KF687311.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
KF530203.1      GTCTAAGAAAAGGATTCTTCAATAGATTAGAAGCAT  525
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KT763060.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  120
KT763055.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  120
KT763056.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  120
KT763057.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  120
KT763058.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  120
AF457102.1      TTATACCTGGTCAAAGAAGTACCGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
M62850.1        TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
KT763053.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
KT763054.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
JQ901971.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
EU346886.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
D01070.1        TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCATATTAGGCCCGAGTGTGACAGATG  69
S38060.1        TTATACCTGGTCAAAGAAGTACCGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
KM190940.1      TTATACCTGGTCAAAGAAGTACCGTTTCTGTCTTCACATTGGGCCCGAGTGTGACAGATG  69
JQ902004.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
KF530212.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
KF687311.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
KF530203.1      TTATACCTGGTCAAAGAAGTACAGTTTCTGTCTTCACATTAGGCCCGAGTGTGACAGATG  69
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KT763060.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  180
KT763055.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  180
KT763056.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  180
KT763057.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  180
KT763058.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  180
AF457102.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  129
M62850.1        ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGACACAGATAAAC  129
KT763053.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCTCACTCATTGGACACAGATAAAC  129
KT763054.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCTCACTCATTGGACACAGATAAAC  129
JQ901971.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCTCACTCATTGGACACAGATAAAC  129
EU346886.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCTCACTCATTGGACACAGATAAAC  129
D01070.1        ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCTCACTCATTGGACACAGATAAAC  129
S38060.1        ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCACTGGATACAGATAAAC  129
KM190940.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCACTGGATACAGATAAAC  129
JQ902004.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGATACAGATAAAC  129
KF530212.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGATACAGATAAAC  129
KF687311.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGATACAGATAAAC  129
KF530203.1      ATGCAGATAAATTATTAATAGCAACCACTTTCTTAGCCCACTCATTGGATACAGATAAAC  129
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KT763060.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    240
KT763055.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    240
KT763056.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    240
KT763057.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    240
KT763058.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    240
AF457102.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGG    189
M62850.1        AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGTCCGG    189
KT763053.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGG    189
KT763054.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGG    189
JQ901971.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGG    189
EU346886.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCTACAGTAGTCCGG    189
D01070.1        AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCCATACGTAGTCCGG    189
S38060.1        AACATTCTCAAAGAGGAGGATTTCTAGTATCACTCCTTGCAATGGCTTATAGTAGCCCAG    189
KM190940.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
JQ902004.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
KF530212.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
KF687311.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
KF530203.1      AACACTCTCAAAGAGGAGGATTTTTAGTATCACTCCTTGCAATGGCTTACAGTAGCCCGG    189
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KT763060.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    300
KT763055.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    300
KT763056.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    300
KT763057.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    300
KT763058.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    300
AF457102.1      AGTTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATACAATATAG    249
M62850.1        AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
KT763053.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
KT763054.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
JQ901971.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
EU346886.1      AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
D01070.1        AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATATAATATAG    249
S38060.1        AATTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATACAATATAG    249
KM190940.1      AGTTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATACAGTATAG    249
JQ902004.1      AGTTATATCTCACTACAAACGGCGTCAATGCTGATGTCAAGTATGTGATATACAGTATAG    249
KF530212.1      AGTTATATCTCACTACAAACGGTGTCAATGCTGATGTCAAGTATGTGATATACAGTATAG    249
KF687311.1      AGTTATATCTCACTACAAACGGCGTCAATGCTGATGTCAAGTATGTGATATACAGTATAG    249
KF530203.1      AGTTATATCTCACTACAAACGGCGTCAATGCTGATGTCAAGTATGTGATATACAGTATAG    249
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Appendix (6) : Multiple sequence alignment of HPIV-3

CLUSTAL O(1.2.1) multiple sequence alignment .

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KT763061.1      TGATACATTTAATGCACGTAGGCAAGAAAACATAACAAAATCAGCTGGTGGAGCTATCAT     60
M11849.1        ------------------------------------------------------TCAT      4
KT763059.1      ------------------------------------------------------TCAT      4
KT763052.1      ------------------------------------------------------TCAT      4
EU346887.1      ------------------------------------------------------TCAT      4
M14552.1        ------------------------------------------------------TCAT      4
X04612.1        ------------------------------------------------------TCAT      4
FJ455842.2      ------------------------------------------------------TCAT      4
KM190938.1      ---------------------------------------------HPIVThailandTCAT     16
U51116.1        ---------------------------------------------------HPIVUSATCAT     11
Z11575.1        ------------------------------------------------------TCAT      4
AB736166.1      ------------------------------------------------------TCAT      4
KJ672605.1      ------------------------------------------------------TCAT      4
KF530245.1      ------------------------------------------------------TCAT      4
                                                                      ****

KT763061.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    120
M11849.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACGA     64
KT763059.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA     64
KT763052.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA     64
EU346887.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA     64
M14552.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA     64
X04612.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA     64
FJ455842.2      TCCTGGACAGAAAAATACTGTCTCTATATTTGCTCTTGGACCGACAATAACTGATGATAA     64
KM190938.1      CCCTGGACAGAAAAATACTGTCTCTATATTCGCTCTTGGACCAACAATAACCGATGATAA     76
U51116.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA     71
Z11575.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA     64
AB736166.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA     64
KJ672605.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA     64
KF530245.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA     64
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KT763061.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  180
M11849.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
KT763059.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
KT763052.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
EU346887.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
M14552.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
X04612.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
FJ455842.2      TGAGAAAATGACATTAGCTCTTCTTTTTTTATCTCATTCACTGGATAATGAGAAACAACA  124
KM190938.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA  136
U51116.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  131
Z11575.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA  124
AB736166.1      TGAGAAAATGACATTGGCCCTTTTGTTTCTATCTCATTCATTAGATAATGAGAAACAACA  124
KJ672605.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA  124
KF530245.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA  124
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KT763061.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  240
M11849.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
KT763059.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
KT763052.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
EU346887.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
M14552.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
X04612.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
FJ455842.2      TGCACAAAGGGCAGGGTTCCTGGTGTCCTTATTATCAATGGCTTATGCCAATCCAGAGCT  184
KM190938.1      TGCACAAAGAGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAACT  196
U51116.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  191
Z11575.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
AB736166.1      TGCACAAAGAGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
KJ672605.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
KF530245.1      TGCACAAAGGGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT  184
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KT763061.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA  300
M11849.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTAGGAA  244
KT763059.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA  244
KT763052.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA  244
EU346887.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA  244
M14552.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA  244
X04612.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA  244
FJ455842.2      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA  244
KM190938.1      CTACTTGACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA  256
U51116.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA  251
Z11575.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA  244
AB736166.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA  244
KJ672605.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA  244
KF530245.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA  244
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KT763061.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  360
M11849.1        AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
KT763059.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
KT763052.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
EU346887.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
M14552.1        AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
X04612.1        AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
FJ455842.2      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGGTATATGA  304
KM190938.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  316
U51116.1        AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  311
Z11575.1        AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
AB736166.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
KJ672605.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
KF530245.1      AGATCTAAAACGGCAAAAGTATGGAGGATTTGTGGTTAAGACGAGAGAGATGATATATGA  304
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KT763061.1      AAAGACAACTGAGTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  420
M11849.1        AAAGACAACTGAGTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
KT763059.1      AAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
KT763052.1      AAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
EU346887.1      AAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
M14552.1        AAAGACAACTGAGTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
X04612.1        AAAGACAACTGAGTGGATATTTGGAAGTGACCTGGATTATGACCAGGAAACTATGCTGCA  364
FJ455842.2      CAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  364
KM190938.1      GAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  376
U51116.1        AAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  371
Z11575.1        AAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  364
AB736166.1      GAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTACGATCAGGAAAATATGTTGCA  364
KJ672605.1      GAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  364
KF530245.1      GAAGACAACTGATTGGATATTTGGAAGTGACCTGGATTATGATCAGGAAACTATGTTGCA  364
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KT763061.1      GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    480
M11849.1        GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
KT763059.1      GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
KT763052.1      GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
EU346887.1      GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
M14552.1        GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
X04612.1        GAACGGCAGAAACAATTCAACGATTGAAGATCTTGTTCACACATTTGGGTATCCATCATG    424
FJ455842.2      GAATGGCAGAAACAATCCAACAATTGAAGACCTTGTCCACACGTTTGGGTATCCATCATG    424
KM190938.1      GAACGGCAGAAACAATTCAACAATTGAAGATCTTGTCCACACGTTTGGGTATCCATCATG    436
U51116.1        GAACGGCAGGAACAATTCAACAATTGAAGACCTTGTCCACACATTTGGGTATCCATCATG    431
Z11575.1        GAACGGCAGGAACAATTCAACAATTGAAGACCTTGTCCACACATTTGGGTATCCATCATG    424
AB736166.1      GAACGGCAGAAACAATTCAACAATTGAAGACCTTGTCCACACATTTGGGTATCCATCATG    424
KJ672605.1      GAACGGCAGAAACAATTCAACAATTGAAGACCTTGTCCACACATTTGGGTATCCATCATG    424
KF530245.1      GAACGGCAGAAACAATTCAACAATTGAAGACCTTGTCCACACATTTGGGTATCCATCATG    424
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KT763061.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCA-------------    527
M11849.1        TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
KT763059.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
KT763052.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
EU346887.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
M14552.1        TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
X04612.1        TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCCATCACTAGCATCTC    484
FJ455842.2      TTTAGGAGCTATTATAATACAGATCTGGATAGTTTTGGTCAAAGCTATCACTAGCATCTC    484
KM190938.1      TTTAGGAGCTATTATAATACAGATCTGGATAGTTTTGGTCAAAGCTATCACTAGCATCTC    496
U51116.1        TTTAGGAGCTCTTATAATACAGATCTGGATAGTTCTGGTCAAAGCTATCACTAGTATCTC    491
Z11575.1        TTTAGGAGCTCTTATAATACAGATCTGGATAGTTCTGGTCAAAGCTATCACTAGTATCTC    484
AB736166.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCTATTACTAGCATCTC    484
KJ672605.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCTATTACTAGCATCTC    484
KF530245.1      TTTAGGAGCTCTTATAATACAGATCTGGATAGTTTTGGTCAAAGCTATTACTAGCATCTC    484
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KT763061.1      ------------------------------------- 527
M11849.1        AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
KT763059.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
KT763052.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
EU346887.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
M14552.1        AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
X04612.1        AGGGTTAAGAAAAGGCTTTTTCACTCGATTAGAGGCTTT 523
FJ455842.2      AGGGTTAAGAAAAGGCTTTTTCACTCGATTGGAAGCTTT 523
KM190938.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTGGAAGCTTT 535
U51116.1        AGGGTTAAGAAAAGGCTTTTTCACCCGATTGGAAGCTTT 530
Z11575.1        AGGGTTAAGAAAAGGCTTTTTCACCCGATTGGAAGCTTT 523
AB736166.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTGGAAGCTTT 523
KJ672605.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTGGAAGCTTT 523
KF530245.1      AGGGTTAAGAAAAGGCTTTTTCACTCGATTGGAAGCTTT 523

KT763061.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    120
M11849.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACGA    64
KT763059.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
KT763052.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
EU346887.1      TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
M14552.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
X04612.1        TCCTGGACAGAAAAATACTGTCTCCATATTTGCCCTTGGACCGACAATAACTGATGACAA    64
FJ455842.2      TCCTGGACAGAAAAATACTGTCTCTATATTTGCTCTTGGACCGACAATAACTGATGATAA    64
KM190938.1      CCCTGGACAGAAAAATACTGTCTCTATATTCGCTCTTGGACCAACAATAACCGATGATAA    76
U51116.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA    71
Z11575.1        TCCTGGACAGAAAAATACTGTCTCTATATTCGCCCTTGGACCGACAATAACTGATGATAA    64
AB736166.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
KJ672605.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
KF530245.1      CCCTGGACAGAAAAATACTGTCTCTATATTTGCCCTTGGACCGACAATAACTGATGATAA    64
                ************************ ***** ** ******** ******** *****  *

KT763061.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    180
M11849.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
KT763059.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
KT763052.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
EU346887.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
M14552.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
X04612.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
FJ455842.2      TGAGAAAATGACATTAGCTCTTCTTTTTTTATCTCATTCACTGGATAATGAGAAACAACA    124
KM190938.1      TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    136
U51116.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    131
Z11575.1        TGAGAAAATGACATTAGCTCTTCTATTTCTATCTCATTCACTAGATAATGAGAAACAACA    124
AB736166.1      TGAGAAAATGACATTGGCCCTTTTGTTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
KJ672605.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
KF530245.1      TGAGAAAATGACATTAGCCCTTCTATTTCTATCTCATTCATTAGATAATGAGAAACAACA    124
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KT763061.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    240
M11849.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KT763059.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KT763052.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
EU346887.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
M14552.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
X04612.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
FJ455842.2      TGCACAAAGGGCAGGGTTCCTGGTGTCCTTATTATCAATGGCTTATGCCAATCCAGAGCT    184
KM190938.1      TGCACAAAGAGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAACT    196
U51116.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    191
Z11575.1        TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
AB736166.1      TGCACAAAGAGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KJ672605.1      TGCACAAAGGGCAGGGTTCTTGGTGTCTTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
KF530245.1      TGCACAAAGGGCAGGGTTCTTGGTGTCCTTATTGTCAATGGCTTATGCCAATCCAGAGCT    184
                ********** ********** ******** ***** ************************ **

KT763061.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    300
M11849.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTAGGAA    244
KT763059.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
KT763052.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
EU346887.1      TTACCTGACAACAAATGGAAGTAATGCAGATGTCAAATATGTCATATATATGATTGAGAA    244
M14552.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    244
X04612.1        TTACCTGACAACAAATGGAAGTAATGCAGATGTTAAATATGTCATATATATGATTGAGAA    244
FJ455842.2      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA    244
KM190938.1      CTACTTGACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    256
U51116.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    251
Z11575.1        CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
AB736166.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
KJ672605.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATCGAGAA    244
KF530245.1      CTACCTAACAACAAATGGAAGTAATGCAGATGTCAAGTATGTCATATACATGATTGAGAA    244
                *** * ************************** ** ********** ***** ***
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Appendixes (7) : Nucleotide Identity Percent estimate HPIV-1.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	KT763060.1	Iraq																		
2	KT763055.1	Iraq	100																	
3	KT763056.1	Iraq	100	100																
4	KT763057.1	Iraq	100	100	100															
5	KT763058.1	Iraq	100	100	100	100														
6	AF457102.1	USA	97.88	97.88	97.89	97.88	97.88													
7	M62850.1	USA	100	100	100	100	100	97.9												
8	KT763053.1	Iraq	99.15	99.15	99.16	99.15	99.15	98.1	99.05											
9	KT763054.1	Iraq	99.15	99.15	99.16	99.15	99.15	98.1	99.05	100										
10	JQ901971.1	USA	99.15	99.15	99.16	99.15	99.15	98.1	99.05	100	100									
11	EU346886.1	Lithuania	99.15	99.15	99.16	99.15	99.15	98.1	99.05	100	100	100								
12	D01070.1	USA	98.31	98.31	98.31	98.31	98.31	97.33	98.29	99.24	99.24	99.24	99.24							
13	S38060.1	Japan	95.55	95.55	95.57	95.55	95.55	97.14	95.81	95.62	95.62	95.62	95.62	95.05						
14	KM190940.1	Thailand	94.92	94.92	94.94	94.92	94.92	96.76	95.05	94.86	94.86	94.86	94.86	94.1	96.57					
15	JQ902004.1	USA	96.4	96.4	96.41	96.4	96.4	97.52	96.57	96.38	96.38	96.38	96.38	95.62	96.95	97.7				
16	KF530212.1	USA	96.4	96.4	96.41	96.4	96.4	97.9	96.57	96.38	96.38	96.38	96.38	95.62	97.33	98.1	99.62			
17	KF687311.1	USA	96.19	96.19	96.2	96.19	96.19	97.71	96.38	96.19	96.19	96.19	96.19	95.43	97.14	97.9	99.81	99.8		
18	KF530203.1	USA	96.19	96.19	96.2	96.19	96.19	97.71	96.38	96.19	96.19	96.19	96.19	95.43	97.14	97.9	99.81	99.8	100	

Appendixes (8) : Nucleotide Identity Percent estimate HPIV-3.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	KT763061.1	Iraq														
2	KT763059.1	Iraq	99.58													
3	KT763052.1	Iraq	99.58	100												
4	M11849.1	Chile	99.36	99.04	99.04											
5	EU346887.1	Lithuania	99.58	100	100	99.04										
6	M14552.1	Chile	100	99.62	99.62	99.43	99.62									
7	X04612.1	India	100	99.62	99.62	99.43	99.62	100								
8	FJ455842.2	China	94.06	94.65	94.65	93.69	94.65	94.26	94.26							
9	KM190938.1	Thailand	92.75	95.41	95.41	94.46	95.41	95.03	95.03	95.98						
10	U51116.1	USA	95.19	96.37	96.37	95.41	96.37	95.98	95.98	96.37	95.85					
11	Z11575.1	USA	96.39	96.37	96.37	95.41	96.37	95.98	95.98	96.37	96.75	100				
12	AB736166.1	Japan	94.69	95.03	95.03	94.07	95.03	94.65	94.65	95.79	96.56	96.75	96.75			
13	KJ672605.1	USA	95.97	96.18	96.18	95.22	96.18	95.79	95.79	96.75	97.32	97.9	97.9	98.47		
14	KF530245.1	USA	95.97	96.18	96.18	95.22	96.18	95.79	95.79	96.75	97.32	97.9	97.9	98.85	99.62	

Appendixes (9): Percent Identity Matrix - created by Clustal2.1 HPIV-1.

1: KT763060.1	100.00	100.00	100.00	100.00	100.00	97.88	100.00	99.15	99.15	99.15	99.15	98.31	95.55	94.92	96.40	96.40	96.19	96.19
2: KT763055.1	100.00	100.00	100.00	100.00	100.00	97.88	100.00	99.15	99.15	99.15	99.15	98.31	95.55	94.92	96.40	96.40	96.19	96.19
3: KT763056.1	100.00	100.00	100.00	100.00	100.00	97.89	100.00	99.16	99.16	99.16	99.16	98.31	95.57	94.94	96.41	96.41	96.20	96.20
4: KT763057.1	100.00	100.00	100.00	100.00	100.00	97.88	100.00	99.15	99.15	99.15	99.15	98.31	95.55	94.92	96.40	96.40	96.19	96.19
5: KT763058.1	100.00	100.00	100.00	100.00	100.00	97.88	100.00	99.15	99.15	99.15	99.15	98.31	95.55	94.92	96.40	96.40	96.19	96.19
6: AF457102.1	97.88	97.88	97.89	97.88	97.88	100.00	97.90	98.10	98.10	98.10	98.10	97.33	97.14	96.76	97.52	97.90	97.71	97.71
7: M62850.1	100.00	100.00	100.00	100.00	100.00	97.90	100.00	99.05	99.05	99.05	99.05	98.29	95.81	95.05	96.57	96.57	96.38	96.38
8: KT763053.1	99.15	99.15	99.16	99.15	99.15	98.10	99.05	100.00	100.00	100.00	100.00	99.24	95.62	94.86	96.38	96.38	96.19	96.19
9: KT763054.1	99.15	99.15	99.16	99.15	99.15	98.10	99.05	100.00	100.00	100.00	100.00	99.24	95.62	94.86	96.38	96.38	96.19	96.19
10: JQ901971.1	99.15	99.15	99.16	99.15	99.15	98.10	99.05	100.00	100.00	100.00	100.00	99.24	95.62	94.86	96.38	96.38	96.19	96.19
11: EU346886.1	99.15	99.15	99.16	99.15	99.15	98.10	99.05	100.00	100.00	100.00	100.00	99.24	95.62	94.86	96.38	96.38	96.19	96.19
12: D01070.1	98.31	98.31	98.31	98.31	98.31	97.33	98.29	99.24	99.24	99.24	99.24	100.00	95.05	94.10	95.62	95.62	95.43	95.43
13: S38060.1	95.55	95.55	95.57	95.55	95.55	97.14	95.81	95.62	95.62	95.62	95.62	95.05	100.00	96.57	96.95	97.33	97.14	97.14
14: KM190940.1	94.92	94.92	94.94	94.92	94.92	96.76	95.05	94.86	94.86	94.86	94.86	94.10	96.57	100.00	97.71	98.10	97.90	97.90
15: JQ902004.1	96.40	96.40	96.41	96.40	96.40	97.52	96.57	96.38	96.38	96.38	96.38	95.62	96.95	97.71	100.00	99.62	99.81	99.81
16: KF530212.1	96.40	96.40	96.41	96.40	96.40	97.90	96.57	96.38	96.38	96.38	96.38	95.62	97.33	98.10	99.62	100.00	99.81	99.81
17: KF687311.1	96.19	96.19	96.20	96.19	96.19	97.71	96.38	96.19	96.19	96.19	96.19	95.43	97.14	97.90	99.81	99.81	100.00	100.00
18: KF530203.1	96.19	96.19	96.20	96.19	96.19	97.71	96.38	96.19	96.19	96.19	96.19	95.43	97.14	97.90	99.81	99.81	100.00	100.00

Appendixes (10): Percent Identity Matrix - created by Clustal2.1 HPIV-3.

1: KT763061.1	100.00	99.36	99.58	99.58	99.58	100.00	100.00	94.06	92.75	95.19	96.39	94.69	95.97	95.97
2: M11849.1	99.36	100.00	99.04	99.04	99.04	99.43	99.43	93.69	94.46	95.41	95.41	94.07	95.22	95.22
3: KT763059.1	99.58	99.04	100.00	100.00	100.00	99.62	99.62	94.65	95.41	96.37	96.37	95.03	96.18	96.18
4: KT763052.1	99.58	99.04	100.00	100.00	100.00	99.62	99.62	94.65	95.41	96.37	96.37	95.03	96.18	96.18
5: EU346887.1	99.58	99.04	100.00	100.00	100.00	99.62	99.62	94.65	95.41	96.37	96.37	95.03	96.18	96.18
6: M14552.1	100.00	99.43	99.62	99.62	99.62	100.00	100.00	94.26	95.03	95.98	95.98	94.65	95.79	95.79
7: X04612.1	100.00	99.43	99.62	99.62	99.62	100.00	100.00	94.26	95.03	95.98	95.98	94.65	95.79	95.79
8: FJ455842.2	94.06	93.69	94.65	94.65	94.65	94.26	94.26	100.00	95.98	96.37	96.37	95.79	96.75	96.75
9: KM190938.1	92.75	94.46	95.41	95.41	95.41	95.03	95.03	95.98	100.00	95.85	96.75	96.56	97.32	97.32
10: U51116.1	95.19	95.41	96.37	96.37	96.37	95.98	95.98	96.37	95.85	100.00	100.00	96.75	97.90	97.90
11: Z11575.1	96.39	95.41	96.37	96.37	96.37	95.98	95.98	96.37	96.75	100.00	100.00	96.75	97.90	97.90
12: AB736166.1	94.69	94.07	95.03	95.03	95.03	94.65	94.65	95.79	96.56	96.75	96.75	100.00	98.47	98.85
13: KJ672605.1	95.97	95.22	96.18	96.18	96.18	95.79	95.79	96.75	97.32	97.90	97.90	98.47	100.00	99.62
14: KF530245.1	95.97	95.22	96.18	96.18	96.18	95.79	95.79	96.75	97.32	97.90	97.90	98.85	99.62	100.00

Appendixes (11) : Sequences producing significant alignments for HPIV3

Sequences producing significant alignments:

Select: All None Selected:14 Show all columns

Alignments Download GenBank Graphics Distance tree of results

Description	Ident	Accession
Human parainfluenza virus 3 strain C-243 nucleoprotein (NP) gene, complete cds	100%	EU346887.1
Human parainfluenza virus type 3 nucleocapsid protein, complete cds	99%	M14552.1
Human parainfluenza type 3 virus gene for major nucleocapsid protein	99%	X04612.1
Human parainfluenza virus 3 nucleocapsid protein gene, complete cds	99%	M11849.1
Human parainfluenza virus 3, mutant cp-45, complete genome	96%	U51116.1
Human parainfluenza virus 3 virus RNA	96%	Z11575.1
Human parainfluenza virus 3 strain HPIV3/Homo sapiens/PER/FLE8246/2010, partial genome	96%	KJ672609.1
Human parainfluenza virus 3 strain HPIV3/Homo sapiens/PER/FLE4204/2009, complete genome	96%	KJ672605.1
Human parainfluenza virus 3 strain HPIV3/Homo sapiens/PER/FLU7236/2007, complete genome	96%	KJ672596.1
Human parainfluenza virus 3 strain HPIV3/Homo sapiens/PER/FPP00635/2011, complete genome	96%	KJ672537.1
Human parainfluenza virus 3 strain HPIV3/Homo sapiens/PER/FPP01456/2012, partial genome	96%	KJ672531.1
Human parainfluenza virus 3 strain HPIV3/MEX/1527/2005, partial genome	96%	KF687323.1

Appendixes (12) : Sequences producing significant alignments for HPIV1

Sequences producing significant alignments:

Select: All None Selected:11 Show all columns

Alignments Download GenBank Graphics Distance tree of results

Description	Ident	Accession
Human parainfluenza virus 1 strain ATCC VR-94, partial genome	100%	JQ901971.1
Human parainfluenza virus 1 strain C35 nucleoprotein (NP) gene, complete cds	100%	EU346886.1
Human parainfluenza virus 1 mRNA for nucleoprotein, complete cds	99%	D01070.1
Human parainfluenza type 1 virus nucleoprotein mRNA, complete cds	99%	M62850.1
HPIV-1 strain Washington/1964, complete genome	98%	AF457102.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D02071/2010, partial genome	96%	JQ902010.1
Human parainfluenza virus 1 strain HPIV1/USA/38081A/2011, complete genome	96%	KF530212.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D01575/2009, partial genome	96%	JQ902004.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D01463/2009, partial genome	96%	JQ902000.1
Human parainfluenza virus 1 strain HPIV1/ZAF/879/2010, complete genome	96%	KF687311.1
Human parainfluenza virus 1 strain HPIV1/USA/38078A/2011, complete genome	96%	KF530203.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D02209/2009, partial genome	96%	JQ902005.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D01790/2009, partial genome	96%	JQ902001.1
Human parainfluenza virus 1 strain HPIV1/WI/629-D01809/2009, partial genome	96%	JQ901995.1

الخلاصة .

يعد فيروس نظير الأنفلونزا البشري (*HPIV*) احد أهم الفيروسات التي تصيب الممرات التنفسية العليا و السفلى في الأطفال كما يعد المسبب الثاني من ناحية الأهمية بعد الفيروس المخلاوي التنفسي (*RSV*) في الأخماج التنفسية و خصوصا في الأطفال و أن كل شخص يتعرض للإصابة به على الأقل مرة واحدة في حياته .

بالنظر للأهمية السريريه لهذا الفيروس صممت الدراسة الحالية لتقييم بعض المظاهر السريرية و الوبائية و التحري عن العتر المحلية السائدة (local predominant genotypes) و ذلك عن طريق التوصيف الجزيئي و التحليل الفيلوجيني (Phylogenetic analysis) و بالمقارنة مع العتر المرجعية المسجلة عالميا .

تم جمع ثلاثمائة مسحة أنفية في الأطفال المصابين بأمراض تنفسية حادة و الذين ادخلوا في مستشفيات الأطفال في محافظة المثنى للفترة من شهر كانون الثاني 2015 إلى آذار 2015 و بعد أخذ الموافقة التحريرية .

و قد سجلت العلامات السريريه المرافقة للإصابة مثل الحمى , صعوبة التنفس , أزيز , السعال , و الطروحات الأنفية , و تم حساب العلامات الأكثر حدوثا بينما سجل أكثر من علامة سريريه في نفس الوقت .

أظهرت الدراسات السريريه إن النسبة للإصابة بالأخماج التنفسية الحادة كانت اعلى نسبة في منطقة الرميثة بنسبة 38% و اقل نسبة إصابة في منطقة الخضر بنسبة 1,33% .. و النسبة الكلية للإصابة للذكور كان 54,33% و النسبة الكلية للإناث 45,66% .

تم استخدام تقنية الاستنساخ العكسي – تفاعل سلسلة البلمرة بالوقت الحقيقي (RT-RT-PCR) للتحري عن جين البروتين النووي (*NP*) لفيروس نظير الأنفلونزا البشري *(HPIV)* و ذلك عن طريق استخدام بادئات متخصصة (primers) و مجس متخصص أيضا (Tag Man prop) .

وقد أظهرت النتائج إن نسبة الإصابة بفيروس نظير الأنفلونزا البشري *(HPIV)* باستخدام تقنية تفاعل سلسلة البلمرة بالوقت الحقيقي (RT-PCR) كانت 45,38% .

نتائج نسبة الإصابة تبعا للجنس أظهرت إن نسبة إصابة الذكور كانت 61,11% بينما نسبة إصابة الإناث 36,66 مع اختلافات معنوية عند ($p\leq0.01$) بين إصابة الذكور والإناث . نتائج معدل الإصابة بواسطة استخدام تقنية الاستنساخ العكسي تبعا لمناطق الإصابة كانت (58,3% , 47,5% , 50,1% , 38,5% , 33,3% , 25% , 0% و 0%) في (السماوة , الرميثة , الهلال , الوركاء , السوير , النجمي , المجد و الخضر) على التوالي . السماوة أظهرت اعلى نسبة إصابة 58,3% بينما النجمي كان مع اقل نسبة إصابة مع 25% مع اختلافات معنوية عند ($P\leq0.01$) .المجد و الخضر لم يسجلا أي نتائج موجبة . وقد و جدت اختلافات معنوية مع بين منطقة الرميثة و الهلال عند مستوى معنوية ($p\leq0.01$) مع المناطق الأخرى .و قد وجدت اختلافات معنوية بين الوركاء و المناطق الأخرى عند مستوى معنوية ($p\leq0.01$).و وجدت اختلافات منوية بين منطقة السوير مع المناطق الأخرى عند مستوى معنوية ($p\leq0.01$) . وجود

اختلافات معنوية بين منطقة النجمي و المناطق الأخرى عند مستوى معنوية ($p\leq0.01$) . ولم توجد اختلافات معنوية بين المجد و الخضر عند مع المناطق الأخرى .

تم تأكيد فحص نماذج عشرة موجبة مختارة و ذلك باستخدام تفاعل سلسلة البلمرة التقليدي (convential PCR) بوجود البادئات المتخصصة للتحري عن جين البروتين النووي (*NP*) (523 bp) .

أظهرت نتائج التحليل الجيني (sequencing) لعشرة من نتائج تفاعل البلمرة و بعد تسجيلها في المركز الوطني للمعلومات الإحيائية (NCBI) و بنك الجينات في الولايات المتحدة (GenBank) و بعد تخصيص عدد انضمام accession NO. سبعة منها كانت HPIV-1 (**KT763053** , **KT763054** , **KT763055** , **KT763056** , **KT763057** , **KT763058** , **KT763060**) و ثلاثة منها كانت HPIV-3 (**KT763052** , **KT763059** , **KT763061**) في حين لم تسجل العتر HPIV-4 , HPIV-2 في هذه الدراسة كما ظهر في التحليل الجيني (phylogentic analysis) وبناء الشجرة الفيلوجينيه (phylogenetic tree) (**KT763053** , **KT763054**) أظهرت نسبة تطابق عالية مع **(EU346886.1,)** و التي عزلت من لتوانيا, و مع (**JQ901971.1** , **D01070.1**) و التي عزلت من الولايات المتحدة . و عزلاتنا كذلك أظهرت نسبة تشابه مع العزلات (**AF457102.1 , JQ902004.1 , KF530212.1 , KF687311.1 , KF530203.1**) و التي عزلت من الولايات المتحدة , **(S38060.1)** والتي عزلت من اليابان و مع **(KM190940.1**) و التي عزلت من تايلاند .

بينما العزلات **KT763058, KT763060 (KT763057 , KT763056 , KT763055**) أظهرت نسبة تطابق عالية مع **(M62850.1)** و التي عزلت مع الولايات المتحدة . **(KT763061)** أظهرت نسبة تطابق عالية مع (**M11849.1 M14552.1**) و التي عزلت من تشيلي ,و مع **(X04612.1)** و التي عزلت من الهند , بينما العزلات (**KT763052 , KT763059**) أظهرت نسبة عالية مع **(EU346887.1)** و التي عزلت من ليتوانيا . كما أظهرت عزلاتنا نسبة تشابه عالية مع العزلات (**KF530245.1 , KJ672605.1 , Z11575.1 U51116.1**) و التي عزلت من الولايات المتحدة ,**(AB736166.1)** و التي عزلت من اليابان , ومع **(FJ455842.2)** و التي عزلت من الصين .

في الختام *HPIV* يعتبر احد اهم المسببات المرضية للجهاز التنفسي في الرضع و الاطفال في المثنى و يعتبر النوع *HPIV1* و *HPIV2* النوع السائد المسجل في مناطق الدراسة المختلفة . التحليل الجيني Phylogenetic analysis اظهرت ترتيب السلالات العراقية من هذه الدراسة مع مناطق اخرى مختلفة مثل الولايات المتحدة و لاتوانيا و تشيلي و غيرها .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة المثنى

كلية العلوم

التحري الجزيئي لفيروس *parainfluenza* في الرضع و الأطفال في محافظة المثنى

رسالة

مقدمة إلى قسم علوم الحياة, كلية العلوم , جامعة المثنى وهي جزء من متطلبات نيل درجة ماجستير علوم في علوم الحياة / الأحياء المجهرية

من قبل الطالبة

أبرار عبدالله كاظم

(بكالوريوس علوم الحياة 2013)

بإشراف

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