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Department of Biology

Molecular Study of Human Metapneumovirus, Human Respiratory Syncytial Virus and Human Parainfluenza Virus Type 1, 3 among Patients with Respiratory Tract Infections in Diyala Governorate, Iraq

A Thesis
Submitted to the Council of the College of Education for Pure Science / University of Diyala in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology

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بسم الله الرحمن الرحيم

ن وَالْقَلَمِ وَمَا يَسْطُرُونَ (١) مَا أَنْتَ بِنِعْمَةِ رَبِّكَ بِمَجْنُونٍ (٢) وَإِنَّ لَكَ لَأَجْرًا غَيْرَ مَمْنُونٍ (٣) وَإِنَّكَ لَعَلَى خُلُقٍ عَظِيمٍ (٤)

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Supervisor Certification

We certify that this thesis (**Molecular study of Human Metapneumovirus, Human Respiratory Syncytial Virus and Human Parainfluenza Virus Type 1, 3 among Patients with Respiratory Tract Infections in Diyala Governorate, Iraq)** was prepared by **Asmaa Haseeb Hwaid Al-Jurani**, under our supervision at the Department of Biology, College of Education for Pure Science, University of Diyala, Iraq. As partial fulfilment of the requirements for the degree of doctorate of philosophy in Biology

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Certification of Examination Committee

We, the members of the examination committee, certify that we have examined the thesis entitled **(Molecular study of Human Metapneumovirus, Human Respiratory Syncytial Virus and Human Parainfluenza Virus Type 1, 3 among Patients with Respiratory Tract Infections in Diyala Governorate, Iraq)** that was prepared by **Asmaa Haseeb Hwaid Al-Jurani** is accepted as partial fulfilment of the requirements for the degree of doctorate of philosophy in Biology.

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Dedication

To who was exhausted and tired by this life,
To who was optimistic in this life,
To who is happy now in the mercy of ***God****,*
To the honest and pure soul,

To my dear brother,

Mohammed

I dedicate this dissertation.

asmaa

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Researcher

Summary

Globally, viral respiratory infections are an important cause of morbidity and mortality and represent a serious threat to human health, among these viruses Human Metapneumovirus (HMPV) which is an emerging respiratory pathogen that was first described in 2001, which is quite similar to the Human Respiratory Syncytial Virus (HRSV) regarding symptoms and clinical signs, Human Parainfluenza viruses type 1 and 3 (HPIV-1 and HPIV-3) are other respiratory pathogens. These viruses are responsible for a wide range of upper and lower respiratory tract infections.

The present study is a cross-sectional study conducted at Baqubah Teaching Hospital and Al-Batool Teaching Hospital for Maternity and Children plus outpatient clinics in Diyala governorate, Iraq, for the period from 16/January/2018 to 30/October/2019. The objects of the current study included, exploration of the prevalence rates of HMPV, HRSV and HPIV type 1 and 3 among patients clinically presented with respiratory tract infections, determination the co-infection with two or more of the respiratory viruses included in this study, figure out the associated socio-demographic risk factors, furthermore, determination the seasonal distribution and genetic diversity (predominant genotype) of the local circulating HMPV and HRSV strains by analyzing the sequencing of nucleotides and phylogenetic tree of (G) and (F) genes of HMPV and genetic subgrouping of (F) gene of HRSV and finally a genetic comparison of the local HMPV isolates with the others from different

countries through the multiple and pairwise alignment of these isolates with international isolates available in the GeneBank database.

Three hundred and twenty-three patients from those clinically suspected as having respiratory tract infections RTIs (children, adults and elderly) were included. The study was extended over two seasons; 185 patients were included in the first season (January, February, March, April, May) and 138 in the second season (November and December). Three different respiratory specimens were collected including nasopharyngeal swabs, nasal swabs and throat swabs. Human privacy was respected through obtaining official written approval from the Research Ethics Committee in Diyala Directory of Health and specimen's collection was done under the direct supervision of specialized doctors and practitioners after approval of the study participants or their parents. Specimens were analyzed by using real-time PCR (RT-PCR/qPCR) assay for (HMPV, HRSV and HPIV-1/HPIV-3) and conventional PCR assay for (HMPV and HRSV). Genotyping for the positive samples of HMPV and genetic subgrouping of HRSV was performed.

The results showed that HMPV single infections was detected in 30(9.3%) and a co-infection with HPIV-3 was (n = 1) that had the same clinical manifestations and single infection with the HRSV was detected in 15(4.6%) and a co-infection with HPIV-3 was (n = 1), while HPIV-3 alone was detected in 17 (5.3%) and no HPIV-1 infections were detected. The highest positivity rates of HMPV appeared in specimens collected during November and December, season II with a significant difference compared to other months (50%, P= 0.0001). Obviously, the highest

HMPV positivity rate was detected among nasal sinus swabs with a statistically significant difference compared to nasopharyngeal and throat swabs (96.7%, P=0.0001). HRSV positivity rate was significantly higher in the first season compare to the second season (93.3% versus 6.7%, P=0.004), March compared to other months of the year shows a highest detection rate (P=0.012), HRSV was detected in (53.3%) of throat swabs which were significantly higher compared to nasopharyngeal swabs (40.0%) and nasal sinus swabs (6.7%) (P=0.003). HPIV-3 positivity rate during the season I was higher compared to season II was (64.7% versus 35.3%) (P=0.525) and November showed the highest detection rate (29.4%, P=0.340); however, the difference was failed to reach the statistical significance for both. Additionally, throat and nasal swabs proved effectiveness in showing the highest positive rate of the HPIV-3 (64.7%, 35.3%) respectively. Regarding the age-related positivity of HMPV, HRSV and HPIV-3 the infections were highest among children under 5 years of age (86.7%, 53.3%, 82.4%) respectively. Clinical pictures most closely related to the higher positivity rate of HMPV and HPIV-3 were Bronchiolitis/bronchitis (86.7%, 88.2%) with a statistically significant difference (P=0.001, 0.003).

Phylogenetic analysis of HMPV attachment glycoprotein (G) gene showed that of the 28 Iraqi isolates collected during the second season, 15 of these isolates belong and cluster within genotype (B), sub-lineages (B2) circulated in this region. These isolates were registered with the (GeneBank) at the National Center for Biotechnology Information (NCBI) and a global accession number was obtained. Multiple sequence

alignment showed high homologies estimated by (98% and 97%) have been observed between Iraqi isolates (MN178608.1, MN178613.1, MN178615.1, MN178617.1, MN178606.1, MN178607.1, MN178619.1, MN178620.1) and HMPV genotype B, sub-lineages B2 prevalent in Spain (GenBank acc. KX829167.1) and Malaysia (GenBank acc. KU320936.1), in the other hand, pairwise sequence alignment showed homologies estimated by (96%) between Iraqi strains (MN178609.1, MN178610.1, MN178611.1, MN178612.1, MN178614.1, MN178616.1, MN178618.1) and Malaysian strains (GenBank acc. KU320936.1). Significant genetic variation in the sequencing of nucleotides and amino acids was observed in the (G) gene, alignment of nucleotides and amino acids sequences of the (G) gene and phylogenetic tree demonstrated that three distinct HMPV variants within the same (B2) sub-lineage were detected as a result of frameshift variations (insertion mutations), the subgroup one included (MN178608.1, MN178613.1, MN178615.1, MN178617.1); the subgroup two included (MN178606.1, MN178607.1, MN178619.1, MN178620.1); and subgroup three included (MN178609.1, MN178610.1, MN178611.1, MN178612.1, MN178614.1, MN178616.1, MN178618.1).

The study concluded that HMPV has an important role as a viral cause with a high rate of RTIs in the Diyala community, especially among children followed by the importance of HRSV and HPIV-3 as respiratory pathogens. Seasonal variation of HMPV can be observed in this study and predominance of the prevalence of HMPV (B) genotype, (B2) sub-lineage compared to other viruses under study. Emerging HMPV strains are continually evolving.

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LIST OF ABBREVIATIONS

Abbreviations	Meaning
AMPV	Avian Metapneumovirus
AOM	Acute otitis media
Asn-X-Ser/Thr/Cys	N-glycosylation sites
CCA	Chimpanzee coryza agent
CDC	Centers for disease control and prevention
CT	Cytoplasmic tail domain
DC	Dendritic cells
DFA	Direct immunofluorescence assay
EE	Extracellular ctodomain
ENT	Ear, Nose and Throat
GAGs	Glycosaminoglycan
HADVs	Human Adenoviruses
HMPV	Human Metapneumovirus
HPIVs	Human Parainfluenza Viruses
HRSV	Human Respiratory Syncytial Virus
ICU	Intensive care unit
IFA	Immunofluorescent assay
IFN	Interferon
LLC-MK2	Rhesus monkey kidney cells
LRTIs	Lower respiratory tract infections
MAVS	Mitochondrial antiviral-signaling protein
MER-CoV	Middle East Respiratory Coronaviruses

MyD88	Myeloid differentiation primary response gene 88
NCBI	National Center for Biotechnology Information
NS1	Nonstructural genes
ORFs	Open reading frames
RIG-I	Retinoic-Inducible Gene-I
RNP	Ribonucleoprotien complex
RTIs	Respiratory tract infections
SARS	Severe Acute Respiratory Syndrome Coronavirus
STAT1	Signal transducer and activator of transcription 1
TM	Transmembrane domain
tMK	Tertiary cynomolgus Monkey Kidney cells
URTIs	Upper respiratory tract infections
VTM	Viral transport medium
WHO	World Health Organization
xg	Relative centrifugal force

CHAPTER ONE

INTRODUCTION

Chapter One

1. Introduction

1.1. Overview:

In the global term, respiratory viral infections are a leading cause of morbidity, hospitalization, and mortality affecting peoples of all ages particularly, infants, young children, the elderly, and immunocompromised individuals (Hijano *et al*., 2018; Li *et al*., 2019). Upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs) are frequently caused by a wide spectrum of viruses causing various clinical syndromes with variable outcomes ranging from common colds, pharyngitis, croup (laryngotracheobronchitis), otitis media, bronchiolitis, and viral pneumonia (Tregoning and Schwarze, 2010; Das *et al*., 2018; Gottlieb, 2019). The most important respiratory viral infections are influenza viruses type A and B, Human Respiratory Syncytial Virus (HRSV), Human Parainfluenza viruses (HPIVs), and Human Adenoviruses (HADVs), plus a number of newly discovered human respiratory viruses for the first time including Human Metapneumovirus (HMPV), Severe Acute Respiratory Syndrome Coronavirus (SARS), Middle East Respiratory Coronaviruses (MERS-CoV), Human Bocavirus, and Human Rhinovirus (Berry et al., 2015; Hasan *et al*., 2018; Tambyah *et al*., 2019; Bradley and Bryan, 2019).

Worldwide, the prevalence rate of respiratory viral infections is very high since viruses were identified as causes of the pediatric acute respiratory tract infections (ARIs) in up to (95%) of cases (Linden *et al*., 2019). Moreover, viruses are the main cause of (90%) of URTIs, and

about (30%) of LRTIs (Korsman *et al*., 2012). In 2013, WHO estimated that ARIs accounted for more than (8%) of all deaths in the Eastern Mediterranean Region (WHO, 2013). HMPV, HRSV and HPIVs are the most common and important causes of LRTIs in infants and children, while, in older children and adults, it causes recurrent-infections that are in the most cases are mild and self-limited in healthy individuals, but can be exacerbated causing serious or fatal diseases in elderly, persons with cardiopulmonary illnesses and immunocompromised patients (Chow *et al*., 2016; Williams *et al*., 2017).

Human Metapneumovirus is a single-stranded RNA virus, belongs to *Metapneumovirus* genus within Pneumoviridae family. Since the first isolation of this virus from (28) Netherlands' children in 2001; HMPV has appeared as one of the important causative agents of ARIs in all ages worldwide. The clinical manifestations of acute and severe RTIs that caused by HMPV are similar and undistinguishable from those caused by of HRSV, which are mostly characterized by mild symptoms to severe cough, bronchiolitis and pneumonia (van den Hoogen *et al*., 2001; Taniguchi *et al*., 2019; Peña *et al*., 2019). HMPV is transmitted by close or direct contact with infected secretions like; droplets, aerosols and fomites. Viral shedding occurs after incubation period of 7-9 days (Vinci *et al*., 2018). Genetic analysis and sequence variability of HMPV (G and F) genes, identified two main genotypes or major genetic sub-groups, designated (A and B), each with two minor sub-lineages (A1, A2, B1 and B2) (Schuster and Williams, 2018). HMPV is responsible for up to 10% of viral respiratory infections and most children under the age of 5 years have already been infected with the virus, the prevalence rate of HMPV infection in different epidemiological studies varies between (5-25%),

suggesting that HMPV is a ubiquitous and globally distributed virus (Schuster and Williams, 2019; Tambyah *et al*., 2019).

Human Respiratory Syncytial Virus is an enveloped virus with negative sense, single-stranded RNA, belongs to *Orthopneumovirus* genus, Pneumoviridae family. Based on the sequence and antigenic differences, HRSV was classified in two main antigenic subgroups A and B (Shi *et al*., 2017). HRSV is one of the most prevalent viruses in the world, infecting children and frequently identified as a major pathogen in adults, particularly the elderly. The clinical picture most closely related to HRSV infection is an upper respiratory infection, but in young children, the bronchiolitis and lower respiratory disease usually appear with obstruction in the small airway, and pneumonia may rarely develop, which leads to respiratory failure, and consequently apnea and death (Sun *et al*., 2019). HRSV is still causing annual outbreaks with no safe and effective vaccine developed yet. Most children are infected at least once in the first two years of age, and it is responsible for a quarter of all pneumonia cases in the first months of life worldwide, ranking as the second common cause of post-neonatal infant death following malaria (Cody Meissner, 2018; Perk and Ozdil, 2018).

Human Parainfluenza Viruses (HPIVs) include several closely related viruses of enveloped single-stranded negative sense RNA viruses belonging to *Respirovirus* genus Paramyxoviridae family, causes many respiratory diseases ranging from the common cold to flu-like syndrome or pneumonia; croup is the most obvious severe clinical manifestations (Branche and Falsey, 2016). By genetic analysis, HPIVs are grouped into four serotypes that are highly transmissible and responsible for up to 10%

of all hospitalized children under 5 years of age due to an acute respiratory infection (Linster *et al*., 2018; Zaki, and Keating, 2018). HPIV-1 and HPIV-3 is the second after HRSV as the main cause of severe respiratory tract illnesses. HPIV-3 infects most of infants during the first year of age, causing seasonal outbreaks leading to a significant burden of illness in children and responsible for 40% of pediatric hospitalizations for pneumonia and bronchiolitis, while, HPIV-1 is responsible of 75% of croup cases. Due to incomplete immunity during childhood, reinfection with HPIV can occur in adults accounting for (15%) of respiratory diseases (Branche and Falsey, 2016; Burrell *et al*., 2017).

Molecular techniques have greatly improved the diagnosis of respiratory pathogens, and are the new gold standard. The developed multiplex PCR amplification techniques and the current increasing use of it for detection of respiratory pathogens in URTIs and LRTIs have provided new data on the epidemiology and genetic diversity of these respiratory pathogens and have shown that most of hospitalized children with ARIs often infected with multiple viruses. On the other hand, it supplied the best understanding of the seasonal distribution of these pathogens and their association with particular clinical manifestations. The prevalence and genetic diversity of these viruses may vary depending on various factors such as geographical location, health and genetic factor of the community, climatic conditions as well as the impact of health reality. Epidemiological studies by using molecular techniques around the world have confirmed the prevalent of HMPV, HRSV and HPIV-1, 3 (Stover and Litwin, 2014; Das *et al*., 2018).

In Iraq, studies reported that the prevalence rates of HMPV infection ranged from (1.33% to 29.74%) (Abduljabbar *et al*., 2018; Hassan *et al*., 2018), HRSV prevalence rates were (1% to 36%) (AL-Bashar *et al*., 2017; Abduljabbar *et al*., 2018; Hassan *et al*., 2018), while, the prevalence rate of HPIV-1 and 3 was (32.17% and 13.21%) (Kadim, 2016). However, virological data concerning the seasonal distribution patterns and circulating genotypes of HMPV, HRSV and HPIV-1, 3 in the Iraqi community and for all age group are currently limited, although many comprehensive studies have investigated the phylogenetic analysis of HMPV isolates, in particular, has been published in many other countries.

In order to establish an epidemiological view and genotypic bases on the current situation of these viruses (prevalence and genetic diversity), particularly HMPV infection in this studied area, the current study was designed to achieve the following objectives,

1.2. Objectives:

1- Exploration of the prevalence rates of HMPV, HRSV and HPIV type 1 and 3 among patients clinically presented with respiratory tract infections.

2- Determination the co-infection with two or more of the respiratory viruses included in this study.

3- Figure out the associated socio-demographic risk factors.

4- Determination the seasonal distribution and genetic diversity (predominant genotype) of the local circulating HMPV and HRSV strains by analyzing the sequencing of nucleotides and phylogenetic tree of (G) and (F) genes of HMPV and genetic subgrouping of (F) gene of HRSV.

5- Genetic comparison of the local HMPV isolates with the others from different countries through the multiple and pairwise alignment of these isolates with international isolates available in the GeneBank database.

CHAPTER TWO

LITRETURE REVIW

Chapter Two

2. Literature Review

2.1. Human Metapneumovirus (HMPV):

2.1.1. Historical Review:

Respiratory tract infections are the most common cause of morbidity and mortality throughout the world, it ranks as the second main cause of death among children under 5 years old, respiratory viruses are one of the most common causes of acute respiratory infections (Monto, 2002; Hanada *et al*., 2018). In addition to human parainfluenza viruses (HPIVs), human respiratory syncytial virus (HRSV) and influenza viruses that are known to be the major cause of "upper and lower respiratory tract infections" in infant and children, In 2001, a team of Dutch researchers detected a new respiratory viral pathogen from the nasopharyngeal aspirates that obtained from children with RTIs clinically similar to those caused by HRSV. The newly discovered virus was named Human Metapneumovirus (HMPV) and it was a new addition to the growing list of infectious agents that were reported as an important cause of RTIs in humans, although retrospective epidemiological studies have confirmed the existence of anti-HMPV antibodies in human since 1958 in the Netherlands (van den Hoogen *et al.*, 2001; Freymuth *et al*., 2003).

Respiratory secretions samples were cultivated in tertiary monkey kidney cells, after 14 days the cytopathic effects were detected. Using electron microscopy, the supernatant of infected cells showed pleomorphic particles, enveloped with short projections like spikes. These

particles lack the hemagglutinating activity toward red blood cells in the guinea pig or turkey and its replication depends on the presence of trypsin. These virological data have made this newly discovered virus within the Paramyxoviridae family as a new member (Bernal *et al*., 2019). HMPV remained unidentified until 2001, although retrospective epidemiological studies have shown existence of anti-HMPV antibodies in human in 1958 in the Netherlands and this virus is not a new virus, van den Hoogen *et al*., (2001), mentioned the reasons of late discovery of HMPV, which were attributed to Firstly, certain virological laboratories employee the continuous cell lines in the isolation of the virus, in which HMPV does not seem to grow and propagate efficiently. Secondly, HMPV express lower replication kinetic classes in vitro, and that may persist undetected. Thirdly, multiplication of HMPV in cell culture appeared to depend on the trypsin and since many of the earlier attempts to detect respiratory pathogens did not use trypsin.

2.1.2. Classification of Human Metapneumovirus:

This virus was earlier classified as a member of the Paramyxoviridae family, which is separated into two subfamilies, Paramyxovirinae and Pneumovirinae – the latter includes two genra, *Metapneumovirus* to which the HMPV belongs, and *Pneumovirus*. However, after the last update taxonomy of the order Mononegavirales in 2016, the paramyxoviral subfamily Pneumovirinae was raised to family rank (Pneumoviridae) (Afonso *et al*., 2016; Amarasinghe *et al*., 2018).

Order: Mononegavirales

Family: Pneumoviridae

Genus: *Metapneumovirus*

Species: 1- *Human Metapneumovirus* (HMPV)

2- *Avian Metapneumovirus* (AMPV)

Depending on sequences homology, morphological and biochemical features, both Human Metapneumovirus and Avian Metapneumovirus - the latter which was identified in 1979 as a major cause of respiratory diseases in poultry- are closely related, based on homology of (Nucleoprotein N, Phosphoprotein P, Matrix M and Fusion F) genes. This genetic identity between the two viruses gave the assumption that HMPV was separated from AMPV-C about 200 years ago (van den Hoogen *et al*., 2002; Biacchesi *et al*., 2004; Yang *et al*., 2009; Piyaratna *et al*, 2011).

Phylogenetic analysis of (F, attachment protein G, P, and N) genes of HMPV have revealed two (main lineage) or main genotypes of HMPV, designated (A and B) each one has two minor sub-lineages (A1, A2a, A2b, B1, and B2), the conservation of F gene was high and relative within minor sub-lineages, (94%-97% nucleotide identity, 97%–99% amino acid identity) and (84%–86% nucleotide identity, 93%-95% amino acid identity) within (main lineage) or main genotypes (A and B) (van den Hoogen *et al*., 2004; Yang *et al*., 2009), in contrast, G and SH genes showed low conservations and greatest genetic variations within two (main lineage) or main genotypes groups (A and B), (50% -57% nucleotide identity, 30% -37% amino acid identity) and (76% –83%

nucleotide identity, 60% –75% amino acid identity) within minor sub-lineages (van den Hoogen *et al*., 2004; Huck *et al*., 2006).

2.1.3. Prosperities of HMPV:

2.1.3.1. Morphological Properties:

By using the electron microscopy, the supernatants of infected cells showed the presence of pleomorphic particles sized from 150-600 nm, varying from spherical particles with diameter of 209 nm to filamentous particles with a size of 282 x 62 nm (Figure 2.1; 2.2), its lipid envelope contain short projections (glycoprotein spikes) sized from 13-17 nm (van den Hoogen *et al*., 2001; Schildgen *et al*., 2011).

Figure (2.1): Negative-stain Electron Micrograph of Human Metapneumovirus. (Peret *et al*., 2002).

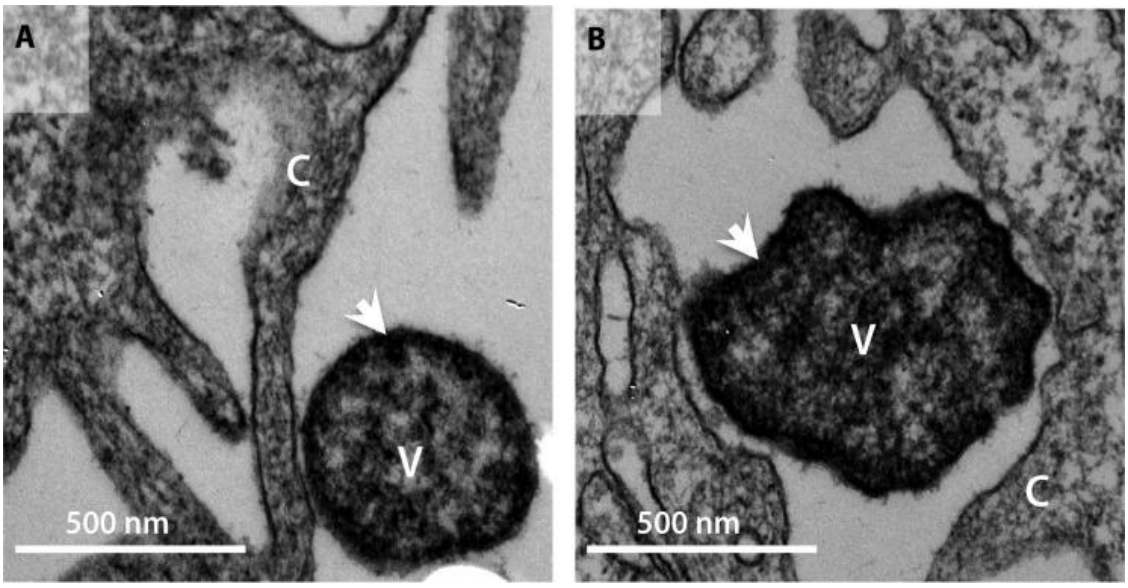

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Figure (2.2): Transmission electron microphotographs of HMPV particles (V) cooperate with monkey kidney cells (C). White arrow shows the envelope with surface glycoproteins. (Lamb and Parks, 2013).

2.1.3.2. Genomic Structure and Proteins of HMPV:

The HMPV genome involves a single-stranded RNA, negative -sense, genetic order of the HMPV genome is (3′-N-P-M-F-M2-SH-G-L-5′), which was presented by van den Hoogen and his colleagues in 2001 indicates the presence of eight genes encodes for nine proteins, Nucleoprotein (N), Large polymerase protein (L), Phosphoprotein (P) which acts as a co-factor for stabilizing (L) protein, allowing the formation of the ribonucleoprotein (RNP) during the replication of the virus (Feuillet et al., 2012), matrix (M) protein which covers the lipid envelope of the interior, M2 gene involved two open reading frames (M2-

1) which encode for transcription elongation factor and (M2-2) encodes for RNA synthesis regulatory factor, and transmembrane surface glycoproteins (fusion protein (F), glycoprotein (G) and small hydrophobic protein (SH) (Figure 2.3), the size of HMPV genome ranges from (13,280 to 13,378) nucleotides in length (van den Hoogen *et al*., 2001; van den Hoogen *et al*., 2002; Biacchesi *et al*., 2007).

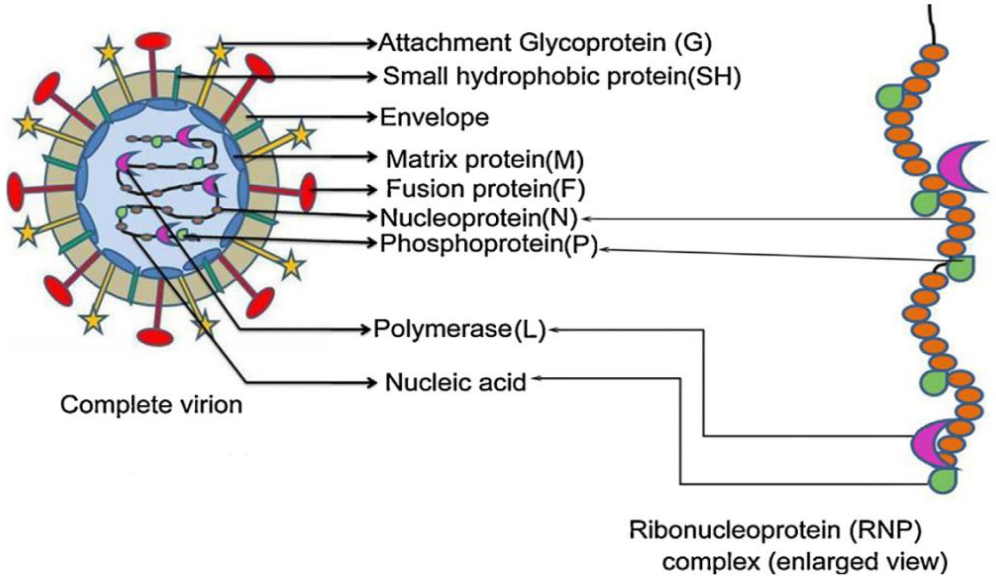


Figure (2.3): Schematic Diagram of Human Metapneumovirus and Ribonucleoprotein Complex (RNP). (Panda *et al*., 2014).

Despite the close similarity of the HMPV genome and HRSV as they share (50%) of the nucleotide sequence identity (figure 2.4), HMPV differs from HRSV in gene order in HMPV, the gene order is (F-M2-SH-G), while in HRSV is (SH-G-F-M2) and lack of HMPV for nonstructural genes (NS1) and (NS2), absence of these genes in HMPV is a notable and important difference between these two viruses, confirmed its classified

into two different genera within Pneumoviridae family (van den Hoogen *et al*., 2002; Lo *et al*., 2005).

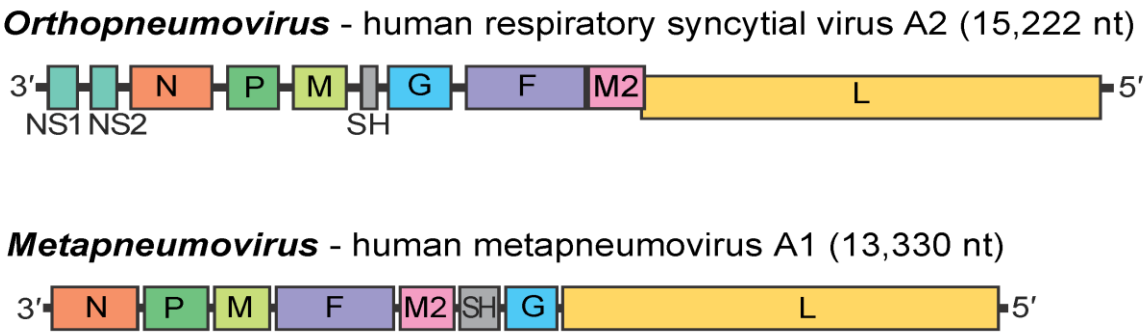


Figure (2.4): Schematic Diagram of HMPV and HRSV Genome (Rima *et al*., 2017).

The fusion (F) open reading frame (ORF) of HMPV is located adjacent to the M ORF and encodes for 539 amino acids (Biacchesi *et al*., 2004). The rate of amino acid sequence identity of (F) protein between HMPV and HRSV is (33%), each of which has 2 cysteine residues in (F2) and 12 in (F1). In addition, HMPV (F) protein is an integral membrane glycoprotein (classic type 1) that contains three potential N-linked glycosylation, cleave-active site and two heptad repeats that facilitated the fusion process between the viral envelope and cell membrane during the early stages of virus entry into the cell (figure 2.5) (van den Hoogen *et al*., 2002; Schowalter., *et al*., 2006).

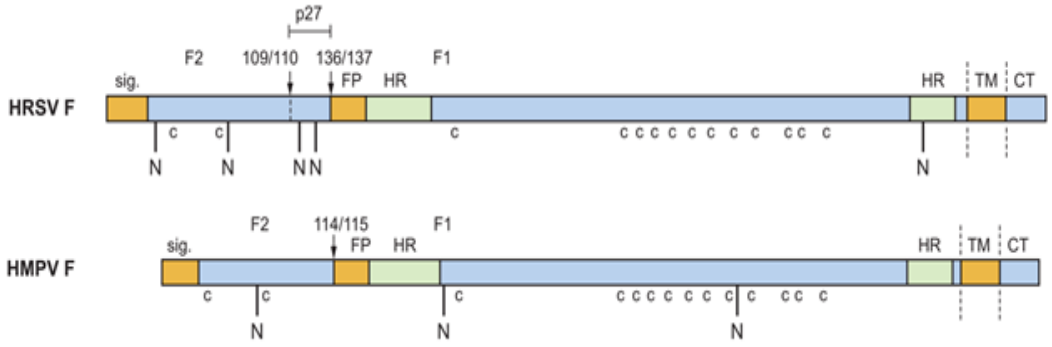


Figure (2.5): Structures of surface glycoproteins for HRSV (F, G, and SH), strain A2 and HMPV, strain CAN97-83. Hydrophobic domains are indicated as brown bars: "Sig., signal peptide; FP, fusion peptide; TM, transmembrane anchor; CT, cytoplasmic tail". Heptad repeats (HR) in (F) protein are green colored and cysteine residues that conserved between (F) proteins of both viruses are indicated underneath (c) (Lamb and Parks, 2013).

Fusion protein binds to cellular surface adhesion receptors α subunit and subunit β1 integrin (αVβ1), which mediate the fusion process between the viral envelope and the plasma membrane of the host cell and characterized as a direct target of neutralizing antibodies, the binding cellular integrin to HMPV (F) protein via specific recognition sequence, (arginine-glycine-aspartate) motif, giving evidence on the role of the (F) protein in the attachment process (Humphries *et al*., 2006; Cseke *et al*., 2009). Fusion proteins are first produced as precursors (F0), that contain one cleavage site, in vitro, require to proteolytically cleaved by exogenous trypsin protease to produce the active (F1-F2) form (disulfide-linked heterodimer) and *in vivo,* the cleavage of (F) protein depends on protease

that secreted from the lumen of the respiratory tract. The cleavage of the HMPV (F) protein is a strict requirement for triggering the fusion between the envelope of the virus and the cellular receptors on host cell in a way that is independent of the HMPV (G) protein (Cox *et al*., 2012; Lamb and Parks, 2013).

The attachment glycoprotein (G) ORF of HMPV, its location is adjacent to the SH gene and encodes for 236 amino acid for A1 and A2 isolates (Ishiguro *et al*., 2004), (G) protein is an integral membrane protein that has a glycosylated type II mucin-like protein features, mediates the attachment to cells by binding to cell surface glycosaminoglycans (GAGs) (Thammawat *et al*., 2008), the sequences of (G) amino acid revealed a high levels of serine, threonine and proline residues than HRSV (G) protein and shorter in length due to lacks cysteine noose structure and conserved central domain that exists in HRSV (G) protein (James and Crowe 2016).

Bao *et al*., (2008), identified HMPV (G) protein as a virulence factor, its importance lies in inhibition of the important immune responses through interacting with RIG-I and blocks it, (retinoic-inducible gene-I) an intracellular molecules which is responsible for response to viral RNA by induction of production antiviral mediators, type I interferons (IFNs) family and pro-inflammatory cytokines, which is an important innate immune system effectors (Matsumiya and Stafforini, 2010). Studies found that recombinant HMPV lacking (G, SH and M) genes have no essential role for HMPV replication in primate host such as hamsters, in the other hand, viruses lacking these genes have high levels of attenuation in

rodents and AMGs and highly immunogenic against wild-type HMPV, inoculation of animals with either the (ΔG) or (ΔSH/G) deletion mutant virus prompted a high titer of neutralizing antibodies to HMPV, these viruses lacking these genes are suitable for developing promising vaccines (Biacchesi *et al*., 2004; Biacchesi *et al*., 2005).

The small hydrophobic (SH) ORF, located nearby to (M2) gene encodes for (177-183) amino acid, is the largest transmembrane glycoprotein (type II) among the Pneumoviruses, all HMPV isolates discovered to date contain an (SH) gene, which indicates the importance of the function of this gene, but the specific role of (SH) protein remains unknown (van den Hoogen *et al.*, 2002).

Biacchesi *et al*., (2004), found that HMPV (SH) is completely unessential *in vivo*, Masante *et al*., (2014), determined that SH protein is a member of viroproteins, which can modulate the cellular membrane permeability to facilitate critical stages of the viral life cycle. HMPV can inhibit type I interferon (IFN) signaling, which is an important mediator of antiviral immunity, Hastings *et al*., (2016), explained the role of the HMPV (SH) protein in the down regulation of IFN signaling through the targeting of "signal transducer and activator of transcription 1" (STAT1) phosphorylation within infected cells.

Human Metapneumovirus (M2) consists of two overlapping ORFs: (M2-1 and M2-2), (M2-1) gene located nearby to the (F) gene, encodes for (187 amino acid), the first AUG start at nucleotide position 14 in mRNA and showed highest sequence identity with (M2-1) ORF of APV-

C (84%), Buchholz *et al*., (2005), demonstrated that HMPV (M2-1) protein was not essential for replication in vitro, in contrast to HRSV, which is critical protein for viral life cycle, a recent study presented by Cai *et al*., (2016), showed that phosphorylation of the HMPV (M2-1) protein is essential for RNA synthesis, replication, and pathogenesis in vivo.

The second ORF of M2 is (M2-2), this gene encodes for (71 amino acids), (M2-2) ORF probably starts with the AUGs at positions (525 and 537), overlapping with (M2-1) ORF by (53 or 41 nucleotides), respectively, HMPV (M2-2) protein plays a role in regulation of viral transcription and replication *in vivo* and *in vitro* (Buchholz *et al*., 2005), Ren *et al*., (2012), (2014), identified that (M2-2) as a novel virulence factor, which mediates immune system evasion through the inhibition of "mitochondrial antiviral-signaling protein"(MAVS)-dependent cellular responses and "myeloid differentiation primary response gene 88" (MyD88)-dependent cellular responses in human dendritic cells (DC), infect of human DC with deleted (M2-2) virus results in the production of IFNs at high levels, cytokines and chemokines, compared to the infected cells with wild-type HMPV, Suggesting the role of (M2-2) protein in inhibition of innate immune response in human dendritic cells.

The nucleoprotein (N) ORF is the first gene in HMPV genomic map, encodes for 394 amino acids, (N) ORF of HMPV shows a high identical with APV-C N ORF in the length and amino acid sequence (88%) and less identity with other paramyxoviruses (7-11%), the (N) protein protects the viral genome from nuclease activity through

encapsidates it, each of N, P (Phosphprotein) and L (polymerase) are proteins responsible for virus replication, and forms a helical ribonucleoprotien complex (RNP) by binding to negative-sense ssRNA genome (Barr *et al*., 1991; van den Hoogen *et al*., 2001). The matrix (M) ORF, is the third ORF in HMPV genome which encodes for 254 amino acids and showed a highest percentage of amino acid sequence identity with APV-C M protein (76-87%) and less identity (37-38%) and (10%) with HRSV and other paramyxoviruses, respectively, (M) protein encircles and surrounds the ribonucleoprotein (RNP) under the envelope and is thought that it is coordinate the viral assembly by interfacing viral nucleocapsids with the surface membrane protein in the other words, this protein play an important role in assembly and budding of the virus (van den Hoogen *et al*., 2002; Biacchesi *et al*., 2005; Leyrat *et al*., 2014b).

2.1.3.3. Life Cycle and Replication of HMPV:

The replication process of HMPV occurs in the cytoplasm and its initiate by the attachment of the glycoprotein (G) to heparin sulphate receptors on the plasma membrane of the host cell, followed by the fusion of the viral envelope with the plasma membrane through HMPV F protein (Schildgen *et al.*, 2011; Lamb and Parks, 2013) after the process of membranes fusion and uncoating of the virion, the viral ribonucleoprotein (RNP) which contains negative-sense RNA genome is released into the cytoplasm, (P, N and L) proteins separate from the viral RNA genome and in turn bind together to form polymerase complex. The viral RNA serve as a template for transcription of mRNA and replicate to synthesis antigenome RNA (+), which is used to produce genome RNA (-), the

genome RNA (-) then used to synthesize additional antigenome RNA (+) for combination into the virions progeny or serve as a template for secondary mRNA transcription, after translation of viral proteins, (N, P and L) are associated to the viral genome to form RNP and (M) protein transported to the plasma membrane, each of (F, G and SH) glycoproteins are transported through the endoplasmic reticulum ER to the Golgi apparatus to the plasma membrane of infected cell, the final step of infection includes assembly of new virions and released from the host cell membrane by budding, (figure 2.6), (Easton *et al*., 2004; Feuillet *et al*., 2012; Lamb and Parks, 2013).

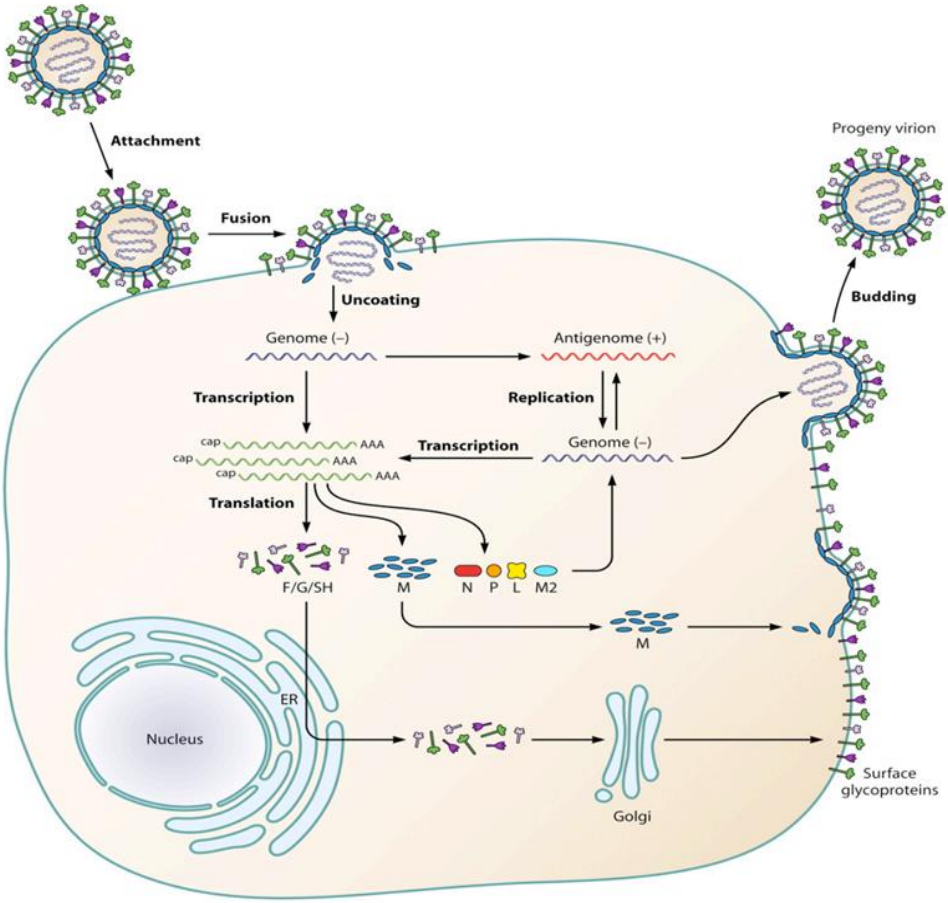


Figure (2.6): Human Metapneumovirus Life Cycle. (Schildgen *et al*., 2011).

2.1.4. Transmission, Pathogenesis and Immune response:

Human Metapneumovirus is probably being transmitted by close or direct contact with infected secretions like; droplets, aerosols or through fomites. Viral shedding occurs after incubation period of (7-9) days (Gralton *et al*., 2013; Talaat *et al*., 2013). Matsuzaki *et al*. (2013), studied HMPV spreading within the families, and they found that there were five days as an interval between the onset of symptom in an HMPV positive-index case and the beginning of symptoms in an exposed contact patient, and that HMPV can survive viable on non-porous surfaces for six hours.

Primary targets of HMPV infection are epithelial cells in the upper and lower respiratory tract and leukocytes in the lung. Infection with HMPV leads to many histopathological changes in the humans and mice lungs, including damage of the respiratory epithelial structure, sloughing and necrosis of respiratory epithelial cells, formation of hyaline membrane, loss of ciliation, roundred cytoplasmic inclusions and exacerbate of mucus production and inflammation of the lung interstitial parenchymal pneumonia or pneumonitis (Vargas *et al*., 2004; Darniot *et al*., 2005). Data concerning the pathogenicity of HMPV in humans are limited, and they were collected from individuals with lung disease. Vargas *et al*. (2004), in their first anatomic description of HMPV infection study, demonstrated that the later stages include:

> *"expansion of peribronchiolar lymphoid tissue, squamous metaplasia, hemosid-erin, and accumulation of intraalveolar foamy macrophages,*

> *indicating chronic/healing airway inflammation with a degree of concomitant airway obstruction and impairment of the mucociliary escalator".*

These features related well with the bronchiolitis and wheezing symptom that noted clinically in patients infected with HMPV.

Alvarez and Tripp (2005), showed that a late and minimal immune response, as well, delayed of cytotoxic T-cells activity with poor clearance of virus during the primary infection, may lead to persistent infection with HMPV. Primary infection of HMPV in BALB/c mouse provokes weak innate and abnormal acquired immune responses in which the induce of aTh2-type cytokines occur at the late stages of infection, this coincides with an increase depression of interleukin-10 and persistent replication of the virus in the lung, Interference HMPV with superantigen-induced T-lymphocyte activation through infection of dendritic cells causing restricted the proliferation CD4+ T cells and result in the production of impaired long-term immunity (Guerrero-Plata, 2013). As evidenced by studies, that comparing vaccinated mice and infected mice experimentally *in vitro*, the acquired immune response provoked by HMPV fails to effectively removing the virus from the respiratory airways, leading to an extreme inflammatory response and damage to the lung. Moreover, after disease resolution, there is a weak development of T and B memory cells, which are thought to have a role in re-infections and circulation the virus in the community (Cespedes *et al*., 2016).

Laham *et al*. (2004), revealed through the examination of nasal washes of hospitalized infants that infection of HMPV induced lower amounts of pro-inflammatory cytokines, IL-12, tumor necrosis alpha (TNF-α), IL-8, IL-6, IL-1β and anti-inflammatory cytokines, IL-10 compared to RSV. Previous studies using (human airway epithelial cell lines) indicated that HMPV, as well, HRSV and HPIVs are capable to block or to modulate the innate immune response by targeting of molecules participated in signaling through blocking type 1 interferon (INF-1) responses in signaling pathway; studies have demonstrated the involvement of G, M2-1, P and SH (Dinwiddie and Harrod, 2008; Ren *et al*., 2012; 2014; Hastings *et al*., 2016). Seroprevalence of HMPV antibodies increases among children from 6 months and reaches approximately (100%) in the age of 5–10 years. Reinfections are common in spite of the high levels of antibody in the adults. Studies have suggested there may be a limited cross-protective immunity between different HMPV strains HMPV, and these antibodies may not be sufficient to prevent infection (Brooks *et al*., 2013).

2.1.5. Clinical Manifestations of HMPV:

Human Metapneumovirus is an important cause of upper and lower respiratory tract infections in children. Clinically, the signs and symptoms that caused by HMPV indistinguishable from those caused by HRSV, especially, in young children (van den Hoogen *et al*., 2001; van den Hoogen *et al*., 2004; Mullins *et al*., 2004; Zhang *et al*., 2009; James and Crowe, 2016). In children, the symptoms of upper respiratory infection include, cough that is present in (90-100%) of cases, rhinorrhea, and fever, fever is more common and account for (52-80%) of HMPV cases than

HRSV. Pharyngitis, conjunctivitis, laryngitis, rhinitis, hoarseness and sore throat are occurring in variable frequencies (Boivin *et al*., 2003; Williams *et al*., 2004; Brooks *et al*., 2013). Acute otitis media (AOM) is another symptom that has been documented in children infected with HMPV (Brooks *et al*., 2013). Schildgen *et al*. (2005), isolated HMPV from (13%) of children diagnosed with (AOM), while, Nokso-Koivisto *et al*. (2012), noted that HMPV appeared in (24%) of children who have upper respiratory tract infection and AOM.

The signs and symptoms of lower respiratory tract are wheezing, with incidence rates ranging from (22%-82%), and the most clinical manifestations among hospitalization children are bronchiolitis in (47-84%), pneumonia in (11-17%), exacerbation of asthma in (11-25%) and croup (Boivin *et al*., 2003; Edwards *et al*., 2013). The clinical features of HMPV infection in adults similar to those in children, it depends on the age and the person's health status, and may be asymptomatic or have symptoms ranging, from mild to severe respiratory infections requiring hospitalization (Walsh *et al*., 2008). However, in immunocompromised patients, the infections of HMPV can be severe and fatal. In this regard, the infection of HMPV has been diagnosed in chemotherapy recipients, solid-organ recipients, stem cell transplant recipients, and chronic cardiopulmonary patients (Englund *et al*., 2006; Hopkins *et al*., 2008; Souza *et al*., 2013).

2.1.6. Epidemiology of HMPV:

Since its initial discovered as new player among respiratory viruses pathogen in 2001, HMPV was detected in most regions of the world and have been isolated in respiratory specimens from patients with respiratory tract infection from all ages (Hamelin *et al*., 2004).

The outbreak of HMPV infection has seasonal differences, in temperate climates, it circulates mainly in the late of winter and early of spring, several studies demonstrated that the peak of HMPV infection may occur following the HRSV and influenza seasons between March and April, while, other studies indicated that the seasons of HMPV infection frequently overlapping with the HRSV infection seasons and other respiratory pathogens in addition, many of evidence revealed that different HMPV sub-lineages can be co-circulate through the same season (van den Hoogen *et al*., 2001; Chan *et al*., 2007; Rafiefard *et al*., 2008; Pilger *et al*., 2011; Mizuta *et al*., 2013). However, Arbefeville and Ferrieri (2017), demonstrated that the peak of HMPV activity was in March and December in Minnesota, USA (figure 2.7).

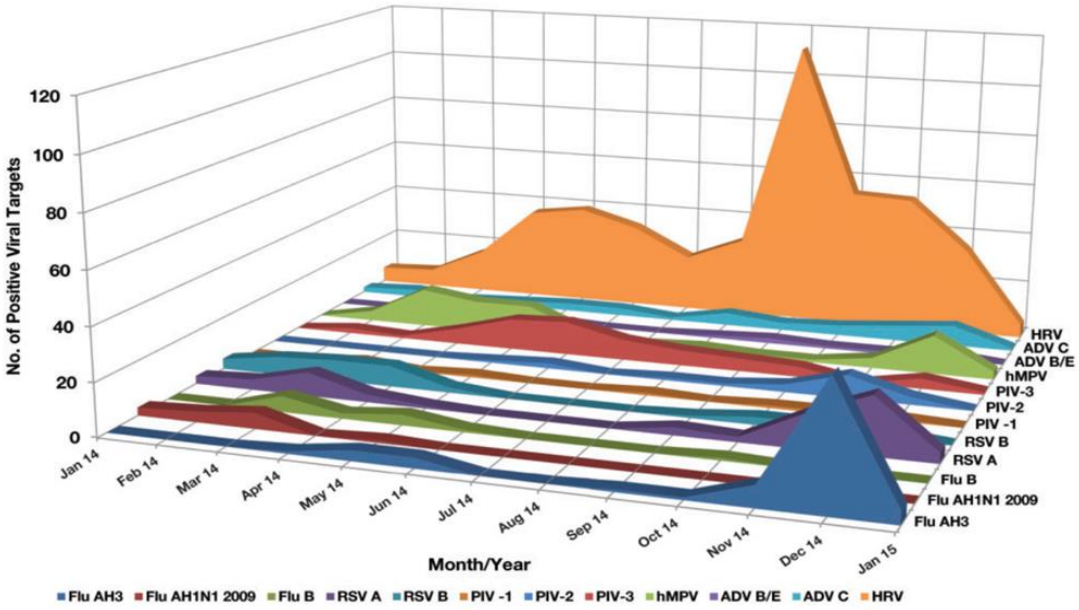


Figure (2.7): Human Metapneumovirus, Human Respiratory Syncytial virus and Human Parainfluenza virus 1 and 3 circulation activity according to the months and years from (2014 to 2015). HMPV had two distinct and separate peaks of circulating activity in March and December. (Arbefeville and Ferrieri, 2017).

Levels of HMPV activity are low during the summer months, a virus has been observed circulating during the summer in the southern hemisphere and the peak activity of HMPV in the sub-tropics regions appear in the spring and early of summer month (Peiris *et al*., 2003). Worldwide, the infections of HMPV are account for at least (5-7%) of hospitalized children with respiratory tract infections, whereas, elderly and immunocompromised individuals as well at risk. In general, HMPV infection is responsible for at least (3%) of patients with RTI, who attend a general practitioner, and children under two years of age are at risk of

developing severe HMPV infection (figure 2.8) (van den Hoogen *et al*., 2004).

The incidence of HMPV infection in different epidemiological studies varies between (5-25%), suggesting that HMPV is a ubiquitous and globally distributed virus (Schuster and Williams, 2019).

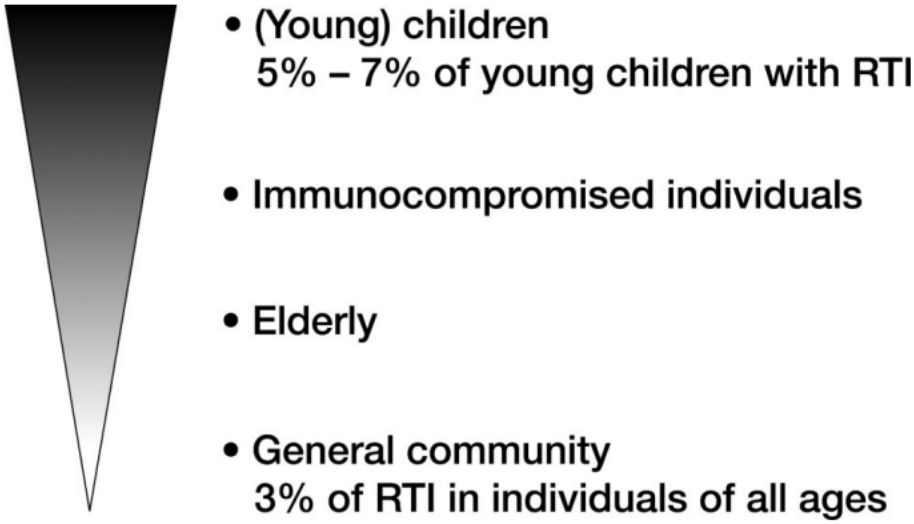


Figure (2.8): Prevalence of HMPV Infection (van den Hoogen *et al*., 2004).

Worldwide epidemlogical studies had shown that the most of children by the age five years have already been infected by HMPV, the infection of HMPV and HRSV in young children are common, by the age of two years, approximately (100%) of children are infected with HRSV, whereas, the primary infection of HMPV occurs in a slightly older children. van den Hoogen *et al*. (2001), showed that about (50%) of children by the age of two years were seropositive for HMPV and (100%) are seropositive by the age of five years. HMPV is not new respiratory

pathogen. Furthermore, the authors found through their studies and serological evidences that HMPV has been prevalent in humans since 1958.

Human Metapneumovirus and HRSV share many epidemiological characteristics, such as: (i) they are prevalent all continents; (ii) they have a seasonal distribution with the major incidence in the winter and spring months; (iii) they are also probably transmitted through airborne respiratory droplets (Kahn, 2006). Similarity in distribution seasons of many viruses causing respiratory diseases leads to co-infection between HMPV and other viruses, but the role of HMPV as a co-pathogen in this co-infections is completely unclear, Therefore, the probability of increased severity of the disease that caused by co-infection with HMPV and HRSV is not known. Greensill *et al*. (2003) and Semple *et al*. (2005), suggest that co-infection with HMPV and HRSV is related to severe bronchiolitis, in the other hand, recent studies indicate that co-infection with MPV plus RSV did not cause differences in the severity of disease or requiring worse clinical manifestations (Escobar *et al*., 2015; Moe *et al*., 2017).

Although the majority of children > 90% are seropositive for HMPV at the age of five years, recurrent infection can occurs frequently during life similar to those caused by HRSV, repeat infection of HMPV has been reported in (1–9%) of adults annually (Falsey, 2008). Recurrent infection of HMPV can occur frequently even during the early childhood in the case of simultaneous co-circulation of different HMPV strains (Ebihara *et al*., 2004). Pavlin *et al*. (2008), revealed that recurrent infection of HMPV

causing disease at rates equal to that of primary infections, HMPV may have a greater impact on older children than previously expected and may cause major outbreaks. The effect protection of HMPV antibodies may vary, in spite of, a high seropositivity in all ages, after the age of 5 years, recurrent infection of HMPV can occur due to insufficient immune response acquired during primary infections, and/or infection with different HMPV genotype and the virus don't induces solid immunity because this virus develops transient or no viremia, so the circulatory Abs (IgM and then IgG) are limited, the prevention of infection is responsibility of local secretory AB (IgA), but unfortunately, the life span of these Abs is short, this explains why high rate of reinfection occurs (van den Hoogen *et al*., 2001; Ebihara *et al*., 2004; Williams *et al*., 2006).

Several studies indicated that populations at high risk to severe HMPV infection are young children and the children under two years in particular (Garcia-Garcia *et al*, 2006; Arnott *et al*., 2013), other have shown that the severity of the disease is greater among children under six months of age than that of older children (Papenburg *et al*., 2012), while, some have demonstrated that infants aged (6-12) months, at a high risk as well (Hahn *et al*., 2013). Children with history of chronic diseases such as (congenital heart diseases, bronchopulmonary dysplasia, asthma, trisomy 21, neuromuscular diseases, and additional to chronic lung diseases) also at high risk for developed severe illness (Hahn *et al*., 2013; Pancham *et al*., 2016). Prematurity has been suggested as an important risk factor that increases the severity of HMPV infection (Schuster and Williams, 2013). Immunosuppressed individuals are at a high risk of severe HMPV

infections, reduced the ability to control virus replication in immunosuppressed hosts may be the basis of increased the severity of HMPV infection (Chu *et al*., 2014), as well, HMPV is an important cause of acute respiratory tract infections in elderly >65 years and adults with a comorbidity disease, such as chronic obstructive pulmonary disease COPD, cancer, asthma, or lung transplantation (van den Hoogen, 2007). Boivin *et al*. (2007), presented histopathological results associated with fatal pneumonia induced by HMPV in an (89) years old woman.

Regarding HMPV phylogenetic analysis, it has been documented that many viruses, in particular RNA viruses may change rapidly their nucleotides due to high and successive mutation rates (Jenkins *et al*., 2002; Schildgen *et al*., 2011). Therefore, new viral genotypes may emerge that are able to escape the human immune system, and this may present an additional challenge related to developing antiviral treatment and vaccines (Papenburg *et al*., 2013). As previously mentioned, HMPV has originated from AMPV about 200 years ago, while the present HMPV appeared approximately (100) years ago. It is clear that each of the major genotypes (A and B) have occurred through the last (30-50) years, while each of the minor subtypes has presumably occurred in less than 30 years old. Therefore, it is important to determine which strains of the virus are circulating in different parts of the world (Schildgen *et al*., 2011; Papenburg *et al*., 2013).

2.1.6.1. Surveillance Epidemiological Studies:

The evolution of molecular diagnostic technologies such as real-time RT-PCR granted more chances to determine the prevalence of diseases caused by HMPV, epidemiological studies from different parts of the world have recorded different prevalence rates of HMPV, and in general the overall prevalence is affected by several important factors such as seasons of infection, geographical region, type of specimens, diagnostic methods and the criteria of patients participating in the study from which the specimens were taken (age, gender, disease status and the risk factors).

Studies have used several types of specimens to detect HMPV such as (nasopharyngeal swabs and aspirates, nasal swabs and aspirates, throat swabs). Limited studies from Iraq have shown using different molecular diagnostic techniques, that the prevalence of HMPV in Kurdistan and Baghdad were (13.4%, 16%, 29.74%) respectively among children patients suffering from respiratory diseases and flu-like illness (Aziz, 2015; Atyah *et al*., 2017; Hassan *et al*., 2018). In Kuwait, HMPV infection was found to be responsible for (5%) of hospitalized patients (Al-Turab *et al*., 2015). Epidemiological studies from Jordan, Saudi Arabia and Egypt reported that HMPV infection rate were (8.6%, 10.9% and 16%) respectively (Schuster *et al*., 2015; Amer, 2016; El-Sawaf *et al*., 2017). By using real-time multiplex RT-PCR for detection (21) respiratory pathogens, Bayrakdar *et al*. (2016), demonstrated for first time low prevalence rates of HMPV infections (2.6%) out of (2900) patients.

Several studies in Iran reported that the prevalence rate of HMPV among children with acute respiratory infections varies from (0.49% to 54.4%) (Arabpour *et al*., 2008; Shatizadeh Malekshahi *et al*., 2010; Parsania *et al*., 2016). In Pakistan, Norway and Brazil, HMPV was detected in (7%, 7.3% and 20%) respectively of hospitalized children with RTIs (Moe, 2017; Yousafzai *et al*., 2018; Gregianini *et al*., 2018). Molecular epidemiology studies in China, Japan, Guatemala and Kenya, demonstrated that HMPV infection in hospitalized children was (10.2%, 3.7%, 9% and 4.8%) respectively (Nidaira *et al*., 2012; Zhang *et al*., 2012; McCracken *et al*., 2014; Owor *et al*., 2016).

2.1.7. Laboratory Diagnosis:

2.1.7.1. Types of the Samples and Handling:

Diagnosis of viral infections in respiratory tracts depends on the type and quality of the specimens and appropriate handling of the samples before laboratory studies. The presence of adequate and appropriates clinical samples avoids the appearance of the false negative diagnosis. Nasal wash, aspirate or mid-turbinate swabs are the best type of specimens for HMPV, HRSV and HPIV diagnosis in infants and also young children (Williams *et al*., 2017). The classical specimens that used for diagnosis of the virus by culture and for cytokines or antibodies detection are nasal washes, while, nasopharyngeal and mid-turbinate swabs are not sensitive like a nasal wash for diagnosis of the virus by culture and direct immunofluorescent antigen detection, but it can give

good diagnostic results in the molecular testing methods (Williams *et al*., 2017).

2.1.7.2. Virus Isolation:

The original isolations of HMPV were cultivated on Tertiary cynomolgus Monkey Kidney cells (tMK) or rhesus monkey kidney cells (LLC-MK2), in a medium containing trypsin, that is required for supporting the growth of the virus (Schildgen *et al*., 2011). Since then, a number of cell lines different in efficacy and sensitivity are used for cultivating the virus, Reina *et al.* (2007), assessed the efficiency of (LLC-MK2), (Hep-2), (MDCK), (Vero cell) and (MRC-5) cell lines, by shell vial technique and the incubation time, in the isolation of HMPV, the study found that the sensitivity of (LLC-MK2) cell line was (100%), (68.7%) for (Hep-2), (28.1%) for (Vero cell), (3.1%) for (MDCK) and (0%) for (MRC-5). Monitoring cultures of clinical specimens should be for 21 days for cytopathic effect (CPE) and may be needed for sub-culture before the appearance of (CPE).

The characteristics of cytopathic effects of HMPV on (LLC-MK2) cell line include, small, granular, refrainment, rounding cells without large syncytia, HMPV infections can be confirmed by using either immunofluorescent assay with HMPV-specific antibodies or RT-PCR (Kolli *et al*., 2008; Falsey, 2015). Cultivation of HMPV in cell lines is difficult due to the slow growth of the virus and because of the limited published information describing the characteristics of the virus's growth

in the laboratory and its capability to replicate in various cell lines; this method has been replaced by more sensitive and rapid methods.

2.1.7.3. Immunofluorescence Assays:

Immunofluorescent assay (IFA) can be used for direct diagnosis of HMPV in respiratory samples and cell culture by using (virus-specific monoclonal antibodies). In the direct immunofluorescence assay (DFA), scales cells or exfoliated cells and other debris are concentrated from the host secretions by centrifugation, and then reacted with a fluorescence-labelled antibody specific to the virus, and visualized by immunofluorescent microscope (Collins and Karron, 2013). In contrast to direct immunofluorescence assay (DFA) tests for detection HRSV that are characterized by high sensitivity and specificity, the current (DFA) test for HMPV detection is characterized by a lack of sensitivity (Landry *et al*., 2008). In a study conducted using two commercial (DFA) test and Reverse Transcriptase PCR (RT-PCR) for detecting HMPV in (515) nasopharyngeal aspirates, it was found that the (DFAs) have sensitivity of (62.5-63.2%) and high specificity (99.8-100%), affirming that both (DFAs) lacked sufficient sensitivity (Vinh *et al*., 2008; Aslanzadeh and Tang, 2011).

2.1.7.4. Serological Assays:

Serological tests are more appropriate in the epidemiological surveys and vaccines studies but less in clinical studies. Sera from acute and convalescent phases are required for serological diagnosis of HMPV or HRSV and HPIV, and because of the seropositivity is approximately common by the age of five years, the serological diagnosis also are required for a fourfold increase in the antibody titer after 2 to 3 weeks or seroconversion to confirm the recent infection. Serological diagnosis is mostly accomplished by the enzyme immunoassay by using complete virus lysates of the descriptive strains of two main genotypes or recombinant HMPV (F) or (N) proteins as antigens (Walsh *et al*., 2008; Feuillet *et al*., 2012). In this regard, two ELISA techniques using the viral protein (N) or (F) expressed in the prokaryotic or "recombinant vesicular stomatitis virus (VSV)" were developed for detection of antibodies against HMPV (Hamelin and Boivin, 2005; Leung *et al*., 2005). Liu *et al*., (2007), demonstrated that HMPV (N) protein expressed in baculovirus can be used for detection of antibodies response against the virus. The nucleocapsid (N) protein of HMPV was selected as the viral antigen candidate for ELISA technique because (N) protein is highly conserved (Bastien *et al*., 2003).

2.1.7.5. Molecular Diagnosis of HMPV:

A part from the difficulties that arise due to slowly growing HMPV on cell culture, molecular diagnosis has become a gold standard for diagnosis of HMPV, and has replaced many other diagnostic methods. Van den Hoogen *et al*. (2001) was the first to use arbitrarily primed-PCR for random amplification of HMPV in respiratory specimens from children. After this initial report, the same author and other researchers developed PCR tests targeting the unique sequence within the HMPV genome including (N, L, M, and F) genes (van den Hoogen *et al*., 2003; Maertzdorf *et al*., 2004; Li *et al*., 2007).

The most sensitive assay for identification HMPV in clinical specimens to date is RT-PCR, many of initial clinical studies used the RT-PCR assay including PCR primers that targeting the polymerase (L) gene sequence of HMPV and the nucleotide sequence of the HMPV (L) gene PCR product was used to identify the virus (van den Hoogen *et al*., 2001; Williams *et al*., 2004). However, this assay was not so equally sensitive to HMPV strains of all four known genetic sub-lineages. Therefore, later, real-time RT-PCR tests were developed, primers and probes that used in these assays targeting the most conserved sequences in the HMPV nucleoprotein (N) gene and designed to match and detect all four genetic sub-lineages of HMPV. Since Real-time RT-PCR technique is characterized by a very high level of sensitivity and specificity, in the real-time PCR system, fluorescence dyes were used for detecting a PCR product that was generated. Thus, the linear correlation between these PCR product that was generated and the intensity of fluorescence can be

monitored, also, it can be measured the PCR amplified product after each PCR thermo-cycle. (Cote *et al*., 2003; Maertzdorf *et al*., 2004; Kessler, 2007; Choudhary *et al*., 2014).

The results of Maertzdorf *et al*. (2004), showed that the sensitivity of this assay for strains of HMPV of all four genetic sub-lineages are similar and approximately 10-fold sensitive more than the previously reported conventional RT-PCR assay that targeting the sequence of HMPV (L) gene. Recent studies have used multiplex real-time RT-PCR for diagnosis the co-infections with multiple respiratory pathogens simultaneously including HMPV (Malhotra *et al*., 2016). Reijans *et al*. (2008), described a new RespiFinder assay to detect (15) respiratory viruses including HPMV in one reaction by using multiplex ligation-dependent probe amplification.

2.2. Respiratory Syncytial Virus (HRSV)

2.2.1. Historical Review:

The first discovery of HRSV was in 1956. Initially, it was not associated with respiratory diseases among infants. Morris and his colleagues (1956) isolated this new virus from a group of 14 chimpanzees which were observed to have symptoms of colds and coryza outbreak, and thus originally was called “chimpanzee coryza agent” (CCA). Subsequently, Chanock *et al*. (1957), confirmed that the virus caused respiratory diseases in humans after they isolated it from two infants, one suffering from laryngotracheobronchites and the other from bronchopneumonia, which cannot be distinguished from (CCA).

Serological studies have shown that the neutralizing antibodies against (CCA) was detected in most infants and school-age children, "Chimpanzee coryza agent" (CCA) was appropriate renaming it as a respiratory syncytial virus to refer to its clinical characteristics and laboratory manifestations of the virus (Chanock *et al*., 1957; Welliver Sr and hall, 2019).

2.2.2. Classification and Structure:

Based on similarities in some properties such as the syncytial appearance in cell culture, internal structure of the particle, eosinophilic inclusions, HRSV was originally classified with the Newcastle diseases and parainfluenza group viruses. However, HRSV is antigenic and does not hemagglutinate red blood cells. Subsequently, nucleocapsid diameter of the HRSV has been determined to be among the largest of the paramyxoviruses compared to those smaller in influenza viruses. Further studies of HRSV structure resulted in the classification of the virus within the order Mononegavirales, Paramyxoviridiae family, Pneumovirinae subfamily and genus *Pneumovirus* (Collins and karron, 2013). Depending on the last update taxonomy of the order Mononegavirales in 2016, HRSV has been classified as a member of Pneumoviridae, and genus *Orthopneumovirus* (Afonso *et al*., 2016; Amarasinghe *et al*., 2018).

Human respiratory syncytial virus has an irregular spherical appearance (figure 2.9), with medium size (120-300 nm), RNA single-stranded, non-segmented, negative-sense genome, the helical nucleocapsid is packaged in an envelope that consists of lipid bilayer

acquired during the budding process from the plasma membrane of the infected cell (figure 2.10). The envelope contains three structural transmembrane surface glycoproteins (spikes) with 11-12 nm in length, and a thistle appearance under electron microscopy. The attachment protein (G), fusion protein (F) and small hydrophobic protein (SH), are played an important role in viral infectivity (attachment the virus to the ciliated epithelial cells, facilitated the fusion of the viral envelope to the plasma membrane, and inserted into infected cells membrane as a viroporins protein that increase the permeability of membrane, respectively). HRSV lacks a hemagglutinin and neuraminidase; HMPV by its (G) protein can agglutinates murine erythrocytes (Wertz and Moudy, 2004; Gan *et al*., 2008; Collins and karron, 2013).

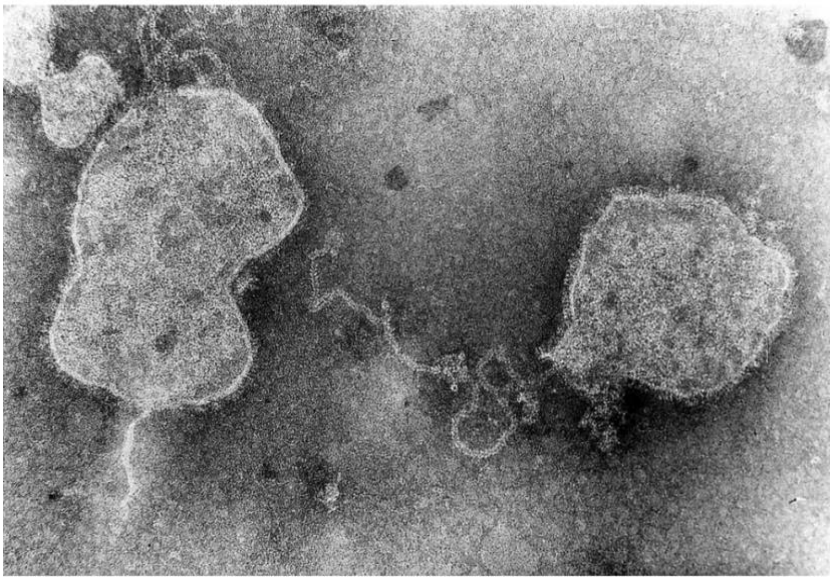


Figure (2.9): Negative-contrast Electron Micrograph of Human Respiratory Syncytial virus (HRSV). (Walsh and Hall, 2015).

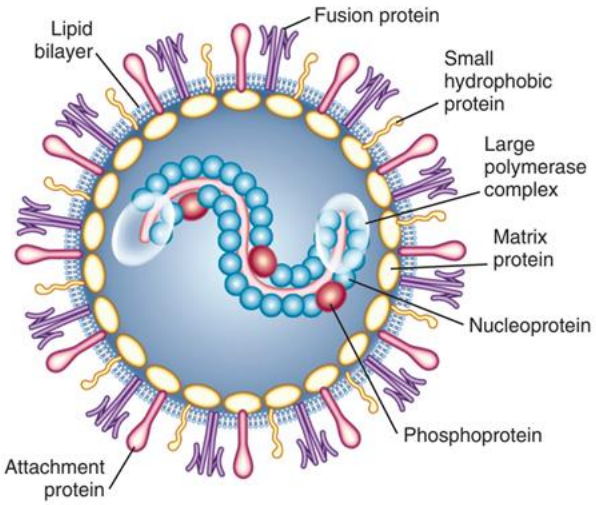


Figure (2.10): Human Respiratory Syncytial Virus (HRSV) Structure. (Hall, 2001).

The RNA genome of HRSV (strain A2), has been entirely sequenced and was found to consist of (15,222) nucleotides and contain (10) genes that encode (11) proteins. The order of genes is 3′NS1-NS2-N-P-M-SH-G-F-M2-L-5′ (Figure, 2.11). The (ORF) of (M2) gene separates into two overlapping (ORFs) which are (M2-1 and M2-2). Functionally, (M2-1 and M2-2) structural proteins have a role in the replication and transcription process as transcription processivity factor and transcriptional regulatory factor respectively.

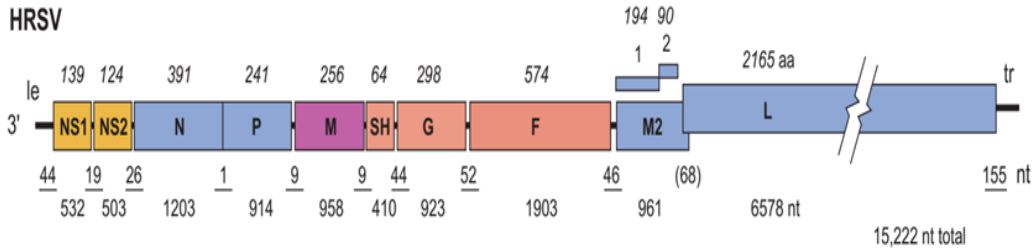


Figure (2.11): Genomic Organization of Human Respiratory Syncytial Virus (HRSV) strain A2 (Collins and karron, 2013).

Four structural proteins nucleoprotein (N), phosphoprotein (P), polymerase (L) and (M2-1) are associated with Ribonucleoprotein complex; all of these proteins have important roles in the encapsidation, RNA replication and transcription process. The larger structural matrix (M) proteins constitute the inner face of the viral envelope and are important in assembly of the virus, (Spann *et al*., 2004; Gan *et al*., 2008; Mitra *et al*., 2012; Collins and karron, 2013). Unlike HMPV, the genome of HRSV contains two nonstructural proteins (NS1) and (NS2) that strongly interferes and inhibits the cellular induction and signaling of the type I and III interferon (IFN) activity (figure, 2.12). This suppresses the components of the innate immunity and subsequently, affects the acquired immune response to HRSV (Bachrach *et al*., 2003; Collins and karron, 2013; WelliverSr and hall, 2019).

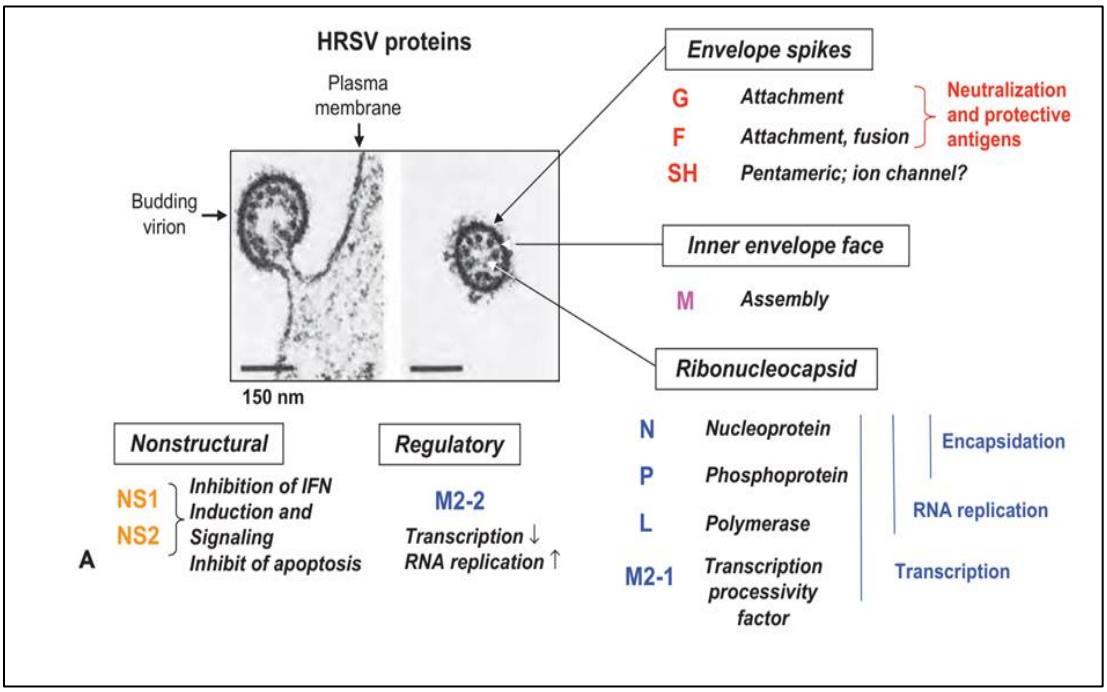


Figure (2.12): Human Respiratory Syncytial virus (HRSV) Proteins and Major Function (Collins and karron, 2013).

Serological studies have confirmed that all HRSV strains belong to single serotype that can be divided into two main antigenic subgroups (A) and (B), each can be further subdivided into multiple subgroups consisting of separate genotypes (Rebuffo-Scheer *et al*., 2011). The genome of HRSV (A) and HRSV (B) subgroups was shared (81%) of nucleotide identity (Collins and karron, 2013). Depending on the phylogenetic analysis of the sequences of the C-terminal region of (G) protein, the second hypervariable regions (HVR2) which is used for HRSV genotyping study and its evolution, the subgroup (A) was classified into (14) genotypes (GA1 to GA7, SAA1, CB-A, NA1 to NA4 and ON1) (Ren *et al*., 2014), while, subgroup (B) was classified into (27) genotypes (BA1 to BA12, BA-C, SAB1 to SAB4, GB1 to GB4, URU1 to

URU2, CB-B, CB-1, BA-CCB and BA-CCA) (Zheng *et al*., 2017).The major genetic variations between two subgroups (A) and (B) reside in (G) protein; a (type II glycoprotein), that is a heavily glycosylated with N- and O-linked sugars, and containing a (cytoplasmic tail), a (transmembrane region) and an ecto-domain with centric conserved region which is flanked by pair of hypervariable regions (HVR), reflecting the relative low in antigen relatedness between (A) and (B) subgroups of only (1% to 7%) for (G) protein, compared to (50%) for (F) protein (Rebuffo-Scheer *et al*., 2011). The amino acid sequencing of HRSV (G) protein differs by approximately (50%) between the HRSV (A) and HRSV (B) strain groups, also the variation between intra-group strains occurs almost within the (G) protein. In contrast, within (F) protein, the sequence of amino acid is conserved approximately well, and therefore neutralizing antibody to HRSV (F) protein is cross-reactive mainly between the two strains. The (F) proteins of prototype HRSV strains from (A and B) groups have homology higher than (90%) in amino acid sequence and a high ratio of antigenic relatedness (Collins and karron, 2013).

Molecular epidemiology studies have shown that the viruses of HSRV subgroup (A) and (B) circulate simultaneously during epidemic season, the presence of two groups and their rotational infections occurrence can increase the ability of HSRV to infect the individuals how previously exposed and bypass pre-existing immune defenses. Different HRSV genotypes can be co-circulate during the season of the epidemic, and one was dominance over another depending on the years and locations (de-Paris *et al*., 2014). Epidemiological reports documented, the

rapid spreading of a new HRSV (A) genotype (A/ON1), replacing the ancestor (A/NA1) in many countries. The rapid circulation of the genotype (ON1) may be associated with duplicated sequence in the (G) gene (Tabatabai *et al*., 2014). Agoti *et al*. (2015), were conducted a phylogenetic analysis of HRSV genomes using a "novel full-genome deep-sequencing process", and they discovered that most HRSV (A) variants have been observed worldwide, while the local HRSV (B) genomes that encoded for high level of variation was less surveillance and description globally.

2.2.3. Clinical Manifestations of HRSV Infection:

Primary infections of HRSV were generally included the lower respiratory tract, especially among infants during the first months of life. Most frequently, bronchiolitis, pneumonia and tracheobronchitis, lanryngotracheobronchitis (crop) was the least frequent and is estimated to be less than (2% to 10%) of cases. In infants with LRI there was a rhinorrhea often associated with anorexia, cough may occur simultaneously, and become clearer and productive, shortly after cough onset, the child may start to wheeze (Borchers *et al*., 2013; CDC. 2019). In more severe cases, the coughing and wheezing progress, and the child becomes dyspneic. Apnea was an important complication of acute HRSV infection, occurs in (1% to 20%) of young infants hospitalized with HRSV illness (Ralston and Hill, 2009). Upper respiratory tract symptoms are always accompanied by lower respiratory tract illness, or infection may be limited to the upper respiratory tract, which is commonly associated with fever and acute otitis media (AOM) in young children.

AOM was a more frequent complication of HRSV infection, HRSV infection have been detected in (74%) of children who developed AOM (Rebuffo-Scheer *et al*., 2011). Adults may have been recurring HRSV infection that occurs during successive years, especially, medical personnel and those individuals taking care for young children, infection usually characterized by pharyngitis, rhinorrhea, cough, sore throat bronchitis, and low grade fever, in elderly adult HRSV causes severe infection and may cause more mortalities among them than in children (Barlam, 2005).

2.2.4. Epidemiology of HRSV:

The main ways of the HRSV transmission are mainly contact closely with infected persons, direct inoculation into the nose and eyes of contagious secretions and contaminated surfaces or materials (fomites), large particles droplets produced by sneezing and coughing of an ill individual may transmit HRSV to other people within the radius is about 3 feet. HRSV can be recovered from contaminated surfaces for up to (6) hours (Hall, 2000; Junge *et al*., 2010). The infection of HRSV occurs worldwide, its main pathogenicity occurs in the infants and young children, in elderly individuals, and in people of all age groups with high-risk conditions, particularly, chronic heart and lung diseases. All the geographical regions of the world have experimented with HRSV activity each year. In general, HRSV outbreaks occur in communities during the cold months in temperate regions and in warm and tropical environments tend to take longer periods, with almost continuous activity (Gamba-Sanchez *et al*., 2016). HRSV infection was observed in (43%) of

those hospitalized patients diagnosed with bronchiolitis and in (25%) of pneumonia patients compared to (11%) of those hospitalized patients diagnosed with bronchitis and (10%) of those with croup. (Collins and Karron, 2013). HRSV infection is more prevalent in infancy than other respiratory viruses, prospective studies showed that 50% to 70% of infants have been infected with HRSV in the first year of life and virtually all of them are infected by the age of 2 years, Worldwide, HRSV is responsible for approximately 33 million case of the lower respiratory tract infections, three million hospitalizations, and nearly 199,000 childhood deaths (Brooks *et al*., 2013; Collins and karron, 2013; Rabarison *et al*., 2018; Welliver Sr and hall, 2019).

Human Respiratory Syncytial Virus (HRSV) is a prevalent and highly infectious virus, it exhibits tropism to the superficial epithelium cells in the respiratory system, where the defenses of the host are relatively ineffective, highly infectivity of HRSV is attributable to its ability to use (G) and (F) proteins in attachment to and fusion with host cells and employs (NS1), (NS2), and (SH) proteins in evading and blunt the host immune response. In particular, HRSV shows antibody trap activity, which provokes expression of type I IFN antagonists, fractalkine and TLR antagonists, which inhibits apoptosis and interfering with macrophage and dendritic cells (DC) function (Cowton *et al*., 2006).

Pathogenesis of HRSV is complicated and variable. The illness range from mild to fatal and comprise a wide spectrum of (acute upper and lower respiratory tract disease) manifestations, which can range from mild rhinitis to bronchiolitis and pneumonia. The major pathologic outcomes observed in the infant's lungs dying from HRSV bronchiolitis, are

> *"peribronchiolar mononuclear infiltration; Necrosis of the epithelium of the small airways; plugging of the Lumina with necrotic epithelial cells, leukocytes, and fibrin; and hyperinflation and atelectasis"* (Johnson *et al*., 2006).

2.2.4.1. Surveillance Epidemiological Studies:

Atyah *et al*. (2017), out of 195 nasopharyngeal swabs specimens, reported low prevalence of HRSV (6.67%) among children < 15 years old in Baghdad, in Tikrit province, AL-Bashar *et al*. (2017), also reported a low prevalent of HRSV (1%) out of 400 hospitalized children suffering from RTIs. Two studies from North Iraq showed that the prevalence of HRSV among hospitalized children with acute respiratory infection was (27% and 20.4%) (Aziz *et al*., 2016; Hassan *et al*., 2018). Furthermore, out of 45 adult with pneumonia, the infection with HRSV was detected in (11.63%) (Fadhil, 2015). The positivity rate of HRSV infection in certain neighboring countries was as follows; (37.9%) in Turkey, (19.38%) in Iran, (26%) in Saudi Arabia and (25.2%) in Kuwait (Hacimustafaoglu *et al*., 2013; Faghihloo *et al*., 2014; Ahmed *et al*., 2016; Madi *et al*., 2018).

In Pakistan, the HRSV infection was detected in (17.8%) out of 169 children with severe pneumonia (Ali *et al*., 2013). During the period 2006-2015, Moe, (2017), reported that the prevalence rate of HRSV infection was (28.7%) among children with acute RTIs. A systematic review and meta-analysis including 21 studies in Iran, 153 in china and 67 studies in Africa found that the prevalence of HRSV infection in people with ARTI was (18.7%, 18.7% and 14.6%) respectively (Zhang *et al*., 2015; Salimi *et al*., 2016; Kenmoe *et al*., 2018).

2.3. Other respiratory viruses associated with RTIs

2.3.1. Human Parainfluenza virus (HPIV):

2.3.1.1. Historical Review:

The first discover of four serotypes of Human Parainfluenza virus types (HPIV-1, HPIV-2, and HPIV-3, HPIV-4) was between 1956 and 1960, following the use of cell culture and hemadsorption techniques in studies of pediatric respiratory infection, HPIV-1, 2, 3 were initially recovered from infants and children with lower respiratory tract infection (LRTI), while, HPIV4 was isolated from children and young adults with moderate upper respiratory tract infection (URI). Shortly after their discovery, HPIV- 1, 2, and 3 were recognized as the main cause of croup, pneumonia and bronchiolitis caused specifically by HPIV-3 (Chanock, 1956; Chanock *et al*., 1958). As a group, HPIV- 1, 2 and 3 are second only to HRSV as an important cause of severe viral respiratory illness in infants and children, while HPV-4 is less common and less severe. The name parainfluenza was originally formulated because some signs of the disease are similar to influenza, plus some viral

morphological characteristics are similar to influenza viruses too. The particle is medium in size, has the same hemagglutination– neuraminidase (NH) activates and lipid envelope (Karron and Collins, 2013).

2.3.1.2. Classification and Structure:

Human Parainfluenza virus serotypes (1 and 3) belongs to the Paramyxoviridea family, *Respirovirus* genus, *Human Respriovirus 1* and *Human Respriovirus 3* species, respectively (Amarasinghe *et al*., 2018). HPIV-1 and HPIV-3 are enveloped viruses, pleomorphic appearance with medium size 150-200 nm, single-stranded negative sense RNA, non-segmented. The genome of all HPIVs consist of approximately 15.000 nucleotides and six genes encode for at least 6 structural proteins 3′-N-P-M-F-HN-L-5′ , depending on the serotypes of HPIVs, (P) gene encodes for one or more of the nonstructural proteins, (C) protein in HPIV-1 and (C and D) proteins in HPIV-3 (figure, 2.13) (Palmer *et al*., 2014; WelliverSr, 2019).

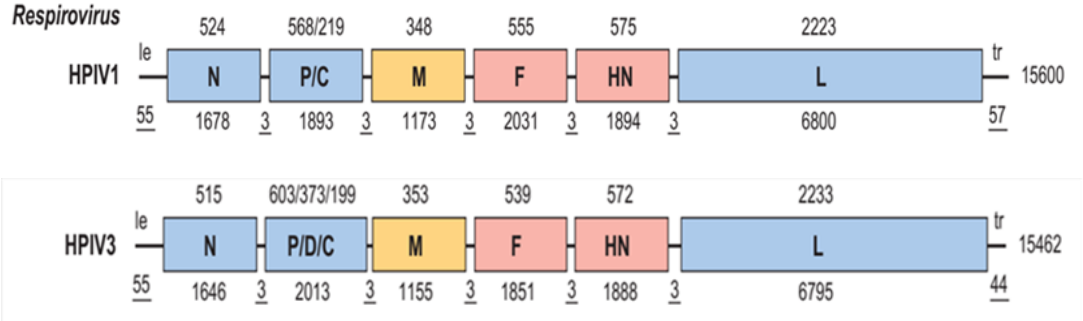


Figure (2.13): Gene Maps of Human Parainfluenza Virus (HPIV-1 and HPIV-3) (Karron and Collins, 2013).

The lipid envelope of the virus is studded and covered with surface glycoproteins spikes, “hemagglutinin-neuraminidase” (HN) and fusion (F) proteins, which play an important role in the pathogenesis of the virus through their roles in the attachment and fusion to the host plasma membrane and facilitates the infection during the entry of the virus to the host cell. Furthermore, these proteins are important immunogenic antigens and form the main antigenic targets for the host neutralizing antibodies. The HPIVs (HN) protein also facilitates the release of progeny virions from the infected cell by cleaving the residual sialic acid (figure 2.14) (Henrickson and Savatski, 1997).

The replication of HPIVs occurs in the cytoplasm, where the nucleocapsid (N) protein binds the viral RNA genome to form a helicalnucleocapsid structure (P), and (L) proteins are then tightly associated with N-RNA complex to form the nucleocapsid core of the virus. The binding of the (N) protein to the viral genome creates a template for the (L) and (P) proteins for transcription and replication of the HPIVs genome (Moscona, 2005; Tamaki *et al*., 2015).

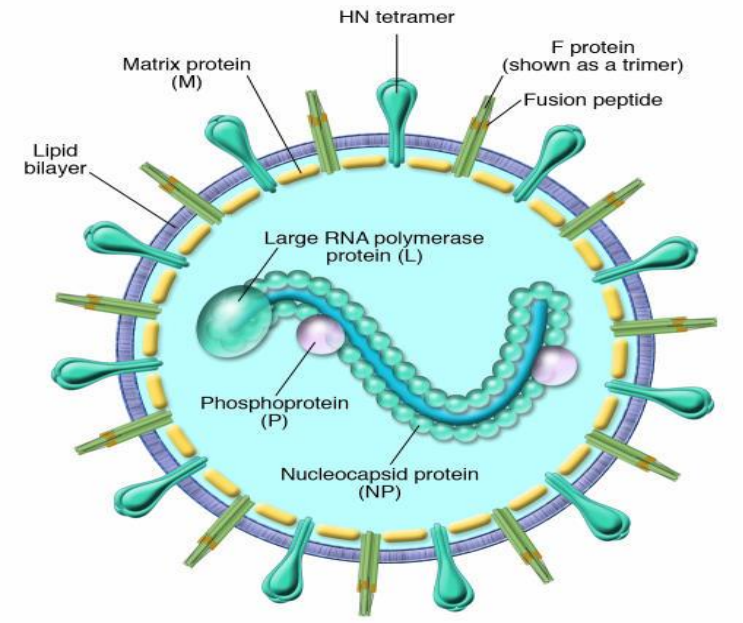


Figure (2.14): A schematic Graph of the Human Parainfluenza Virus (HPIV). (Moscona, 2005).

2.3.1.3. Clinical Manifestations of HPIV-1 and HPIV-3 Infection:

Although there was no particular virus associated with classic clinical manifestations, specific HPIV serotypes are more associated with certain clinical signs. HPIV-1 was associated with laryngotracheobronchitis (croup), in children. Croup is the term used to include a different group of diseases that infect the larynx, trachea, and bronchi, usually appears with fever, hoarseness, laryngeal obstruction, rhinorrhea, dyspnea and Pharyngitis. Occasionally, HPIV-3 serotype may be responsible for acute croup in the adult (Bjornson and Johnson, 2008). Bronchiolitis, which is the most dominant symptoms and include fever, wheezing, tachypnea, and air trapping. Bronchiolitis was most commonly observed in children under one year of age. While all serotypes of HPIV

were capable of causing bronchiolitis, HPIV-1 and HPIV-3 are more frequently noted. Moreover, HPIV-3 is responsible for many cases of bronchiolitis among hospitalized children more than HPIV-1 (Lee *et al*., 2011; Brooks *et al*., 2013). Pneumonia was a major clinical manifestations of lower respiratory tract infection that can be caused by all HPIV serotypes, but HPIV-1and HPIV-3 were accountable for nearly (10%) of outpatient pneumonia cases and (7% to 15%) of hospitalized children cases of all ages, as with bronchiolitis. HPIV-3 causing more high percentage of pneumonia cases among hospitalized patients than that caused by HPIV-1 (Abedi *et al*., 2014; Jain *et al*., 2015). Additionally, Otitis media and pharyngitis may increase the complications of upper respiratory tract infection caused by Human Parainfluenza viruses in young infants and older children. HPIVs are frequently isolated from children with and without a previous infection of otitis media, from cases of the otitis media that have been related with HPIV, the serotypes HPIV-1 and HPIV-3 are the most frequently detected (Wiertsema *et al*., 2011).

Several reports have documented the occurrence of outbreaks of HPIV infection on transplant units and children and adults who are immunocompromised, particularly those with cellular immune deficiency, who were highly susceptible for the development of severe, and occasionally fatal, HPIV infections (Chemaly *et al*., 2012).

2.3.1.4. Epidemiology of HPIV-1 and HPIV-3:

Human parainfluenza viruses, especially HPIV-3, are highly communicable. Like HRSV, the most common ways of transmitting HPIVs are direct contact, (close person-to-person) by large infectious respiratory droplets, and by contaminated fomites with respiratory secretions, as well as through self-inoculation by hand contaminated with infectious respiratory secretions (Englund and Moscona, 2014). HPIVs replicates solely in the respiratory epithelium cells, resulting in infect the nose and nasopharynx epithelial cells and the clinical signs appear after an incubation period of 2 to 4 days. HPIVs mostly infect the large airways of the lower respiratory tract, resulting in croup. In the most severe cases, the infection of HPIVs may disseminate to the distal airways and cause bronchiolitis or pneumonia. Significant replication of HPIVs had been detected in the nose and lungs within 24 hours of infection, with peak replication occurring between 2 to 5 days. Necrosis and sometimes proliferation of the bronchiolar epithelium associated with the destruction of respiratory ciliated epithelial cells in children with bronchiolitis and pneumonia (Bjornson and Johnson, 2008; do Carmo Debur *et al*., 2010).

Human parainfluenza viral serotypes have a distinct seasonal and epidemiological patterns; depending on the location and years. In temperate regions, the occurrence of HPIVs infections appears correlated with seasonal climatic conditions, while, in tropical regions, HPIVs do not appear seasonal variation, HPIV-1 usually occurs in the autumn of odd-numbered years and frequently causes the largest outbreaks of croup among children by the age 3-5 years and account for (75%) of pediatric

hospitalizations of croup cases (Henrickson *et al*., 2003). HPIV-3 is the most prevalent, and endemic strain. The infection occurs mostly in the spring and summer and continues in autumn, especially, in the absent of other HPIV outbreaks, serological studies have shown that HPIV-3 is responsible for (50%) of pediatric hospitalizations of respiratory tract infection in (bronchiolitis and pneumonia) in children during the first year of life (Weinberg *et al*., 2009; Branche and Falsey, 2016). In adult's hospitalization with pneumonia, HPIV-3 was detected in (3.1%) of patients, and HPIV-1 in (2.5%) (Marx *et al*., 1999).

2.3.1.4.1. Surveillance Epidemiological Studies:

There were limited epidemiological studies of the HPIVs in Iraq, Kadim, (2016), found that the prevalence of HPIVs was (45.38%) among hospitalized infants and preschool children suffering from acute respiratory infections in AL-Muthanna province, Iraq, HPIV-1 was responsible for (32.17%) of infections, while, HPIV-3 was responsible for (13.21%). In Kuwait, Essa *et al*. (2017), found that the prevalence of HPIVs was 2.6% among pediatric intensive care and intensive care unit patients, HPIV-3 was the most frequently (71.8%) followed by HPIV-1, 23.1%. In Saudi Arabia, the prevalence rate of HPIV-3 during two consecutive seasons was (9.59%) and (2.8%) (Almajhdi *et al*., 2012). HPIVs were detected in (10.4%) of Brazilian children, (57.7%) was positive for HPIV-3 and (11.5%) for HPIV-1 (Thomazelli *et al*., 2018). Studies from Viet Nam and Taiwan reported high prevalence rates of HPIV-3, (13.87%, 50% and 73.4%) in compared to HPIV-1, (1.55%,

11.17% and 9.8%) respectively (Xiao *et al*., 2016; Linster *et al*., 2018; Wu *et al*., 2018).

2.3. Vaccines

Human paramyxoviruses including Human Respiratory Syncytial Virus, Human Metapneumovirus, and Human Parainfluenza virus - 3, are responsible for most of "acute upper and lower respiratory tract" diseases in humans, especially in infants, children, elderly, and immunocompromised patients, which leads to an important economic loss, higher costs of health care, and passionate burdens. Despite great efforts, there are no current licensed vaccines possible for these viruses (Zhang *et al*., 2014).

Use of formalin-inactivated vaccine against these viruses is not suitable, it causes enhancing in lung damage when the reinfection occurs with the same viruses, enhanced diseases may be caused by (1) “Th2-biased T-cell-memory responses”, (2) hypersensitivity to formaldehyde, and/or (3) production of immature antibodies and its weak ability to recognized the HRSV epitopes of natural infections, (Delgado *et al*., 2009). Other types of vaccines such as subunit vaccine although it’s safe and promising and boosting immune responses in the individuals with previously infected with HMPV, however, it provokes short protecting responses to primary infection (Herfst *et al*., 2008). Live attenuated vaccines of HMPV are promising, for both infants and young children, however, it may be difficult to find a balance between a satisfactory level of attenuation of the virus and obtaining a sufficient degree of

immunogenicity. Ren *et al*. (2015) believe that an adequate and best understanding of the important roles of viral proteins in host immune responses may lead to the development of effective prophylactic vaccines.

Recombinant vaccine candidates against HMPV or HRSV that have been designed up to date by using other viruses to express HMPV antigens, or vice versa can contribute to overcoming some major difficulties in developing new HMPV vaccines, and achieve a good balance between immunization capacity of immunogenicity and attenuation of the live recombinant vaccine, by reverse genetics, can be easily modified the genomic of the virus in the form of cDNA according to the design of the vaccine, This technique is based on the transfecting of permissive cells with a plasmid coding for the of the viral genome and satellite plasmids that coding for all the proteins required to form a ribonucleoprotein complex (RNP) that responsible for initiating the transcription of viral genes (figure, 2.15) (Yu *et al*., 1995; Beaty *et al*., 2017).

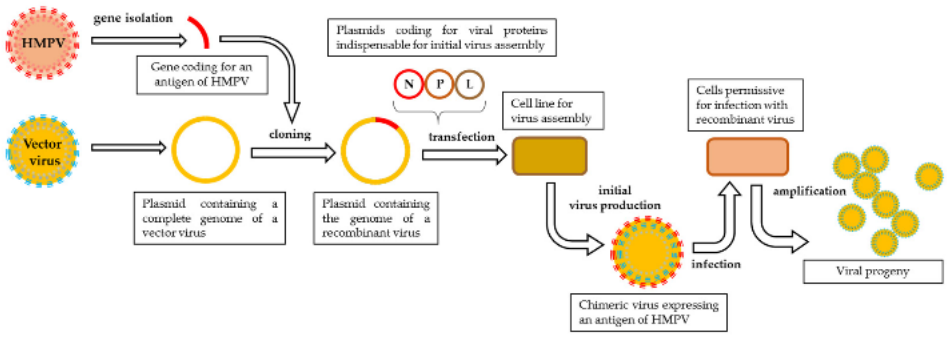


Figure (2.15): Schematic Representation of Reverse Genetics Pipeline. (Ogonczyk Makowska *et al*., 2020).

CHAPTER THREE

SUBJECTS, MATERIALS AND METHODS

Chapter Three

3. Subjects, Materials and Methods

This chapter includes a description of the subjects, materials as methods plus the statistics analysis applied on data accumulated throughout this study.

3.1. Timing and Conducting Area:

According to the approved study proposal, this study was conducted in the Diyala province, Iraq, for the period from 16/January/2018 to 30/October/2019. Collection of the samples was extended throughout two seasons; the first one was from 16/January/2018 to 15/may/2018 and the second one from 1/November/2018 to 16/December/2018. Therefore it was a case comparative study. The study samples were collected at Baqubah Teaching Hospital, Department of Respiratory and Chest Diseases and Department of Ear, Nose and Throat (ENT) plus Al-Batool Teaching Hospital for Maternity and Children, Department of Pediatrics (attended and hospitalized) and from patients attending outpatient clinics. It was worthy to mention that samples were collected after obtaining the written and official approval documented by the Research Ethics Committee in the Diyala Directory of Health and under the direct supervision of specialized doctors as well as the approval of the study participants or their parents.

3.2. Subjects:

3.2.1. Study Population:

Three hundred twenty three patients from those clinically suspected as having RTIs were enrolled; (185 patients were in the first season and 138 in the second season). Clinical data were obtained at the time of clinical examination by doctors as well as from medical records. Based on clinical, X-ray and laboratory findings, these cases were categorized as follows:

1. **Upper respiratory tract infections (URTIs)**; Tonsillitis, otitis media, coryza, rhinorrhea, pharyngitis, acute laryngitis, sinusitis and sore throat.
2. **Lower respiratory tract infections (LRTIs)**; Includes five diagnoses based on clinical manifestations and radiological finding**s,** bronchiolitis, bronchitis, pneumonia, influenza-like illness, dyspnea/tachypnea, cough, wheezing.
3. **Chronic.** Samples were collected from patients suffering from chronic respiratory conditions, such as asthma exacerbation (Matu, 2015; Moe, 2017). The details of clinical information's was listed in Appendix (1)

The sample was taken from the patients under the supervision of the specialist doctor and after the approval of the study participants or their parents' consent. Data of patients participating in the study were collected by using a questionnaire specially designed for this purpose as seen in Appendix (1). This questionnaire was divided into two parts, first part

included socio-demographic information and the second part included clinical information.

3.2.2. Sample Collection and Transportation:

In this study, three types of respiratory samples were collected and used for molecular detection of HMPV, HRSV and HPIV-1/3, these are throat, nasal and nasopharyngeal swabs, for this purpose a standard ∑-Virocult swabs (mwe /UK) with polyurethane foam tip was used, Throat swabs were collected from pharynx and tonsillar areas, the nasopharyngeal swabbing was achieved through the entrance of the swab carefully into one nostril and pushing it straight back along the floor of the nasal canal for several centimeters until reaching the nasopharynx. Because of the pain caused by nasopharyngeal swabs insertion during specimen collection from patients of different ages, especially for children, who have small and narrow nasal passages, so the researcher used the nasal swabs. Ipp *et al*., (2002) showed for the first time, that nasal swabs were painless and provoke much less discomfort for children than nasopharyngeal swabs and are accurate, suitable and for viral detection. The swabs were gently rotated for (5-10) seconds to loosen and capture large numbers of epithelial cells during specimen collection from the throat cavity, the nasal turbinate and nasopharynx passages, after swabbing, swab plastic applicator shaft was broken off from the breakpoint, and each cellular foam-bud tip (absorbent) swab was placed into the vial containing (1 ml) of the viral transport medium (VTM) (mwe / UK and *E.coli* Ltd. / Russia) and transported by cooled box to the blood

bank department in the hospital and stored at (-80°C) until molecular analysis was carried out.

3.3. Materials.

3.3.1. Instruments and Equipment:

Table (3.1): Instruments and Equipment used with Manufacturing Company and Country of Origin.

No.	Equipment & instrument	Company/ Origin
1.	Standard Sigma Virocult®/ swab MW951S	Medical wire (mwe/ UK)
2.	Sterile Swab Stick (nylon shift)	RP BIOTECH / India
3.	Micro spin 12, High-speed Mini-centrifuge	Bio San /Latvia
4.	V-1 plus, Personal Vortex	Bio San /Latvia
5.	Micropipettes (2-20 µl)	Germany
6.	Sensitive Balance	KERN KFB/Germany
7.	Incubator	Jrad/ china
8.	MultiGene™ OptiMaxPCR Thermal Cycler	Labnet / USA
9.	Electrophoresis	CBS Scientific / USA
10.	Gel documentation system	
11.	Real Time PCR thermocycler	SaCycler-96 / Italy
12.	UV Trans illuminator	VilberLourmat / France
13.	Microwave	Gosonic /

14.	Water Distiller	HENAN / China
15.	Combi-spin	Bio San / Latvia
16.	Microspin	Bio San / Latvia
17.	Bio TDB-100, Dry block thermostat built	Bio San / Latvia
18.	Deep freeze (-80, -20)	ALS / Italy
19.	Refrigerator for 2–8 °C	Shark
20.	AURA TM PCR Cabinet	Italy
21.	PCR premix tubes	
22.	Eppendorf tubes	
23.	Cooled box	China

3.3.2. Biochemicals:

Table (3.2): The Biochemicals used with Manufacturing Company and Country of Origin.

No.	Chemical	Company and Origin
1.	Agarose gel	Conda / USA
2.	Red safe staining solution	Intron / Korea
3.	Ladder (100bp)	Kapa /USA
4.	Pre-mix PCR	Intron / Korea
5.	TBE buffer 10 X	Conda / USA
6.	Primers	Integrated DNA technologies /USA
7.	DNase/RNase-Free Water	Zymo Research/ USA

8.	Ethanol	Zymo Research/ USA
9.	small vial with (1.0) ml of Viral Transport Media	Medical wire (mwe/ UK)
10.	AmpliSens® Transport Medium	*E.coli* Ltd. / Russia

3.3.3. Diagnostic Kits:

Table (3.3): The Molecular Extraction and Diagnostic Kits with the Manufacturing Company and Country of Origin.

No.	Kits	Company and Origin
1.	The Quick-RNA™ Viral RNA Extraction kit	Zymo Research / USA
	Viral RNA Buffer 1 Viral Wash Buffer 2 DNase/RNase-Free Water Zymo-SpinTM IC Columns	
2.1.	REVERTA-LReverse Transcriptase kit	Ecoli Ltd./Russia
	RT-G-mix-1 RT-mix Revertase (MMlv) DNA-buffer	
2.2.	GoScript™ Reverse Transcription System	Pro-mega / USA
	GoScript™ Reverse Transcriptase GoScript™ 5X Reaction Buffer	

	MgCl2 (25mM) PCR Nucleotide Mix Oligo(dT)$_{15}$ Primer Random Primers Nuclease-Free Water Recombinant RNasin® Ribonuclease Inhibitor	
3.	AmpliSens® ARVI-screen-FRT Multiplex Real-Time PCR kit	Ecoli Ltd. /Russia
	Internal Control STI-rec (IC) PCR-mix-1-FL-F HRSV – HMPV PCR-mix-1-FL-F HPIV 1/3 PCR-mix-2-FRT Polymerase (TaqF) Positive Control cDNA HRSV – HMPV (C+$_{HRSV - HMPV}$) Positive Control cDNA HPIV 1/3 (C+$_{HPIV\ 1/3}$) Positive Control STI-88 (CS+) TE-buffer Negative Control (C–)	
4.	GoTaq® Probe qPCR System	Promega / USA
	GoTaq® Probe qPCR Master Mix CXR Reference Dye Nuclease-Free Water	

5.	Maxime ™ PCR PreMix Kit (i-Taq™) (Conventional PCR)	Boca Scientific / USA
	i-Taq DNA Polymerase DNTPs Reaction buffer (10X) Gel loading buffer	

3.3.4. Primers and Probes:

All primers and probes used in this study were selected from online published literatures after careful and thorough review. The suitability and specificity of these primers and probes has been confirmed in the NCBI primer basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Two sets of PCR primers for Attachment (G) and Fusion (F) Human Metapneumovirus genes has been used in the conventional PCR amplification in order to get PCR products used in the sequencing method for genotyping of the virus and phylogenetic tree analysis (Zhang et al., 2012; Aziz, 2015). Set of primers and probes were used in Real-Time PCR assay for Fusion (F) genes for determined the antigenic subgroups of Human Respiratory Syncytial virus and another set of primers used in the conventional PCR amplification for Attachment (G) gene for determined genotype of HRSV (Hibino *et al*., 2018). These primers were used for positive samples detected by a real-time Polymerase Chain Reaction (RT-PCR), Multiplex Detection. Primers and probes were provided by (IDT Company/ USA), table (3-4).

Table (3.4): Primers and Probes used in this Study.

Primer	Sequence (5′ – 3′)	TM (°C)	Amplicon size (bp)
HMPV (G) gene			
Forward	5′- GAGAACATTCGAGCAATAGACATG -3′	53.8	1035
Reverse	5′- AGATAGACATTAACAGTGGATTCA -3′	51.5	
HMPV (F) gene			
Forward	5′- ATGTTGGAGAACCGTGCGA -3′	57.0	465
Reverse	5′- CCCTACTCTGTTGCTGCCAA -3′	57.4	
HRSV-A (F) gene			
Forward	5′- ATCAGAAAAAGTTAATGTCCA -3′	47.4	118
Reverse	5′- ACACAATATAGTGGTAATTGT -3′	48.7	
Probe	5′-FAM/TCAAATAGTTAGACAGCAAAGTTACTCT/3BHQ1-3′	54.3	
HRSV-B (F) gene			
Forward	5′- GTTTAACAAGGACTGATAGAG -3′	48.0	153
Reverse	5′- TGTTACAAAGGCTGACTT-3′	48.6	
Probe	5′-FAM/ACTGATCCTGCATTATCACARTACCA/3BHQ1-3′	56.9	
HRSV-A (G) gene			
Forward	5′-GAAGTGTTCAACTTTGTACC -3′	49.0	487
Reverse	5′-GGCAAATAACAATGGAGTTG -3′	49.8	
HRSV-B (G) gene			
Forward	5′-AAGATGATTACCATTTTGAAGT -3′	48.0	507
Reverse	′5- GGCAAATAACAATGGAGTTG -3′	49.8	

3.4. Methods:

3.4.1. Viral RNA Extraction:

3.4.1.1. Assay Principle:

Viral respiratory ribonucleic acid (RNA) from the throat, nasal and nasopharyngeal swabs specimens was extracted by a commercial kit (Zymo Research/USA) according to the manufacturer's protocol (The Quick-RNA™ Viral RNA Extraction kit). The ZR Viral RNA Kit™ employs a single buffer system that facilitates viral particle lysis and allows for RNA adsorption onto the matrix of the Zymo-SpinTM Column. The RNA was washed then eluted with DNase/RNase-Free Water. The eluted RNA is suitable for use in various subsequent procedures including RT-PCR.

3.4.1.2. Buffer Preparation and Protocol:

Before starting, beta-mercaptoethanol was added to the Viral RNA Buffer to a final dilution of (0.5%), (26 ml 95%) ethanol was added to the (6 ml) Viral Wash Buffer concentrate.

1. All samples and reagent were centrifuged before additions started centrifugation steps should be performed between (10,000–16,000 xg).
2. One hundred microliters of the throat, nasal and nasopharyngeal swabs specimens (from the viral transport medium) were added to 300 µl of Viral RNA lysis Buffer and mix.

3. Ten microliters of Internal Control STI-rec (IC) were added to each sample lysate.
4. All mixture of (lysate sample buffer) was transfer to the Zymo-Spin™IC Column[2] (filter) was placed in a collection tube and centrifuged for 1-2 minutes.
5. Five hundred microliters of Viral Wash Buffer were added to the column and centrifuge for 2 minutes. Then the column was carefully transferred into the DNase/RNase-free tube.
6. Twenty microliters of DNase/RNase-free water were added directly to the column matrix and centrifuged for 30 seconds.
7. Eluted RNA was then ready to convert to cDNA.

3.4.2. Reverse Transcription:

3.4.2.1. Assay Principle:

Two steps reverse transcription was done to convert the template RNA into (complementary DNA or cDNA) before it was amplified with real-time PCR, by REVERTA-L Reverse Transcriptase kit (Ecoli Ltd, Russia) first kit and GoScript™ Reverse Transcription System (Pro-mega, USA) second kit, reverse transcription reaction was carried out using a random sequence of (6) bases (hexamers) (random primers) as polymerization primers. The reverse transcription reaction synthesizes cDNAs from all the different RNA molecules existing in RNA extracted from the specimens being tested.

3.4.2.1.1. Protocol for First Kit:

The total reaction volume was 20 µl, and the volume of RNA sample was10 µl.

1- Five microliters of RT-G-mix-were added to the tube containing RT-mix and carefully mixed by vortex for (3 seconds) and then centrifuged for 5-7 second for removing the drops that attaches to the inner surface of the test tube covers.

2- Six microliters of Revertase (MMlv) were added into the tube containing reagent mix and then pipetted for 5 times and mixed by vortex for 3 seconds, and then centrifuged for 5-7 seconds to remove any drops attaches to the inner surface of the test tube covers.

3- Ten microliters of prepared reagent mix were distributed or dispensed into each prepared test tubes which are (0.5 ml) disposable polypropylene microcentrifuge tubes).

4- Ten microliters of RNA-sample were added to the appropriate test tube containing prepared reagent mixture and then carefully mixed by the pipette.

5- All test tubes containing samples and reagent mix were placed into thermocycler apparatus and incubate at 37 °C for 30 minutes.

6- After the end of incubation period each cDNA sample was diluted in the ration (1:1) with DNA-buffer by added (20 µl) DNA buffer to each test tube and then carefully mixed by the pipette for 10 times.

Due to RT-PCR amplification was preceding in a two-step process, the reverse transcriptase was thermally inactivated by using ice before amplification.

3.4.2.1.2. Protocol for Second Kit:

1- Five microliters of RNA and primer mix were prepared by adding RNA sample (up to 5µg), Random Primer (0.5µg) and Nuclease-Free Water after centrifuged each of these component before use it.

2- All the tubes was placed into a preheated (70°C) heat block for (5) minutes and immediately cold in ice water for at least (5 minutes), each tube was centrifuged for (10 seconds) in a microcentrifuge to collect the condensates and preserve the original volume, the tubes were remained closed on ice until adding the reverse transcription reaction mix.

3- Five microliters of the reverse transcription reaction mix were prepared by mixing of following component of (the GoScript™ Reverse Transcription System), table (3.5) in a sterile tube on ice, then vortex the tube gently in order to mix well, and was kept on ice before distributing into the reaction tubes.

Table (3.5): The Reverse Transcription Reaction Mix Component.

No.	Component	(Final volume)15 µL
1.	Nuclease-Free Water	Xµl
2.	GoScript™ 5X Reaction Buffer	4.0 µl
3.	MgCl2	1.2–6.4µl

4.	PCR Nucleotide Mix	1.0µl
5.	Recombinant RNasin® Ribonuclease Inhibitor (optional)	20u
6.	GoScript™ Reverse Transcriptase	1.0 µL

4- Fifteen microliters of aliquots part of (the reverse transcription reaction mix) were added to each tube on the ice, then (5µl) of (RNA and primer mix) was added to each tube to be the final reaction volume (20µl) per tube.

5- For annealing, all the tubes were placed in controlled-temperature heat block at (25°C), and incubated for 5 minutes.

6- The extension was performed by incubation all the tubes in a controlled-temperature heat block at (42°C) for up to 1 hour. The optimization of the extension temperature may be between (37°C) and (55°C).

Due to RT-PCR amplification was proceeding in a two-step process, the reverse transcriptase was thermally inactivated before the amplification process by incubating the reaction tubes in the controlled-temperature heat block at (70°C) for 15 minutes.

3.4.3. Real-Time Polymerase Chain Reaction, Multiplex Detection:

In this study, real-time RT-PCR hybridization-fluorescence amplification was used to multiplex detected and identified of specific nucleic acid fragments for the seven RNA/DNA viruses and its genotypes that causing acute respiratory tract infections (ARTIs), this assay was

performed on (SaCycler-96, Italy) using (AmpliSens® ARVI-screen-FRT Multiplex Real-time PCR kit, Ecoli Ltd, Russia). These viruses are Human Metapneumovirus (HMPV) RNA; Human Respiratory Syncytial virus (HRSV) RNA; Human Parainfluenza Virus 1, 3 (HPIV) and four other viruses not included in this study, in a clinical material such as (nasal and oropharyngeal swabs, sputum, aspirate of trachea, bronchoalveolar lavage, bronchial washing fluid, and autopsy material).

3.4.3.1. Method Principle:

The amplification products in Real-time PCR were detected using fluorescent dyes; these dyes are attached to oligonucleotide probes that are specifically binding to the amplification products during thermo-cycling. Monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulated amplification products without re-opening the tubes after running of PCR. Channel of fluorescence detection of internal control and viruses understudy were listed in the table, (3-7).

3.4.3.2. Assay Protocol:

The total reaction volume was (25 µl), and the volume of cDNA sample is (10 µl).

1- Tubes of (cDNA HRSV – HMPV positive control) and (cDNA HPIV 1/3 positive control) with the corresponding PCR-mix-1-FL were thawed, and the (PCR-mix-1-FL-F, PCR-mix-2-FRT, and polymerase (TaqF) tubes were mixed by vortex and centrifuged for a short time.

2- For amplification of the (20) cDNA samples, (20) sterile tubes were prepared.
3- To prepare PCR Master Mix for HRSV-HMPV and HPIV-1/3, two tubes were used for each.
4- Two hundred microliters of PCR-mix-1-FL-F of HRSV-HMPV and (200 µl) of PCR-mix-1-FL-F of HPIV-1/3 was added.
5- One hundred microliters of PCR-mix-2-FRT of HRSV-HMPV and (100 µl) of PCR-mix-2-FRT of HPIV-1/3 were added.
6- Ten microliters of polymerase (TaqF) of HRSV-HMPV and (10 µl) of polymerase (TaqF) of HPIV-1/3 were added.

In the case of the use of a number of samples more or less the volume of Real-time PCR Master Mix was calculated according to the following equation.

For (N) of samples of each multiplex of nucleic acid fragments of viruses, add to the new tube:

10*(N+1) µl of PCR-mix-1-FL-F

5*(N+1) µl of PCR-mix-2-FRT

0.5*(N+1) µl of polymerase (TaqF)

7- Two tubes containing PCR Master Mix were vortex and then centrifuged shortly.
8- Fifteen microliters of the prepared PCR Master Mix were transferred to new each tube.

9- Ten microliters of cDNA that obtained from the (RNA reverse transcription) step were added into the each tubes containing PCR Master Mix to be the total reaction (25µl).

10- Ten microliters of TE-buffer were added to the NCA tube (Negative control of amplification) and (10 µl) of positive control were added to C+ tubes (C+HRSV-HMPV) and (C+HPIV-1/3).

Table (3-6): Thermal Cycler Conditions of Real-Time PCR.

Temperature, °C	Time	cycles
95	15min	1
95	10sec	10
54	25sec	
72	25sec	
95	10sec	35
54	25sec Fluorescence detection	
72	25sec	

Table (3.7): Channel of fluorescence detection

FAM	HEX	ROX
Internal control IC	HRSV	HMPV
Internal control IC	HPIV-3	HPIV-1

3.4.4. Analysis of Real-Time PCR Results:

RNA of HRSV, HMPV, HPIV-1 and HPIV-3 were detected in the case of the Ct value for these samples were determined in the results network in the corresponding channel. The results of amplification were positive when the curve of the fluorescence for these samples crossed the threshold line at 35 cycles or earlier in the field of the exponential growth of the fluorescence.

3.4.5. Real-Time Polymerase Chain Reaction, HRSV Genotyping:

3.4.5.1. Real-Time PCR Reaction Mixture Preparation:

The preparation of RT- PCR Master Mix was done by using "one step Reverse Transcription and Real-Time PCR detection"(GoTaq® Probe qPCR Master Mix Kit, Promega/USA) and according to the company instructions, (10μL) of the Master Mix which are provided as ready-to-use and include all components, including GoTaq® Hot Start Polymerase, dNTPs, MgCl2 and reaction buffer were added to prepare the RT-qPCR reaction mixture, table (3-8). The reaction were done for each genotype (A and B) separately, using different forward, reverse (primers), and probe.

Table (3.8): RT-PCR Reaction Mixture Components.

No.	Component	(Final volume)20 µL
1.	GoTaq® Probe PCR Master Mix	10 µL
2.	Forward primer	1 µL
3.	Reverse primer	1 µL
4.	Probe	0.4 µL
5.	Reverse transcriptase	0.4 µL
6.	Nuclease-free water	Up to 20 µL
7.	RNA Sample Volume	2 µL

3.4.5.2. Thermal Cycler Conditions of Real-Time PCR:

Thermal cycler conditions of real-time PCR were set according to the primer temperature and (RT-qPCR TaqMan kit) instructions, table (3.9).

Table (3.9): Thermal Cycler Conditions of Real-Time PCR.

Step	Temperature °C	Time	Cycle	Scanning
Reverse transcription	42 °C	10 min	1 / Hold	
Enzyme deactivation	95 °C	3 min	1/ Hold	
Denaturation	95 °C	15 sec	40	
Annealing	50 °C	15 sec		
Extension	72 °C	15 sec		

3.4.5.3. Analysis of Real-Time PCR Results:

RNA of HRSV main antigenic sub-groups (A and B) were detected in the case of the Ct value for these samples were determined in the results network in the corresponding channel. The results of amplification were positive when the curve of the fluorescence for these samples crossed the threshold line at (35) cycles or earlier in the field of the exponential growth of the fluorescence.

3.4.6. Conventional PCR, Sequencing of the HMPV (F) and (G) gene:

Sequencing of HMPV (F) and (G) genes was conducted to determine the HMPV main genotypes and main minor sub-lineages, HMPV-positive samples that were detected in the multiplex RT-PCR assay were genotyped by conventional PCR and DNA was sequenced by using two primers targeting the (F) and (G) gene of HMPV.

3.4.6.1. Preparation of Conventional PCR Reaction Mixture:

Master Mix of conventional PCR was prepared by using (Maxime ™ PCR PreMix, i-Taq™ Kit, Boca Scientific / USA) according to company instructions, (5) microliter of Master Mix content (i-Taq DNA Polymerase, dNTPs, Reaction buffer, Gel loading buffer) was added to prepare the PCR reaction mixture, table (3-10),

Table (3.10): PCR Reaction Mixture Component.

No.	Component	volume
1.	PCR Master Mix	5µl
2.	G gene forward primer	10 picomole/µl (1 µl)
3.	G gene reverse primer	10 picomole/µl (1 µl)
4.	cDNA template	1.5µl
5.	Distill water	16.5 µl
6.	Final volume	25µl

After preparing the PCR reaction mixture, PCR tubes were transferred into combi-spin vortex centrifuge at (3000) rpm for 3 minutes, and then placed in PCR Thermal Cycler.

3.4.6.2. Thermal Cycler Conditions of Conventional PCR:

Thermal cycler conditions for (G) gene amplification were set by using conventional PCR thermal cycler system, table (3-11):

Table (3.11): Thermal Cycler Conditions of Conventional PCR for (G) Gene.

PCR steps	Temperature (^{o}C)	Time	No. of cycle
Initial Denaturation	95^{o}C	5 min.	1 Cycle
Denaturation	95^{o}C	45 sec	45 Cycle
Annealing	52^{o}C	45 sec	

Extension-1	72°C	1 min	
Extension -2	72°C	7 min.	1 Cycle

Table (3.12): Thermal Cycler Conditions of Conventional PCR for (F) Gene.

PCR steps	Temprture (°C)	Time	No. of cycle
Initial Denaturation	95°C	5 min.	1 Cycle
Denaturation -2	95°C	45 sec	45 Cycle
Annealing	50°C	45 sec	
Extension-1	72°C	1 min	
Extension -2	72°C	7 min.	1 Cycle

3.4.6.3. Agarose Gel Electrophoresis of PCR Amplification Products and Analysis of the Results:

The PCR products of HMPV (G) and (F) gene were separated by using gel electrophoresis on (1.5%) agarose gels and stained with Red Safe™ Nucleic acid staining (Intron/Korea) and visualized under UV light.

1- Two percent (2%) of agarose was prepared in 100 ml of pre-prepared TBE buffer and this solution was mixed well by swirling and it was then heated in the microwave until all agarose dissolves completely.

2- The agarose gel was left to cool at (45-50^{o}C) for a few minutes and (3µl) of Red Safe stain was added to the agarose gel solution,

3- Then the agarose gel solution was poured into an electrophoretic tank after fixing the comb inside it to form the holes in which the samples will be added within left to fix for 30 minutes to solidify at room temperature.

4- After removing the comb carefully, (5 µl) of the PCR products were added and then loaded onto the wells.

5- A DNA marker (5 µl) of 100bp Ladder were added into the first lane of the wells.

6- The tank was loaded with TBE buffer to covers the surface of the gel after the gel plate was fixed in electrophoresis chamber.

7- The tank was linked into an electric current and has been turned on at (70) Volts/ (65) Amp for about 1-1.5 hours.

8- The PCR products of the HMPV (G) gene (870bp) and (F) gene (1100bp) were visualized using UV transilluminator.

3.4.7. DNA Sequencing Method:

The amplified PCR products of HMPV (G) gene amplicons (870bp) of fifteen positive samples and (F) gene amplicons (1100bp) of eleven positive samples were purified and sequenced from the forward direction using forward primers for both genes, by DNA sequencing system, (Macrogen Inc. Geumchen, Seoul, South Korea) using the automated sequencer (ABI 3100/Applied Biosystems) and (Big Dye Terminator Kit/ Applied Biosystems) according to the manufacturer's instructions, genotyping of HMPV were determined by comparing the

DNA sequences of local samples with retrieved sequenced data of HMPV that obtained with the nucleotide BLAST database in NCBI online (www.ncbi.nlm.nih.gov/BLAST/) and BioEdit Sequence Alignment Editor Software Version 7.1, (multiple and pairwise sequences alignment), (DNASTAR, Madison, WI, USA). Online server for nucleic acid translation, (https://web.expasy.org/translate/), was used to convert the variations into amino acid residues in the HMPV surface attachment glycoprotein.

3.4.8. NCBI-Gene Bank Submitting of HMPV Isolates:

Fifteen local Iraqi isolates of HMPV have been sequenced in this study and submitted to NCBI- GeneBank in order of global recorded and published in this website and to obtain specific accession number after phylogenetic comparison of these isolates which recovered from Iraqi patients with other international strains. Regarding the sequencing results of HMPV (F) gene samples, none shows good sequences analysis, while (15) of (G) gene samples show good sequences. The (G) gene sequences were submitted to NCBI which gave the published accession number on NCBI, Appendix (2); it can be found on the website;

https://www.ncbi.nlm.nih.gov/popset/?term=MN178607&utm_source=gquery&utm_medium=search

(HMPV1, MN178606.1; HMPV2, MN178607.1; HMPV3, MN178608.1; HMPV4, MN178609.1; HMPV5, MN178610.1; HMPV6, MN178611.1; HMPV7, MN178612.1; HMPV8, MN178613.1; HMPV9, MN178614.1; HMPV10, MN178615.1; HMPV11, MN178616.1; HMPV12, MN178617.1; HMPV13, MN178618.1; HMPV14, MN178619.1; HMPV15, MN178620.1).

3.4.9. Phylogenetic Tree Analysis:

A phylogenetic tree was constructed according to the protocol defined by Al-Shuhaib *et al*. (2019). The HMPV local isolates were compared with the homologous reference sequences using (NCBI-BLASTn) (Zhang *et al*. 2000). Then, the blast results of the (G) gene variants were combined and aligned together using a (Clustal Omega based tools). A full phylogenetic tree, including the HMPV local isolates, was designed on the distance method and visualized as polar cladogram using (Figtree tool)/ (http://tree.bio.ed.ac.uk/software/figtree/).

3.4.10. Statistical Analysis:

Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25). Data was presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of the difference of different means (quantitative data) was tested using Students-t-test for the difference between two independent means or ANOVA test for difference among more than two independent means. The significance of the difference of different percentages (qualitative data) was tested using the Pearson Chi-square test (c2-test) with an application of Yate's correction or Fisher Exact test whenever applicable. Statistical significance was considered whenever the P value was equal or less than 0.05 (Daniel and Cross, 2010).

CHAPTER FOUR

RESULTS AND DISCUSSION

Chapter Four

4. Results and Discussion

The results presented in this chapter are obtained from statistical analysis of raw data accumulated throughout the study period which was extended from January 2018 till October 2019. The importance of this study surly emerge from its uniqueness in Diyala province and its comprehensiveness as it covers the most common viruses causing respiratory tract infections that are detected by recent molecular techniques. In Iraq, infectious diseases are a leading cause of mortality and morbidity. Three of the major killers that cause of mortality in children less than 5 years are neonatal causes, acute respiratory infections (ARIs), diarrheal diseases (Denno, 2013). Both of ALRI and diarrhea are responsible for (70%) of deaths among children under five.

The frequency rates of respiratory infections increased in the early 1990s and have continued high during the last decade. This is mainly due to international sanctions, food insecurity and malnutrition, lack of quality health services, (WHO, 2003), other factors that are worsening include the presence of displaced persons and refugees complaining of poor living conditions and general neglect of their health status that are still going on to this day. Furthermore, what is complicating the viral respiratory tract infections is its exacerbation by superimposed bacterial pathogens particularly those causing lower respiratory tract infections and pneumonia (Hanada *et al*., 2018).

4.1. Period of Specimens Collection:

The collection of study specimens was done over two seasons; the first season was extend over the months of January, February, March, April and May 2018, while the second season was extended over the months of November and December 2018. The number and percentage over the entire period was shown in table (4.1).

Table (4.1): Number and Percentage of Collected Specimens by Seasons.

Month of year	Total		Season I		Season II	
	No.	%	No.	%	No.	%
January	40	12.4	40	21.6	-	-
February	28	8.7	28	15.1	-	-
March	75	23.2	75	40.5	-	-
April	24	7.4	24	13.0	-	-
May	18	5.6	18	9.7	-	-
November	98	30.3	-	-	98	71.0
December	40	12.4	-	-	40	29.0

4.2. Number and Types of Specimens:

During the study period, total of (323) specimens were collected over two seasons. These include (71) nasopharyngeal swabs, (161) nasal sinus swabs and (91) throat swabs. Season I include (68) nasopharyngeal swabs, (26) nasal sinus swabs and (91) throat swabs while season II include (3) nasopharyngeal swabs and (135) nasal sinus swabs. Therefore, the rate of nasal sinus swabs was significantly higher

as compared to nasopharyngeal swabs and throat swabs as shown in table (4.2).

Table (4.2): Types and Number of Collected Specimens by Seasons.

Type of specimens	Total		Season I		Season II		P value
	No.	%	No.	%	No.	%	
Nasopharyngeal swabs	71	22.0	68	36.8	3	2.2	0.0001*
Nasal sinus swabs	161	49.8	26	14.1	135	97.8	
Throat swabs	91	28.2	91	49.2	-	-	
Total	323	100	185	100	138	100	

***Significant difference between proportions by Pearson Chi-square test at 0.05 levels.**

4.3. Distribution of Participants by Age:

Table (4.3) revealed the distribution of age groups over the two seasons. It is clearly evident that the rate of participants less than 5 years old was significantly higher (58.2%) compared to other age groups (p= 0.0001). Furthermore, within this group, the rate during the season II was higher than that of season I (73.2% *versus* 47.0%). The second age group was those with 5 years old (14.9%) with higher rate during the season II compared to season I (22.5% *versus* 9.2%). These were followed by the age group of 10 years (9.3%). The least rate of participants was among those with 40 years old (2.8%). Additionally, none of participants over the age of 30 years were included during season II.

Table (4.3): The Number and Rate of Different Age Groups Included.

Age groups (Ys)	Total		Season I		Season II		P value
	No.	%	No.	%	No.	%	
< 5	188	58.2	87	47.0	101	73.2	0.0001*
5	48	14.9	17	9.2	31	22.5	
10	30	9.3	25	13.5	5	3.6	
20	13	4.0	12	6.5	1	.7	
30	11	3.4	11	5.9	-	-	
40	9	2.8	9	4.9	-	-	
50	12	3.7	12	6.5	-	-	
= > 60	12	3.7	12	6.5	-	-	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

4.4. Distribution of Participants by Gender:

In general, the rate of males was insignificantly higher compared to female (54.8% *versus* 45.2%, P= 0.322). Similarly, the rate of male participants was slightly higher than that of female in both season I (52.4% *versus* 47.6%) and season II (58.0% *versus* 42.0%), table (4.4).

Table (4.4): The Gender Distribution of Participants over Seasons.

Gender	Total		Season I		Season II		P value
	No.	%	No.	%	No.	%	
Male	177	54.8	97	52.4	80	58.0	0.322
Female	146	45.2	88	47.6	58	42.0	

***insignificant difference between proportions by Pearson Chi-square test at 0.05 levels.**

4.5. Distribution of Clinical Signs and Symptoms:

The distributions of clinical signs among participants over seasons were shown in table (4.5). It is important to remind that season I include (January, Februarys, March, April and May) months while the season II include (November and December) months. It is clear that cough was significantly recorded in season II versus season I (71.7% *Vs* 32.4%, P= 0.0001). Similarly fever/chill was significantly recorded during season II compared to season I (32.6% *Vs* 20.0%, P= 0.01). None of dyspnea were reported during the season II while 5 (2.7%) were recorded during season I, However, the difference was failed to reach the levels of statistical significance (P= 0.052).

The wheezing, rhinitis, Pharyngitis, pneumonitis, sinusitis, Asthma exacerbation showed no significant difference between season I and II (P= 0.193, P= 0.408, P= 0.144, P= 0.296, P= 0.400, P= 0.265) respectively. Clinical signs and symptoms showed significant increase in season I compared to season II were sore throat (6.5% *Vs* 0.7%, P= 0.009) and otitis media (10.3% *Vs* 2.2, P= 0.004). On the contrary, those showed significantly increase during season II compared to season I were laryngitis (6.5% *vs* 1.1, P= 0.008), bronchiolitis/bronchitis (72.5% *vs* 37.8%, P= 0.0001), tonsillitis (5.8% *vs* 1.6%, P= 0.041) and severe flu since no cases were recorded during the season I while 4 (2.9%, P= 0.020).

Table (4.5): Distribution of Clinical Signs and Symptoms over Seasons.

Sign or symptom		Total		Season I		Season II		P Value
		No.	%	No.	%	No.	%	
Cough	Yes	159	49.2	60	32.4	99	71.7	0.0001*
	No	164	50.8	125	67.6	39	28.3	
Fever/chill	Yes	82	25.4	37	20.0	45	32.6	0.010*
	No	241	74.6	148	80.0	93	67.4	
Tachypnea/ Dyspnea	Yes	5	1.5	5	2.7	-	-	0.052
	No	318	98.5	180	97.3	138	100	
Wheezing	Yes	6	1.9	5	2.7	1	0.7	0.193
	No	317	98.1	180	97.3	137	99.3	
Rhinorrhea/ Rhinitis	Yes	13	4.0	6	3.2	7	5.1	0.408
	No	310	96.0	179	96.8	131	94.9	
Sore throat	Yes	13	4.0	12	6.5	1	0.7	0.009*
	No	310	96.0	173	93.5	137	99.3	
Laryngitis	Yes	11	3.4	2	1.1	9	6.5	0.008*
	No	312	96.6	183	98.9	129	93.5	
Otitis media	Yes	22	6.8	19	10.3	3	2.2	0.004*
	No	301	93.2	166	89.7	135	97.8	
Pharyngitis	Yes	86	26.6	55	29.7	31	22.5	0.144
	No	237	73.4	130	70.3	107	77.5	
Bronchiolitis/ Bronchitis	Yes	170	52.6	70	37.8	100	72.5	0.0001*
	No	153	47.4	115	62.2	38	27.5	
Pneumonitis	Yes	50	15.5	32	17.3	18	13.0	0.296
	No	273	84.5	153	82.7	120	87.0	

sinusitis	Yes	3	0.9	1	0.5	2	1.4	0.400
	No	320	99.1	184	99.5	136	98.6	
tonsillitis	Yes	11	3.4	3	1.6	8	5.8	0.041
	No	312	96.6	182	98.4	130	94.2	
Asthma exacerbation	Yes	14	4.3	6	3.2	8	5.8	0.265
	No	309	95.7	179	96.8	130	94.2	
Severe flue	Yes	4	1.2	-	-	4	2.9	0.020*
	No	319	98.8	185	100	134	97.1	

***Significant difference between proportions using Pearson Chi-square at 0.05 levels.**

Months of November and December are the time of gradual decrease in climate temperature and the beginning of the winter season in Iraq. Therefore, it is the time for the spread and upsurge of respiratory tract infections which are usually associated with symptoms of cough, fever, laryngitis and bronchiolitis. Furthermore, these months are part of the school season, during which the crowdedness of children in the class rooms, poor ventilation and weak surveillance system may act as risk factors facilitating the transmission of respiratory infections among children and peak of these infections at different times during these seasons (CDC, 2019). Additionally, due to shortage in the electricity, most families depend largely on kerosene heaters that may produce gases and fumes irritant for upper respiratory tract mucous membrane and thus increase the rate of respiratory tract infections (WHO, 2015).

4.6. Molecular Detection of Respiratory Viruses:

Regarding the molecular detection of different respiratory viruses included in this study is shown in table (4.6). The results revealed that out of 323 specimens, 30(9.3%) specimens were positive for HMPV, 2 (1.1%) were in season I and 28 (20.3%) were in season II, with obviously significantly higher infection rate during season II (P= 0.0001), furthermore, 15(4.6%) were positive for HRSV which were distributed as 14 (7.6%) in the season I and 1(0.7%) in the season II, with a statistically significantly higher infection rate during season I (P= 0.004). Concerning the HPIV-3, a total of 17 (5.3%) specimens were positive, of these 11 (5.9%) specimens were in season I and 6 (4.3%) specimens were in season II, with insignificantly higher infection rate during season I (P= 0.525). On the other hand, all of these specimens were negative for HPIV-1 during the two seasons.

Table (4.6): Positivity Rate of Different Respiratory Viruses by Seasons.

Type of virus	Status	Total		Season I		Season II		P value
		No.	%	No.	%	No.	%	
HMPV	Positive	30	9.3	2	1.1	28	20.3	0.0001*
	Negative	293	90.7	183	98.9	110	79.7	
HRSV	Positive	15	4.6	14	7.6	1	0.7	0.004*
	Negative	308	95.4	171	92.4	137	99.3	
HPIV3	Positive	17	5.3	11	5.9	6	4.3	0.525
	Negative	306	94.7	174	94.1	132	95.7	
HPIV1	Positive	-	-	-	-	-	-	-
	Negative	323	100	185	100	138	100	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

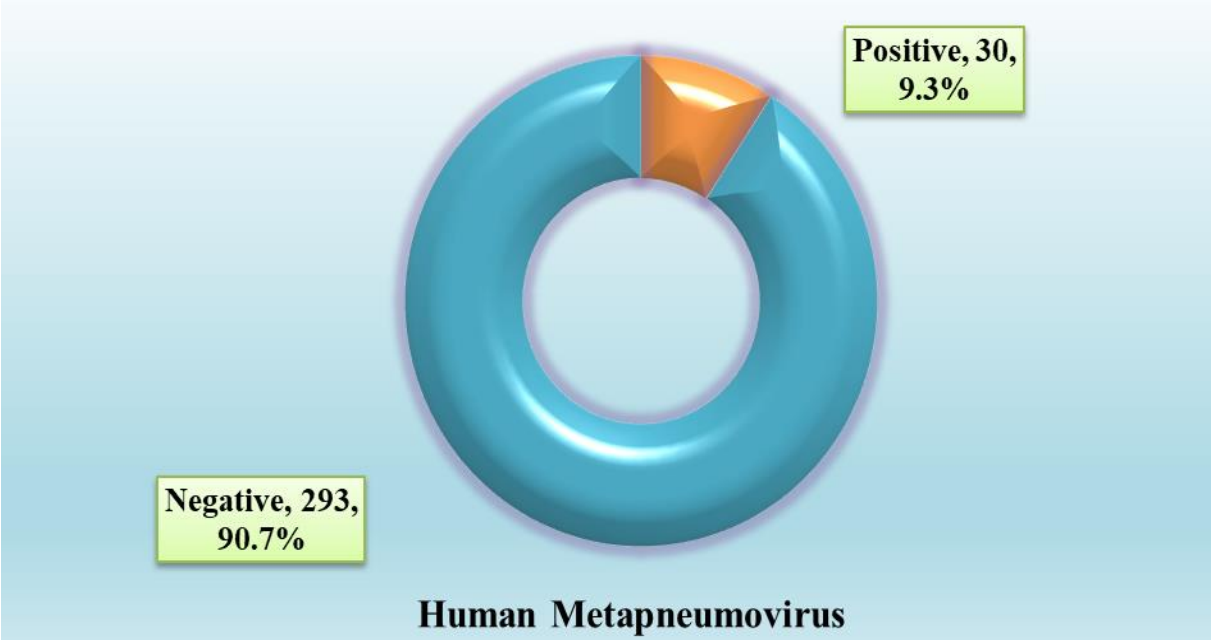


Figure (4.1): Positivity and Negativity Rates of Human Metapneumovirus.

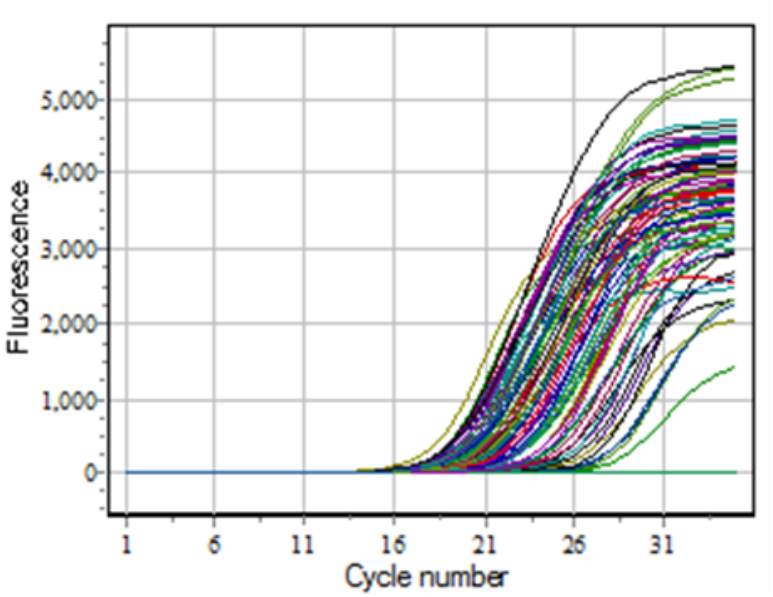


Figure (4.2): Real-Time PCR amplification log plot showed cycles of internal control results ranged from CT:13.1 to CT:28.

One of the limitation in viral detection by genetic amplification techniques is the false negative results, which may be the result of poor quality RNA / RNA extraction due to insufficient initial sample quantity and degradation of the genetic material of the virus due to improper sample storage or loss of it during extraction, the other explanation may be the presence of “inhibitors of the enzyme mixture (reverse transcriptase and DNA polymerase)” in the samples (respiratory secretions, sputum, etc.) (Kouni et al., 2013). However, the molecular assay used in this study eliminated these false negatives by the addition of internal control that was extracted with any possible viruses existing in the sample and subsequently amplified in the PCR reaction tubes, confirming the perfect and correct efficiency both of RNA extraction and PCR amplification.

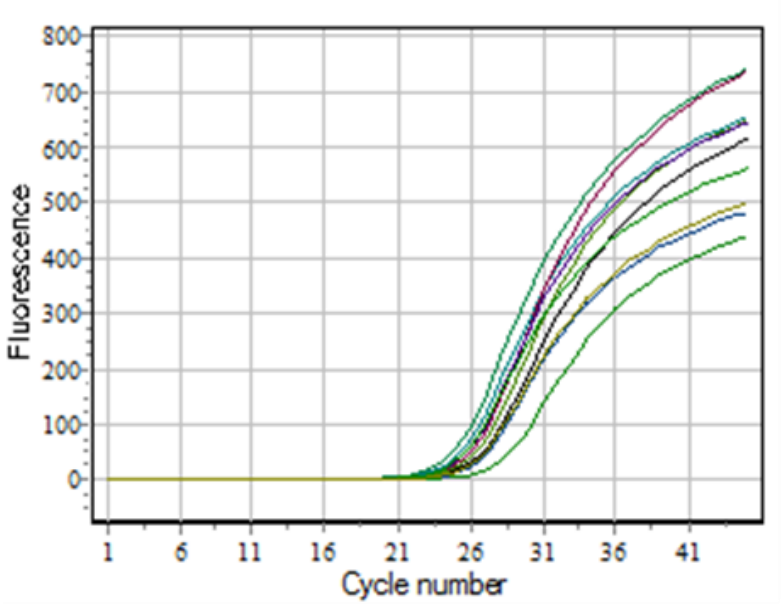


Figure (4.3): Real-Time PCR amplification log plot showed cycles of HMPV positive results ranged from CT:20.4 to CT:26.9.

The rates of different human viruses responsible for major respiratory tract infections are important determinants for public health policies in Iraq over the past years. During the current research period it was noticed that respiratory tract infections are one of the biggest health problems experienced by health authorities particularly in children hospitals in the studied area. In this regard, limited studies were conducted in Iraq which reported the rate of HMPV infection, for instance, in Baghdad, two studies were reported low and high rates of HMPV (1.33% and 16%) respectively, while in Kurdistan, the rate of HMPV infection among children suffering from respiratory diseases and flu-like illness was high as compared with the results of current study (13.4% to 29.74%) (Aziz, 2015; Atyah *et al*., 2017; Abduljabbar *et al*., 2018; Hassan *et al*., 2018).

In Saudi Arabia, Ali *et al*. (2019), had reported a detection rate of HMPV similar to that obtained in the current study (9.9%), while in Egypt, El-Sawaf *et al*. (2017), reported a higher detection rate of HMPV (16%). Studies from around the world had yielded variable detection rates of HMPV, for example in Bangkok, Pakistan, Norway and Brazil, the HMPV was detected among hospitalized children with RTIs in (3.6%, 7%, 7.3% and 20%) respectively (Moe, 2017; Yousafzai *et al*., 2018; Gregianini *et al*., 2018; Thongpan *et al*., 2019). The positivity rate of HMPV detection is affected by several important factors such as seasons of infection, age of the patients, geographical region, different methods of sampling, type of specimens, diagnostic methods, different viral strain and the criteria of patients participating in the study from which the specimens were taken (Amer, 2016).

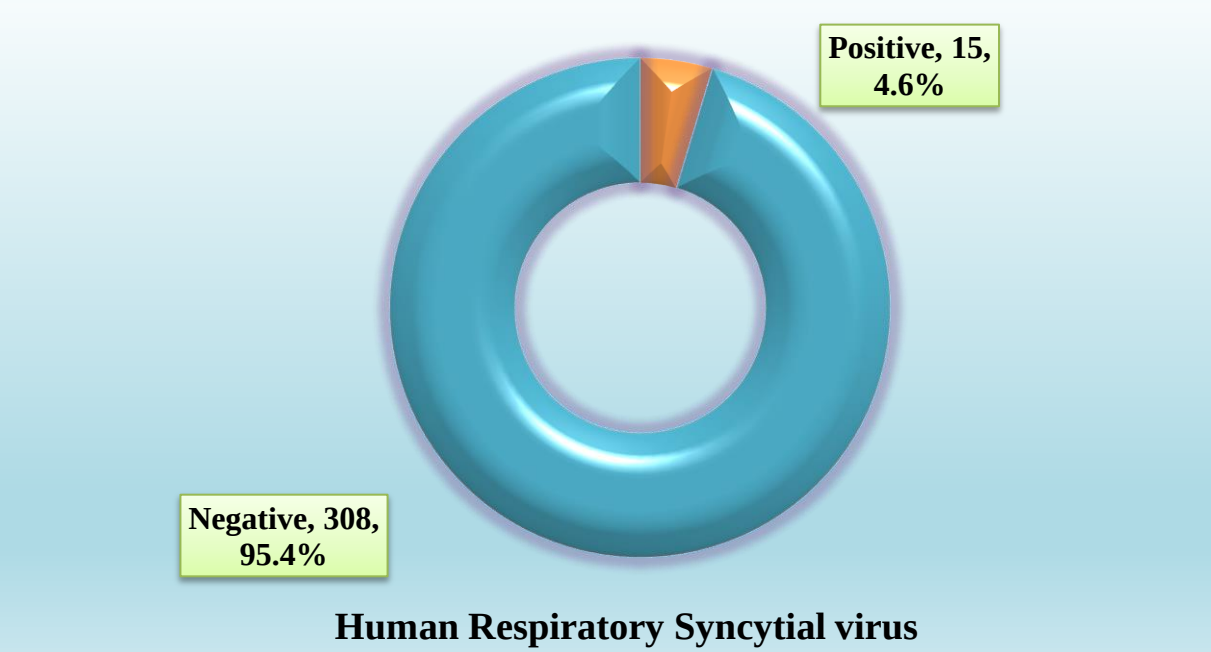


Figure (4.4): Positivity and Negativity Rates of Human Respiratory Syncytial Virus.

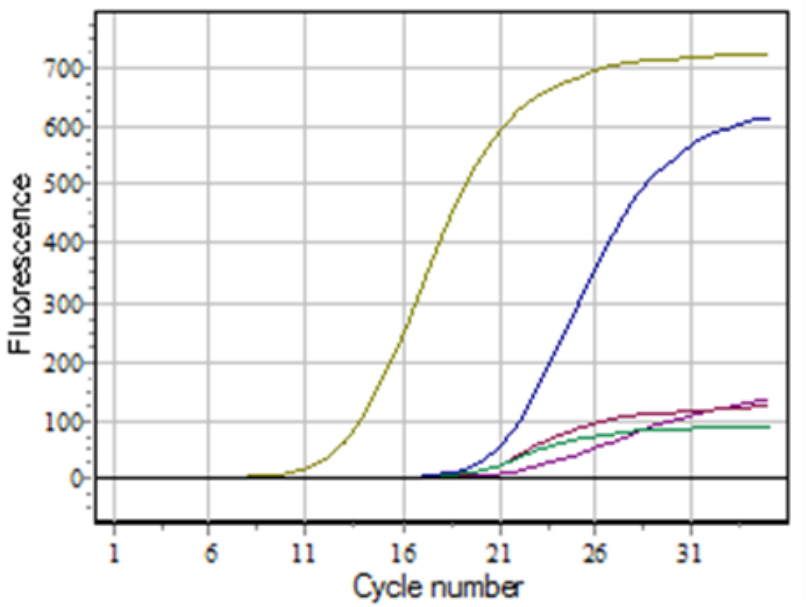


Figure (4.5): Real-Time PCR amplification log plot showed cycles of HRSV positive results ranged from CT:7 to CT:16.4.

Although the molecular diagnostic assays have been revealed in a wide range of studies that Human Respiratory Syncytial Virus (HRSV), is one of the common causes of acute respiratory bronchiolitis and the most prevalent viral agent of death associated with acute respiratory infection among the pediatric age group (Brini *et al*., 2017; Oumei *et al*., 2018). However, The results of the current study are in agreement with other two studies conducted in Baghdad and Tikrit, that also showed a low HRSV detection rates (6.67% and 1%) (Atyah *et al*., 2017; AL-Bashar *et al*., 2017). On the contrary, many other studies that were conducted in different regions of Iraq were reported a higher detection rates of HRSV (17.33% and 20.4%) (Hassan *et al*., 2018; Abduljabbar *et al*., 2019). Similar higher detection rates were reported from other countries, for instance (37.9%) was reported in Turkey, (19.38%) was registered in Iran, (26%) was documented in Saudi Arabia and (25.2%) was reported in Kuwait (Hacimustafaoglu *et al*., 2013; Faghihloo *et al*., 2014; Ahmed *et al*., 2016; Madi *et al*., 2018). A higher rate of detection was also reported in Taiwan, Pakistan, and Southern India (Ali *et al*., 2013; Chen *et al*., 2014; Kini *et al*., 2019).

The negativity rate of HRSV or other viruses in current study among patients with respiratory tract infection may be explained by the fact that RTIs may be caused by other viral infections, since many viruses are responsible for respiratory diseases in humans, such as rhinoviruses, adenovirus, coronavirus, and bocavirus, besides many of bacterial agent that play a major role in respiratory infections (Eccles, 2005).

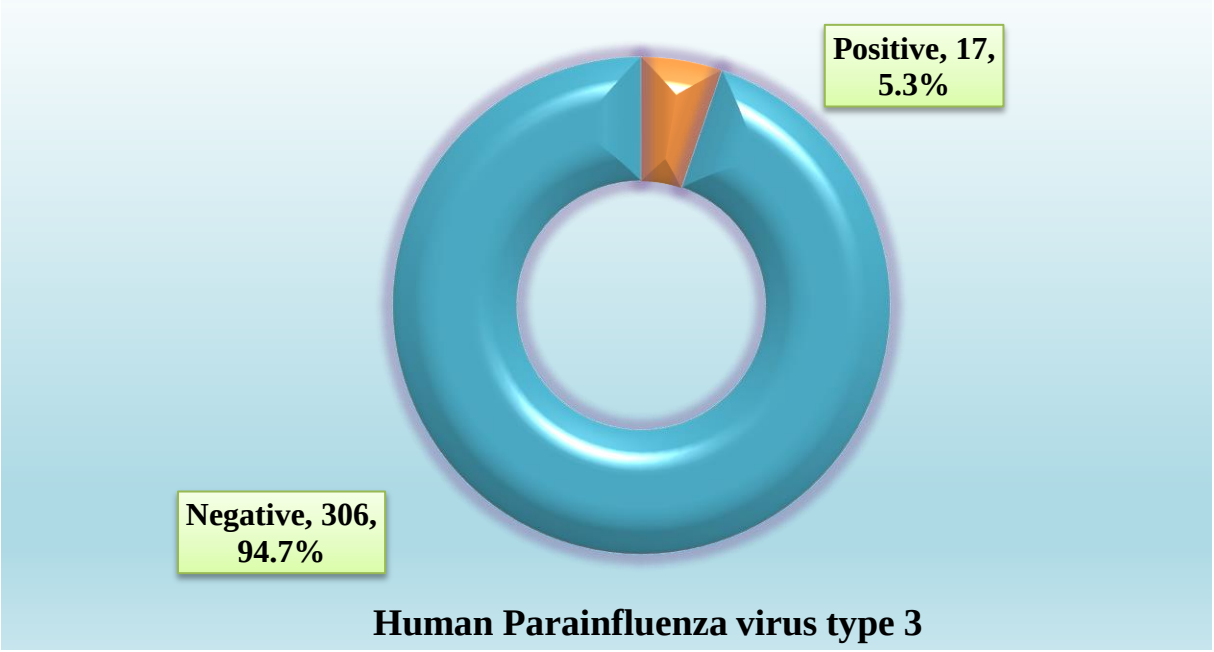


Figure (4.6): Positivity and Negativity Rates of Human Parainfluenza Type 3.

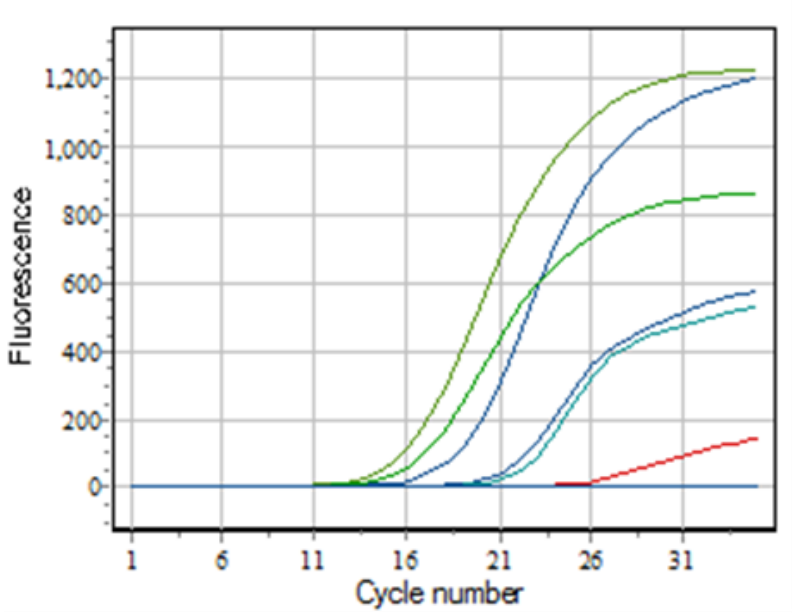


Figure (4.7): Real-Time PCR amplification log plot showed cycles of HPIV-3 positive results ranged from CT: 15.4 to CT: 25.5.

A limited number of studies in Iraq had assessed the impact of HPIV-3 among the pediatrics and other age groups, in Diyala province, this is the first study conducted on hospitalized patients and outpatients to investigate the molecular detection rate of HPIV-3 and HPIV-1. In this regard, the current results showed that the detection rate of HPIV-3 was 17(5.3%), which is less than the results obtained by Kadim, (2016) who reported a detection rate of (13.21%) in Al-Muthanna province, while in Kurdistan, Hassan *et al*., (2018), reported low 4(1.5%) detection rate of HPIV-3 among children.

Epidemiological studies from different parts of the world reported different detection rates of HPIV-3, some are similar to the current study, others obtained higher or lower detection rates, for instance (5.1%) detection rate was reported from Qatar, (3.4%) from Israel, (2%) from Catalan community, Spain, (4.48%) from Beijing, China, and (73.4%) from Southern Taiwan (Godoy *et al*., 2016; Pan *et al*., 2017; Janahi *et al*., 2017; Jornist *et al*., 2018; Wu *et al*., 2018). Upon reviewing the literature, Data of most studies including the current one showed that HPIV3 is the predominant type and constitutes the bulk of respiratory infections compared to other HPIVs types in different regions of the worldwide (Wang *et al*., 2015).

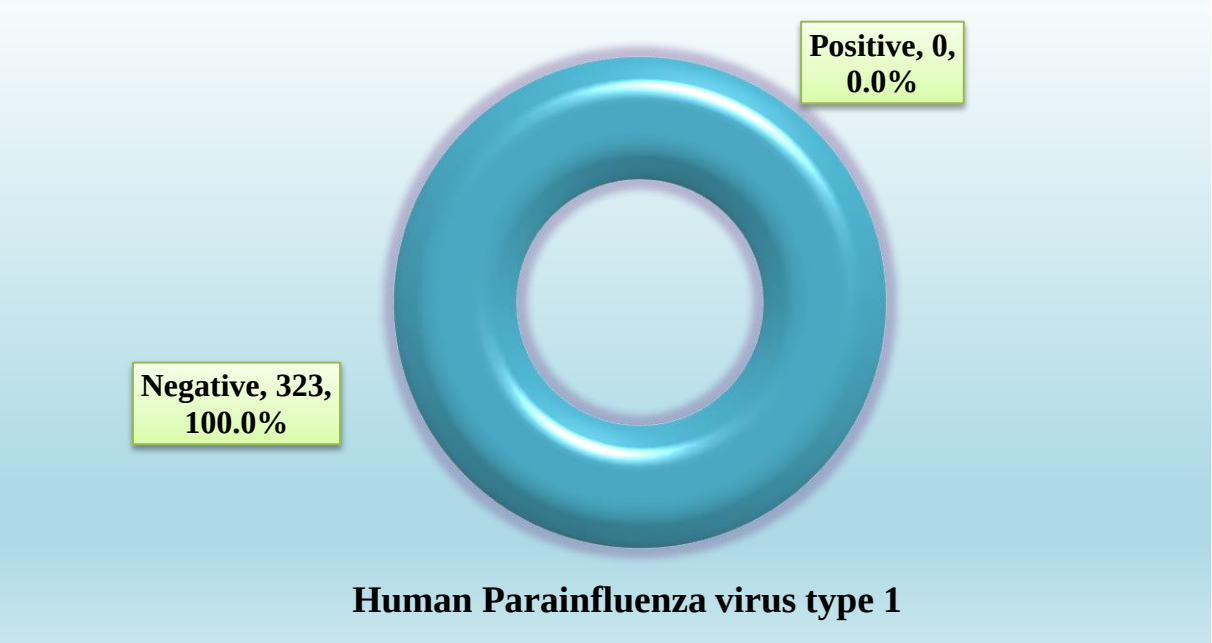


Figure (4.8): Positivity and negativity rates of human parainfluenza type 1.

HPIV-1 is the major cause of croup and less frequent than HPIV-3 among hospitalized children (El Feghaly *et al*., 2010), however, the present study didn't reported positivity rates of HPIV-1 in all patients with RTIs included in this study, in contrast to this result, Kadim, (2016), found that the prevalence of HPIV-1 was 32.17% of infections among hospitalized infants and preschool children suffering from acute respiratory infections in AL-Muthanna province, Iraq.

2.7. Human Metapneumovirus (HMPV):

2.7.1. Distribution of HMPV by Types of Specimens:

The distribution of HMPV positivity rate according to the type of specimen collected was shown in table (4.7). It is clearly evident that the highest HMPV positivity rate was detected among nasal sinus swabs with a significant difference (96.7%, P= 0.0001).

Table (4.7): The HMPV Positivity Rate by Type of Specimens.

Type of specimen	HMPV Positive		HMPV Negative		P value
	No	%	No	%	
Nasopharyngeal swabs	1	3.3	70	23.9	0.0001*
Nasal sinus swabs	29	96.7	132	45.1	
Throat swabs	-	-	91	31.1	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

It is a fact that the diagnosis of viruses causing respiratory diseases depends largely on the source of clinical specimens taken. Most epidemiological studies use nasopharyngeal or nasal swabs as conventional samples, as NP wash, aspirate and swab are the 'gold standard' for viral isolation, but it is cumbersome of performance and unpleasant to patients especially for children, who have small and narrow nasal passages, which may induce severe pain that cannot tolerated by the children, so the researcher used nasal swabs for most children (Ortiz de la Tabla *et al*., 2010). Therefore, the current study showed that the detection rate of HMPV was recorded among nasal sinus swabs with a significant difference (96.7%, P= 0.0001) versus (3.3%) of nasopharyngeal swabs which were taken mostly from older age groups.

Few studies used nasal swabs for detection of HMPV, in this regard, studies from Finland, Jordan and Bangladesh using nasal swab had demonstrated that the detection rate of HMPV was (3.5%, 6% and 5.4%) respectively (Heikkinen *et al*., 2008; Ali et al., 2010; Rahman *et al*., 2019). Although nucleic acid amplification methods were able to detect a low concentration of viruses in throat samples, this study was

failed to recover any of HMPV in all throat samples during the first season. These results are consistent with a previous study which compared the viral yield of NP specimens and throat swab specimens (Robinson *et al*., 2008). However, other studies showed that HMPV was detected in (3.7%) and (7%) of throat swabs (Nidaira *et al*., 2012; Yousafzai *et al*., 2018). Seasonal distribution and age of the patient may play a role in the absence of this virus during the first season in all throat samples.

4.7.2. Distribution of HMPV by Season and Months of the Year:

Table (4.8) showed the HMPV positivity rate according to the season and months of the year. The results revealed that the positivity rate was significantly higher in season II compared to season I (93.3% *vs* 6.7%, P= 0.0001). Regarding distribution of HMPV positivity rate, the results showed that all specimens collected during January, February and March were negative for HMPV and the highest positivity rate was appeared in specimens collected during November with a significant difference compared to other months (50%, P= 0.0001).

Table (4.8): The HMPV Positivity Rate by Season and Months of the Year.

Season	HMPV Positive		HMPV Negative		P value
	No	%	No	%	
Season I	2	6.7	183	62.5	0.0001*
Season II	28	93.3	110	37.5	
Month of year					
January	-	-	40	13.7	0.0001*
February	-	-	28	9.6	
March	-	-	75	25.6	
April	2	6.7	22	7.5	
May	-	-	18	6.1	
November	15	50.0	83	28.3	
December	13	43.3	27	9.2	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

For the best of our knowledge, this is the first study conducted in Diyala governorate hospitals to determine the detection rate of these viruses and its seasonal distribution. In spite of that there is no clear definition of the seasonal distribution of respiratory viruses in Iraq as a whole; however in this study a clear seasonally distribution of HMPV infection was observed, since a high rate of HMPV detection in the second season (autumn period) with a peak in November and December with a highly significant difference in constant to the first season in which only two cases were detected in March. This seasonal pattern is not coinciding to data from other regions and countries. For instance, Atyah *et al*. (2017), found that the detection of HMPV was in

the October and November with a peak of cases in January. Furthermore, , Hasan *et al*. (2018), demonstrated that the majority of respiratory infections among children were recorded between November and March, with a peak in January and February, confirming that late autumn to early spring is the flourishing season for respiratory viruses in Kurdistan. In Iran, Moattari *et al*. (2010), revealed that the rate of HMPV detection was increasing in winter and spring.

Previous studies reported variable seasonal distribution of HMPV around the world. In Kuala Lumpur- Malaysia, a higher detection rate of HMPV was documented in March 2012, April and November 2013 (Chow *et al.*, 2016). Previous studies from Egypt, Kurdistan and Pakistan, showed that the majority of HMPV infection was detected in the winter months with peak rates in February (Ali *et al*., 2013; Aziz, 2015; Mahdy El-Wakil *et al*., 2017). High prevalence rates of HMPV during the second season compared to the first one may be due to the sample type which differs from those taken in the first season, age of patients and/or this season may be the usual seasonal distribution of HMPV in this region.

4.7.3. Distribution of HMPV by Age Groups:

Regarding the distribution of HMPV positive rate according to age, data presented in table (4.9) clearly revealed that the positivity was highest in children under 5 years of age, limited in up to 5 years old children and no positive rate were appeared in other age groups, However, the difference was failed to reach the levels of statistical significant (P= 0.060).

Table (4.9): HMPV Positivity Rate by Age Groups.

Age groups (Ys)	HMPV positive		HMPV Negative		P value
	No.	%	No.	%	
<5	26	86.7	162	55.3	0.060
5	4	13.3	44	15.0	
10	-	-	30	10.2	
20	-	-	13	4.4	
30	-	-	11	3.8	
40	-	-	9	3.1	
50	-	-	12	4.1	
=>60	-	-	12	4.1	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

Previous serologic analyses have indicated that the majority of children less than 5 years in Europe and North America have been infected with HMPV, probably because they are highly susceptible to infections with this virus (Panasik and Pancer, 2009; Falsey, 2015; Tambyah *et al*., 2019). Additionally, the current results are consistent with most previous studies suggesting that the infection with HMPV was a highest among children <5 years old and decreased gradually with increased age (Atyah *et al.,* 2017), children below 5 years of age, lost passively transferred maternal Abs which gradually rapidly wane during the first months of life, leaving the children vulnerable to infection and since they have no previous exposure to this virus, so they have no protective Abs (Healy *et al*., 2004; Nunes *et al*., 2016).

Despite the fact that some of these studies were reported a few cases of HMPV infection in other age groups, (Williams *et al*., 2010; Gregianini *et al*., 2018; Thongpan *et al*., 2019).

4.7.4. Distribution of HMPV by Gender.

In this regard, the results showed that the HMPV positivity rate was equally distributed between both sexes with logically insignificant difference (P=0.579), table (4.10).

Table (4.10): HMPV Positivity Rate by Gender.

Gender	HMPV positive		HMPV Negative		P value
	No.	%	No.	%	
Male	15	50.0	162	55.3	0.579
Female	15	50.0	131	44.7	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

These results are consistent with most other studies which documented instatistically significant effect of sex on the rate of infection (Arabpour *et al*., 2008; Hahn *et al*., 2013; Al-Turab *et al*., 2015; Zhang *et al*., 2018; Safamanesh *et al*., 2018; Gregianini *et al*., 2018). Thus the current findings and previous studies are in agreement that gender is not associated with an increased risk of disease (Moe *et al*., 2017).

4.7.5. HMPV Co-Infection with Other Viruses:

Results in table (4.11) revealed that none of the HMPV positive patients were positive for HRSV infection while 15 patients who were HMPV negative were positive for HRSV infection with insignificant difference (5.4%, P= 0.204). Furthermore, none of positive or negative patients for HMPV were positive for HPIV-1. On the other hand, 1(3.3) patient was co-infected by both HMPV and HPIV-3 plus 16 (5.5%) of negative HMPV patients were positive for HPIV-3, with insignificant difference (P= 0.619).

Table (4.11): HMPV Co-Infection Rate with Other Respiratory Viruses.

Other viruses		HMPV positive		HMPV negative		P value
		No.	%	No.	%	
HRSV	Positive	-	-	15	5.4	0.204
	Negative	30	100	278	100	
HPIV-3	Positive	1	3.3	16	5.5	0.619
	Negative	29	96.7	277	94.5	
HPIV-1	Positive	-	-	-	-	-
	Negative	30	100.0	293	100.0	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

Regarding multiple infections, although the role of co-infections of respiratory viruses is uncertain and controversial, there are several reports confirming no relationship between severity of disease and co-viral infections (Lim *et al*., 2016; Moe *et al*., 2017). On the contrary, other previous studies were showed that co-infection of these viruses

was notably associated with increasing duration of symptoms (Cho *et al*., 2012; Harada *et al*., 2013); However, multiplex RT-PCR revealed that HMPV was not co-detected with HRSV, so the current findings are inconsistent with most previous studies that reported a potential dual infections between these viruses in most patients (Oliveira *et al*., 2009; Malekshahi *et al*., 2010; Ali *et al*., 2010; Kouni *et al*., 2013; Atyah *et al*., 2017; Zhang *et al*., 2018; Thongpan *et al*., 2019).

The main reason for the absence of co-infection in this study probably due to that the seasonal distribution of HMPV was not overlapped with HRSV in this geographic region, Pinky and Dubrovolny, (2016), suggested that preventing one virus infection through the presence of another virus could be clarified simply through resource competition, and other reasons suggested that other mechanisms, such as interfering through viral proteins or the host's immune response are responsible for the growing interference between two viruses and for different dominant patterns (Wiegand *et al*., 2015). On the other hand, one patient had co-infection with HMPV and HPIV-3, Thus the current results are in agreement with Zang *et al*., (2018), and Williams *et al*., (2010), who were demonstrated that co-viral infection was detected in one patient and with (Mao *et al*., 2012) who found coinfection in three patients. Therefore, overlap of seasonal distribution between these two viruses was the cause of appearing the double infection.

4.7.6. Association of HMPV by Clinical Picture:

Table (4.12) showed the association of HMPV positivity rate with certain clinical signs and symptoms. The results revealed that 22 (73.3%) of HMPV positive patients had cough while 8 (26.7%) had no cough with a significant difference (P=0.006). Similarly, Bronchiolitis/bronchitis has a significant association with HMPV infection (86.7% vs 13.1%, P= 0.001). On the contrary, Fever/chill, pneumonitis, Pharyngitis had a significantly negative association with HMPV positivity (30% vs 70%, P= 0.542, 13.3% vs 86.7, P= 0.733, 10% vs 90%, P= 0.034 and 3.3% vs 96.7%) respectively. Other clinical sings had neither positive nor negative association with HMPV infection.

Table (4.12): Association of HMPV Positive Patients with Clinical Picture.

Sign or symptom	Status	HMPV positive		HMPV negative		P value
		No.	%	No.	%	
Cough	Yes	22	73.3	137	46.8	0.006*
	No	8	26.7	156	53.2	
Fever/chill	Yes	9	30.0	73	24.9	0.542
	No	21	70.0	220	75.1	
Tachypnea/ dyspnea	Yes	-	-	5	1.7	0.471
	No	30	100.0	288	98.3	
Wheezing	Yes	-	-	6	2.0	0.429
	No	30	100.0	287	98.0	
Rhinorrhea/ Rhinitis	Yes	1	3.3	12	4.1	0.840
	No	29	96.7	281	95.9	

Sore throat	Yes	-	-	13	4.4	0.239
	No	30	100.0	280	95.6	
Laryngitis	Yes	1	3.3	10	3.4	0.982
	No	29	96.7	283	96.6	
Otitis media	Yes	1	3.3	21	7.2	0.427
	No	29	96.7	272	92.8	
Pharyngitis	Yes	3	10.0	83	28.3	0.034
	No	27	90.0	210	71.7	
Bronchiolitis/ bronchitis	Yes	26	86.7	144	49.1	0.001*
	No	4	13.3	149	50.9	
Pneumonitis	Yes	4	13.3	46	15.7	0.733
	No	26	86.7	247	84.3	
sinusitis	Yes	-	-	3	1.0	0.578
	No	30	100.0	290	99.0	
tonalities	Yes	-	-	11	3.8	0.280
	No	30	100.0	282	96.2	
Asthma exacerbation	Yes	1	3.3	13	4.4	0.777
	No	29	96.7	280	95.6	
Severe flue	Yes	1	3.3	3	1.0	0.276
	No	29	96.7	290	99.0	

***significant difference between proportions using Pearson Chi-square at 0.05 levels.**

The symptoms of HMPV infection as a new pathogen of the most pediatrics viral respiratory infections, and those caused by other respiratory viruses are similar and include cough, fever, sore throat, runny nose, wheezing; bronchiolitis and pneumonia are the most frequent clinical manifestation associated with HMPV infections

(Shafagati and Williams, 2018). In concurrence with the most other studies (Arabpour *et al*., 2008; Ali *et al*., 2010; Safamanesh *et al*., 2018; Zang *et al*., 2018) the results of the current study showed that most patients with cough are the most infected with HMPV. In addition, the current results showed a high rate of HMPV-positive specimens was isolated from children with bronchiolitis; this finding is consistent with data from previous studies which showed that bronchiolitis was the most frequent clinical diagnosis among HMPV positive cases (Wei *et al*., 2013; Zeng *et al*., 2015), In a related context, Williams *et al*., (2010), reported that bronchiolitis, and pneumonia are the most frequent discharge diagnoses were observed among HMPV positive children.

4.8. Human Respiratory Syncytial Virus (HRSV):

4.8.1. Distribution of HRSV Positivity Rate by Type of Specimens:

The distribution of HRSV positivity rate according to the type of swabs collected was shown in table (4.13). Among the total of 15 HRSV positive swabs, 8(53.3%) were throat swabs which were significantly higher (P= 0.003) compared to nasopharyngeal swabs (40.0%) and nasal sinus swabs (6.7%).

Table (4.13): The HRSV Positivity Rate by Type of Specimens.

Type of specimens	HRSV Positive		HRSV Negative		P value
	No	%	No	%	
Nasopharyngeal swabs	6	40.0	65	21.1	0.003*
Nasal sinus swabs	1	6.7	160	51.9	
Throat swabs	8	53.3	83	26.9	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

Although Jansen *et al.*, (2013), reported that nasal swabs have a significant efficiency in detecting HRSV compared to throat swabs. Furthermore, Robinson *et al*., (2008), mentioned that throat swab are inferior to nasopharyngeal specimens in respiratory viruses detection. However, the results of the current study demonstrated that HRSV was detected in (53.3%) of throat swabs which were significantly higher compared to nasopharyngeal swabs (40.0%) and nasal sinus swabs (6.7%).

Studies from different parts of the world detected HRSV using throat swabs alone (Almasri *et al*., 2013; Faghihloo *et al*., 2014; Li *et al*., 2014; Arjeyni *et al*., 2017). Additionally, Rahman *et al*., (2014), reported that throat swabs were more efficient in detecting the HRSV than nasopharyngeal aspirate (62.90% *vs* 37.10%), while other researchers had suggested that throat swabs alone may not be enough for surveillance for respiratory viruses and its value may be an as complementary assay (Ali *et al*., 2015). Current results proved the efficiency of throat swabs, which slightly outperformed nasopharyngeal swabs in the detection of HRSV during the first season, which differed from the second season, may be due to seasonal distribution of this virus.

4.8.2. Distribution of HRSV Detection Rate by Season and Months of the Years:

Results in table (4.14) are clearly evident that the HRSV positivity rate in the first season is significantly higher than that in season two (93.3% *versus* 6.7%, P = 0.004). Although the HRSV positivity rate is generally increased during the three months of the year: However, table (4.5) revealed that the HRSV positivity rate was

significantly higher in March compared to other months (January, February, April, May, November and December) (P= 0.012).

Table (4.14): The HRSV Positivity Rate by Season and Months of the Years.

Season	HRSV Positive		HRSV Negative		P value
	No	%	No	%	
Season I	14	93.3	171	55.5	0.004*
Season II	1	6.7	137	44.5	
Month of year					
January	4	26.7	36	11.7	0.012*
February	2	13.3	26	8.4	
March	8	53.3	67	21.8	
April	-	-	24	7.8	
May	-	-	18	5.8	
November	-	-	98	31.8	
December	1	6.7	39	12.7	

***Significant difference between proportions using Pearson Chi-square test at 0.05 levels.**

As shown, the highest detection rate of HRSV was found during the first season of the study period i.e. January, February with the peak in the March, these results are consistent with (Atyah *et al*., 2017; Abduljabbar *et al*., 2018). Other studies demonstrated that the seasonal distribution of HRSV has been observed in the winter with a peak in January (Aziz *et al*., 2016; Bimouhen *et al*., 2016; Albogami *et al*., 2018). According to most previous studies, the seasonal distribution of HRSV was during winter, this may be one of the reasons why HRSV infection does not appear during the second season except in one

patient. On the other hand, the controversy about HRSV infection rates in terms of differences between the two seasons or in comparison to other studies can be explained by several reasons. Of these, HRSV may be cleared before sampling and sampling delay may reduce the rate of actual viral infection, the virus or its RNA may have degraded due to freezing and thawing during storage in freezers in hospitals and laboratories as well as, sample types, duration of hospitalization, circulating strains, seasonal pattern and age of the patient may also affect the results of the study (Salimi *et al*., 2016).

4.8.3. Distribution of HRSV Positivity Rate by Age Groups:

Results concerning the HRSV positivity rate according to age groups showed that the children under 5 years of age were mostly affected (53.3%) with an insignificant difference compared to other age groups (P= 0.099), table (4.15)

Table (4.15): HRSV positivity by Age Groups.

Age groups (Ys)	HRSV positive		HRSV Negative		P value
	No.	%	No.	%	
<5	8	53.3	180	58.4	0.099
5	1	6.7	47	15.3	
10	1	6.7	29	9.4	
20	2	13.3	11	3.6	
30	-	-	11	3.6	
40	2	13.3	7	2.3	
50	-	-	12	3.9	
=>60	1	6.7	11	3.6	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels**

Human respiratory syncytial virus is a regular and highly contagious winter visitor among people of all ages (Hall, 1983). Since the largest number of samples were collected from children under 5 years (58.2%, 188/323), suggesting that the burden of occurrence of respiratory infections is higher in this group (Nair *et al*., 2013). Analysis of samples of participants suffering from respiratory infections in this study observed that the positive rate of HRSV was 4.6% (15/323). Obviously the current results found that the largest number of HRSV positive individuals was among children less than five year and there are fewer cases in other age groups older than 5 years, suggesting that the rate of HRSV infection decreases with increasing age (Obodai *et al*., 2018). These results are consistent with other studies from different parts of the world (Gamino-Arroyo *et al*., 2016; Fieldhouse *et al*., 2018; Gregianini *et al*., 2018; Tsagarakis *et al*., 2018).

There are several factors responsible for the higher rate of viral infection among young children. Among these the reduced maternal immunity as infants age, along with a naive immune system, this may increase the vulnerability of infants to viral infections. Other important factors, there is no control and prevention programs and effective vaccine for most viral infections during childhood. Hospital-acquired infection and direct contact among children, through attending school, high density among a household population and presence of different viral infectious strains may exaggerate the problem (Anderson *et al*., 2013; Dadashi *et al*., 2018).

4.8.4. Distribution of HRSV Positivity Rate by Gender:

Although the HRSV positivity rate was higher among male versus female (60% *Vs* 40%): However, the difference between them was statistically insignificant (P= 0.679), Table (4.16).

Table (4.16): HRSV Positivity by Gender.

Gender	HRSV positive		HRSV Negative		P value
	No.	%	No.	%	
Male	9	60.0	168	54.5	0.679*
Female	6	40.0	140	45.5	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels**

The results of the present study showed that gender did not appear as a risk factor increasing the infection rate of HRSV among males and females. these results are consistent with most published studies from different countries, (Lamarao *et al*., 2012; Rahman *et al*., 2014; Aziz *et al*., 2016; Kenmoe *et al*., 2018; Hibino *et al*., 2018).

4.8.5. Co-Infection Rate of HRSV:

Table (4.17) revealed that one patient who was positive for HRSV was positive for HPIV-3 (6.7%) with insignificant association (P= 0.803), on the other hand, none of the HRSV positive patients were positive for HPIV-1.

Table (4.17): HRSV Co-Infection Rate among other Respiratory Viruses.

Other viruses		HRSV positive		HRSV negative		P value
		No.	%	No.	%	
HPIV 3	Positive	1	6.7	16	5.2	0.803*
	Negative	14	93.3	292	94.8	
HPIV 1	Positive	-	-	-	-	-
	Negative	15	100.0	308	100.0	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels**

Studies had demonstrated that simultaneous respiratory infection with two or more viruses were widespread among hospitalized patients, although it is unclear whether these infections are less serious or more than a single virus infection (Pinky and Dobrovolny, 2016). Many studies had reported mixed infections: However, the co-infection between HRSV and other respiratory viruses varied from (4% to 53%) (Cai *et al*., 2014). The present study showed that one patient was positive for both HRSV and HPIV-3. Mao *et al*., (2012); found that HRSV co-infection with HPIV-3 was detected in three patients suffering from pneumonia and bronchiolitis. Furthermore, Liu *et al*., (2013), found that HRSV was the major co-detected pathogen with other viruses, it appeared concurrent with HPIV-3 in five patients. The

overlapping of seasonal distribution of these viruses was the reason behind the appearance of co-infection. As previously known that simultaneous appearance of viral infections showed a phenomenon known as viral interference in which one virus prevents the growth of another virus through resource competition (Pinky and Dobrovolny, 2016). Therefore, the concurrent respiratory infection needs further investigations.

4.8.6. The HRSV Associated Clinical Pictures:

Table (4.18) summarized the association of HRSV infection and certain clinical signs and symptoms. Data presented showed that 40% versus 60% of patients with positive HRSV and negative HRSV respectively had cough and Bronchiolitis/bronchitis with insignificant association (P= 0.464, P= 0.316) respectively. On the other hand, only 4(26.7%) of HRSV positive complaining fever with insignificant association (P = 0.907). Additionally, 4(26.7%) the HRSV positive patients had Pharyngitis, and 4(26.7%) had pneumonia with insignificant association (P= 0.997, P= 0.220) respectively.

Table (4.18): Association of HRSV Positive Patients with Clinical Pictures.

Sign or symptom	Status	HRSV positive		HRSV negative		P value
		No.	%	No.	%	
Cough	Yes	6	40.0	153	49.7	0.464
	No	9	60.0	155	50.3	
Fever/chill	Yes	4	26.7	78	25.3	0.907
	No	11	73.3	230	74.7	
Tachypnea/dyspnea	Yes	-	-	5	1.6	0.619
	No	15	100.0	303	98.4	
Wheezing	Yes	-	-	6	1.9	0.585
	No	15	100.0	302	98.1	
Rhinorrhea/Rhinitis	Yes	1	6.7	12	3.9	0.594
	No	14	93.3	296	96.1	
Sore throat	Yes	1	6.7	12	3.9	0.594
	No	14	93.3	296	96.1	
Laryngitis	Yes	-	-	11	3.6	0.456
	No	15	100.0	297	96.4	
Otitis media	Yes	-	-	22	7.1	0.284
	No	15	100.0	286	92.9	
Pharyngitis	Yes	4	26.7	82	26.6	0.997
	No	11	73.3	226	73.4	
Bronchiolitis/ bronchitis	Yes	6	40.0	164	53.2	0.316
	No	9	60.0	144	46.8	
Pneumonitis	Yes	4	26.7	46	14.9	0.220
	No	11	73.3	262	85.1	
sinusitis	Yes	-	-	3	1.0	0.701

	No	15	100.0	305	99.0	
tonalities	Yes	-	-	11	3.6	0.456
	No	15	100.0	297	96.4	
Asthma exacerbation	Yes	1	6.7	13	4.2	0.650
	No	14	93.3	295	95.8	
Severe flue	Yes	-	-	4	1.3	0.657
	No	15	100.0	304	98.7	

Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.

Most HRSV detected in this study were observed among patients with cough and bronchiolitis/bronchitis followed by fever, pharyngitis and pneumonia in contrast to other signs and symptoms. Additionally, these results showed that HRSV had an important pathogenic role of bronchiolitis. Similarly, other studies reported a higher HRSV detection rate were noticed among patients with cough and bronchiolitis (Cangiano *et al*., 2016; Janahi *et al*., 2017; Ali *et al*., 2017).

4.9. Human Parainfluenza Virus type 3 (HPIV-3)

4.9.1. Distribution of HPIV-3 by types of specimens:

Table (4.19) revealed that none of the nasopharyngeal swabs were positive for HPIV-3. Among the nasal sinus swabs, 11(64.7%) were positive for HPIV-3 and 6 (35.3%) of the throat swabs were also positive for HPIV-3. The difference among the types of specimen was statistically insignificant (P= 0.080).

Table (4.19): The HPIV-3 Positivity Rate by Type of Specimens.

Type of specimen	HPIV-3 Positive		HPIV-3 Negative		P value
	No	%	No	%	
Nasopharyngeal swabs	-	-	71	23.2	0.080
Nasal sinus swabs	11	64.7	150	49.0	
Throat swabs	6	35.3	85	27.8	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

As in HMPV, nasal swabs were superior in detecting HPIV-3 compared to nasopharyngeal swabs that were failed in detection of these viruses in any of the samples studied. This may be due to the fact that all positive samples of the HPIV-3 as well as HMPV appeared in children under the age of 5 years, in whom the researcher used for this age group nasal swabs and throat swabs because of the difficulty of using nasopharyngeal swabs for these ages, due to inconvenience and pain induction. Thus these swabs were used only for older patients. In similar circumstances in terms of the use of nasal and throat swabs, Kaveri *et al*. (2015), showed that 26 (11.2%) of patients with influenza like illness were positive for HPIV-3 serotype.

4.9.2. Distribution of HPIV-3 by Season and Month of the Year:

Results in table (4.20) showed that the HPIV-3 positivity rate during season I was (64.7%) while that of season II was (35.3%), However, the difference was failed to reach the levels of statistical significance (P= 0.525). regarding the distribution of HPIV-3 detection rate over the months of the study period, the results revealed that the highest detection rate was found during November (29.4%), but without statistical difference (P= 0.340).

Table (4.20): The HPIV-3 Positivity Rate by Season and Month of Year.

Season	HPIV-3 Positive		HPIV-3 Negative		P value
	No	%	No	%	
Season I	11	64.7	174	56.9	0.525
Season II	6	35.3	132	43.1	
Month of year					
January	-	-	40	13.1	0.340
February	2	11.8	26	8.5	
March	4	23.5	71	23.2	
April	3	17.6	21	6.9	
May	2	11.8	16	5.2	
November	5	29.4	93	30.4	
December	1	5.9	39	12.7	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

As Hassan *et al*. (2018) had mentioned that a large number of viral respiratory infections among children were appeared between November and March, meaning late autumn to early spring in Kurdistan. Similarly, high detection rate of HPIV-3 was observed during these months with a peak in March. The appearance of cases in both April and May, plus seasonal distribution of HPIV-3 based on published studies from worldwide countries are varies from region to region. It depends on data from the samples collected during the study period, some of which reveal that the seasonal outbreaks occur during spring following the epidemics of the influenza, while others studies

reported the peaks of HPIV-3 appear during spring and summer (Fry *et al*., 2006; Zaki and Keating, 2018; Wu *et al*., 2018). Upon reviewing of the scientific literature, no previous studies had revealed the seasonal distribution of HPIV-3 in Iraq in general and the region of the current study in particular. Therefore, this is the leading study in clarifying the outlines of the activity of this virus.

4.9.3 Distribution of HPIV-3 by Age Groups:

Table (4.21) clearly showed that the majority of HPIV-3 positivity rate was in the 5 years old group (82.4%). Nevertheless, the difference among the age groups was statistically insignificant (P= 0.504).

Table (4.21): HPIV-3 Positivity Rate by Age Groups.

Age groups (Ys)	HPIV-3 Positive		HPIV-3 Negative		P value
	No.	%	No.	%	
< 5	14	82.4	174	56.9	0.504
5	2	11.8	46	15.0	
10	-	-	30	9.8	
20	-	-	13	4.2	
30	-	-	11	3.6	
40	-	-	9	2.9	
50	-	-	12	3.9	
=> 60	1	5.9	11	3.6	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

Previous studies have confirmed that HPIV-3 was known to be the most commonly diagnosed in hospitalized patients, especially among young children (Henrickson, 2003; Gomez *et al*., 2009). It is worth noting that similar results were obtained in this current study. Additionally, similar results were reported by other researchers, (Godoy *et al*., 2016; Wu *et al*., 2018). Keeping in the same context, Goya *et al*., (2016), from Argentina found that HPIV-3 was the second cause of respiratory viral infection among children following the HRSV and Moa *et al*., (2012), reported that HPIV-3 was the fifth commonly virus responsible for respiratory infection in pediatric patients in China.

4.9.4. Distribution of HPIV-3 by Gender:

Although the HPIV-3 positivity rate was higher in female compared to males (58.8% vs 41.2%): However, the difference was failed to reach the levels of statistical significant (P= 0.246), table (4.22).

Table (4.22): HPIV-3 Positivity Rate by Gender.

Gender	HPIV-3 Positive		HPIV-3 Negative		P value
	No.	%	No.	%	
Male	7	41.2	170	55.6	0.246
Female	10	58.8	136	44.4	

***Insignificant difference between proportions using Pearson Chi-square at 0.05 levels.**

Compared with the results of the current study which showed a slight increase of HPIV-3 positivity rate among females, which consistent with a study conducted in Korea (Park *et al*., 2018), most

other studies showed that the HPIV-3 infection was higher among males compared to females, however these rates of infection was not a significant difference between the sexes (Mao *et al*., 2012; Shi *et al*., 2015).

2.9.5. Association of HPIV-3 by Clinical Pictures:

Table (4.23) revealed the association of HPIV-3 positivity and certain clinical signs and symptoms. It was obvious that none of the patients with positive HPIV-3 had dyspnea, Wheezing, Rhinitis, Sore throat, Otitis media, sinusitis, tonalities, Asthma exacerbation, and severe flue with insignificant association. On the other hand, Bronchiolitis/bronchitis was found to be the only significantly associated signs among patients with positive HPIV-3 (88.2% vs 11.8%, P= 0.003). Cough (41.2%) and pneumonia (29.4%) were the second moderately associated signs but without significance (P= 0.495 and 0.103) respectively. Other signs and symptoms including Fever, Laryngitis, Pharyngitis had the least insignificant association (P= 0.058, 0.051 and 0.154) respectively.

Table (4.23): Association of HPIV-3 Positive Patients with Clinical Pictures.

Sign or symptom	Status	HPIV-3 positive		HPIV-3 negative		P value
		No.	%	No.	%	
Cough	Yes	7	41.2	152	49.7	0.495
	No	10	58.8	154	50.3	
Fever/chill	Yes	1	5.9	81	26.5	0.058
	No	16	94.1	225	73.5	
Tachypnea/	Yes	-	-	5	1.6	0.595

dyspnea	No	17	100.0	301	98.4	
Wheezing	Yes	-	-	6	2.0	0.560
	No	17	100.0	300	98.0	
Rhinorrhea/ Rhinitis	Yes	-	-	13	4.2	0.386
	No	17	100.0	293	95.8	
Sore throat	Yes	-	-	13	4.2	0.386
	No	17	100.0	293	95.8	
Laryngitis	Yes	2	11.8	9	2.9	0.051
	No	15	88.2	297	97.1	
Otitis media	Yes	-	-	22	7.2	0.252
	No	17	100.0	284	92.8	
Pharyngitis	Yes	2	11.8	84	27.5	0.154
	No	15	88.2	222	72.5	
Bronchiolitis/ bronchitis	Yes	15	88.2	155	50.7	0.003*
	No	2	11.8	151	49.3	
Pneumonitis	Yes	5	29.4	45	14.7	0.103
	No	12	70.6	261	85.3	
sinusitis	Yes	-	-	3	1.0	0.682
	No	17	100.0	303	99.0	
tonalities	Yes	-	-	11	3.6	0.426
	No	17	100.0	295	96.4	
Asthma exacerbation	Yes	-	-	14	4.6	0.367
	No	17	100.0	292	95.4	
Severe flue	Yes	-	-	4	1.3	0.635
	No	17	100.0	302	98.7	

***significant difference between proportions using Pearson Chi-square at 0.05 levels.**

The current results revealed that HPIV-3 was the predominant and main subtype that was highly detected in patients with bronchiolitis/bronchitis. These findings are consistent with those of (Schomacker *et al*., 2012; CDC, 2019) which documented that HPV-3 is more frequently associated with clinical manifestations, bronchiolitis/bronchitis and pneumonia. Similarly, Wang *et al*., (2015), and Villaran *et al*. (2013) had reported a high detection rate of HPIV-3 among patients with severe bronchiolitis/bronchitis and pneumonia in contrast to other HPIVs types.

4.10. Phylogenetic Analysis and Genetic Diversity of HMPV local Isolates

4.10.1. Amplifying of HMPV (G) and (F) Genes by Conventional PCR:

The PCR products after amplification using specific primers for (G) and (F) genes have been presented to the gel electrophoresis, which showed the bands of both genes, as shown in the figures (4.9) and (4.10a and b) respectively.

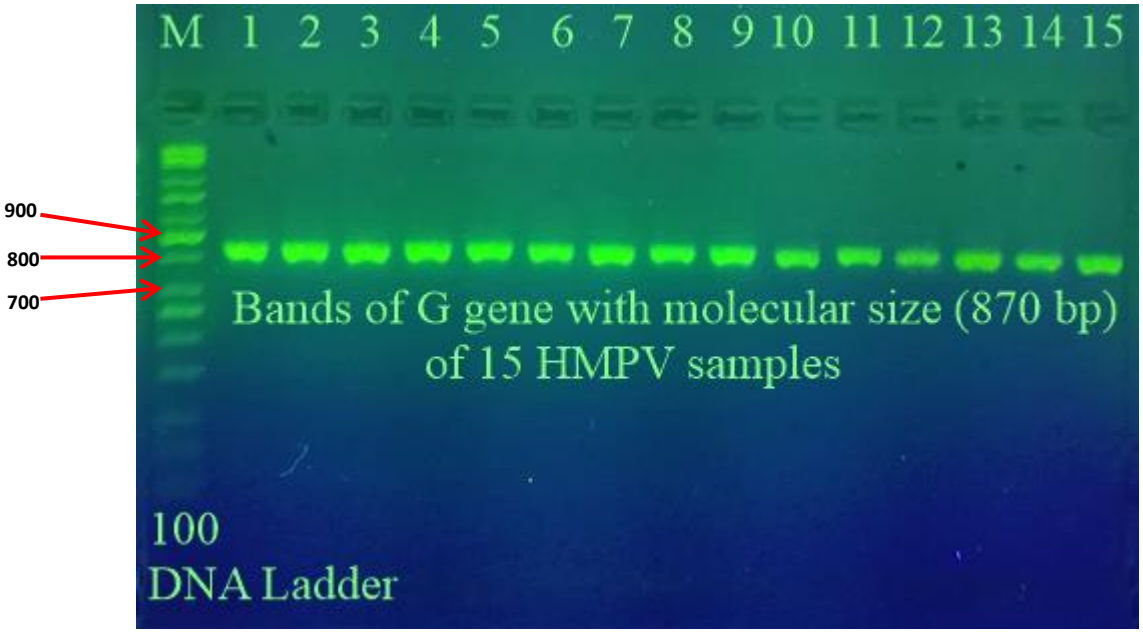


Figure (4.9): Gel Photo of (G) Genes Bands with Product Size, (2%) of agarose gel electrophoresis of PCR product at (70) Volts/ (65) Amp for about (1-1.5) hours and visualized by UV transilluminater. (Lane M: ladder, 100bp). Lane of 1-15 PCR products with 870bp.

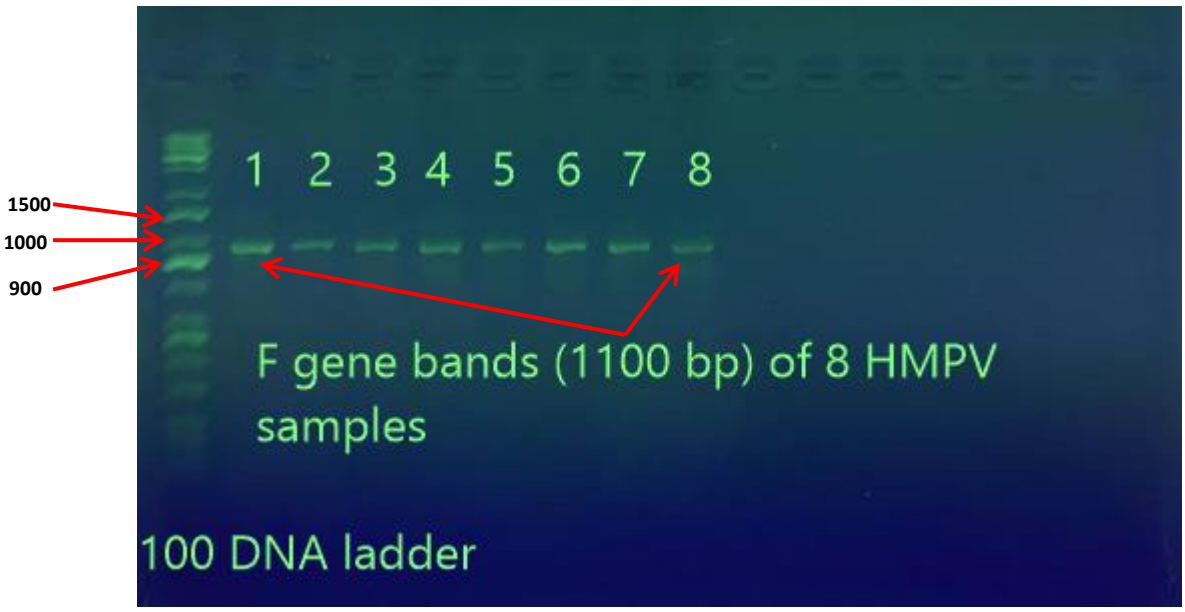


Figure (4.10.a): Gel Photo of (F) Genes Bands with Product Size, (2%) of agarose gel electrophoresis of PCR product at (70) Volts/ (65) Amp for about (1-1.5) hours and visualized by UV transilluminater. (Lane M: ladder, 100bp). Lane of 1-8 PCR products with 1100bp.

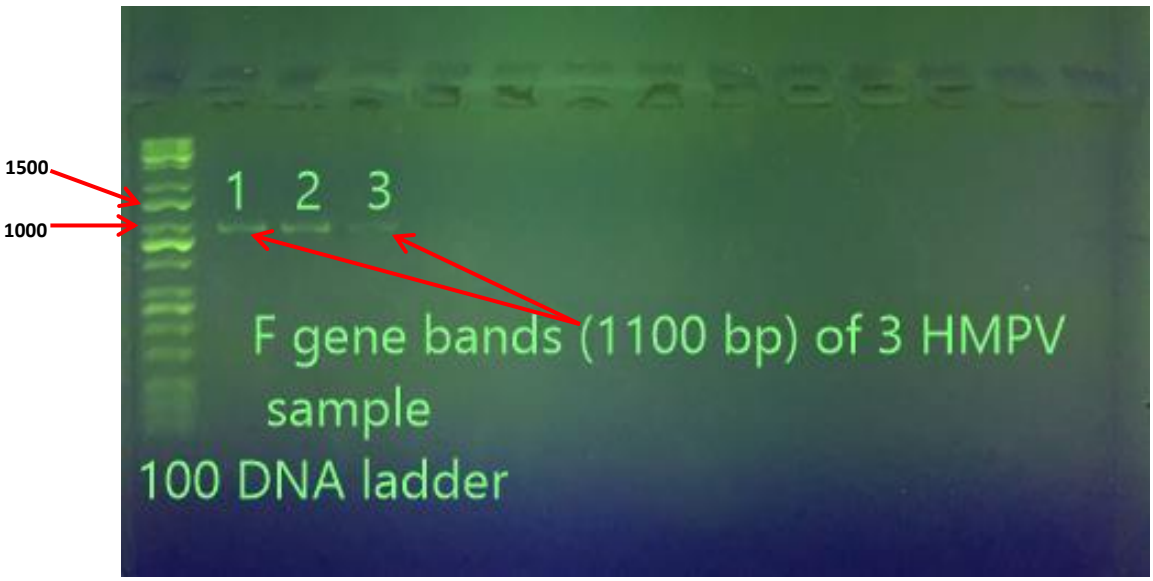


Figure (4.10.b): Gel Photo of (F) Genes Bands with Product Size, (2%) of agarose gel electrophoresis of PCR product at (70) Volts/ (65) Amp for about (1-1.5) hours and visualized by UV transilluminater. (Lane M: ladder, 100bp). Lane of 1-3 PCR products with 1100bp.

4.10.2. Sequencing of HMPV (G and F) Genes:

In spite of the detection of the HMPV (F) gene (figure. 4.10), none of these showed good sequences analysis results, while (15) of (G) gene samples showed good sequences. The (G) gene sequences were submitted to NCBI which gave the published accession number on NCBI, Appendix (2); it can be found on the website;

https://www.ncbi.nlm.nih.gov/popset/?term=MN178607&utm_source=gquery&utm_medium=search

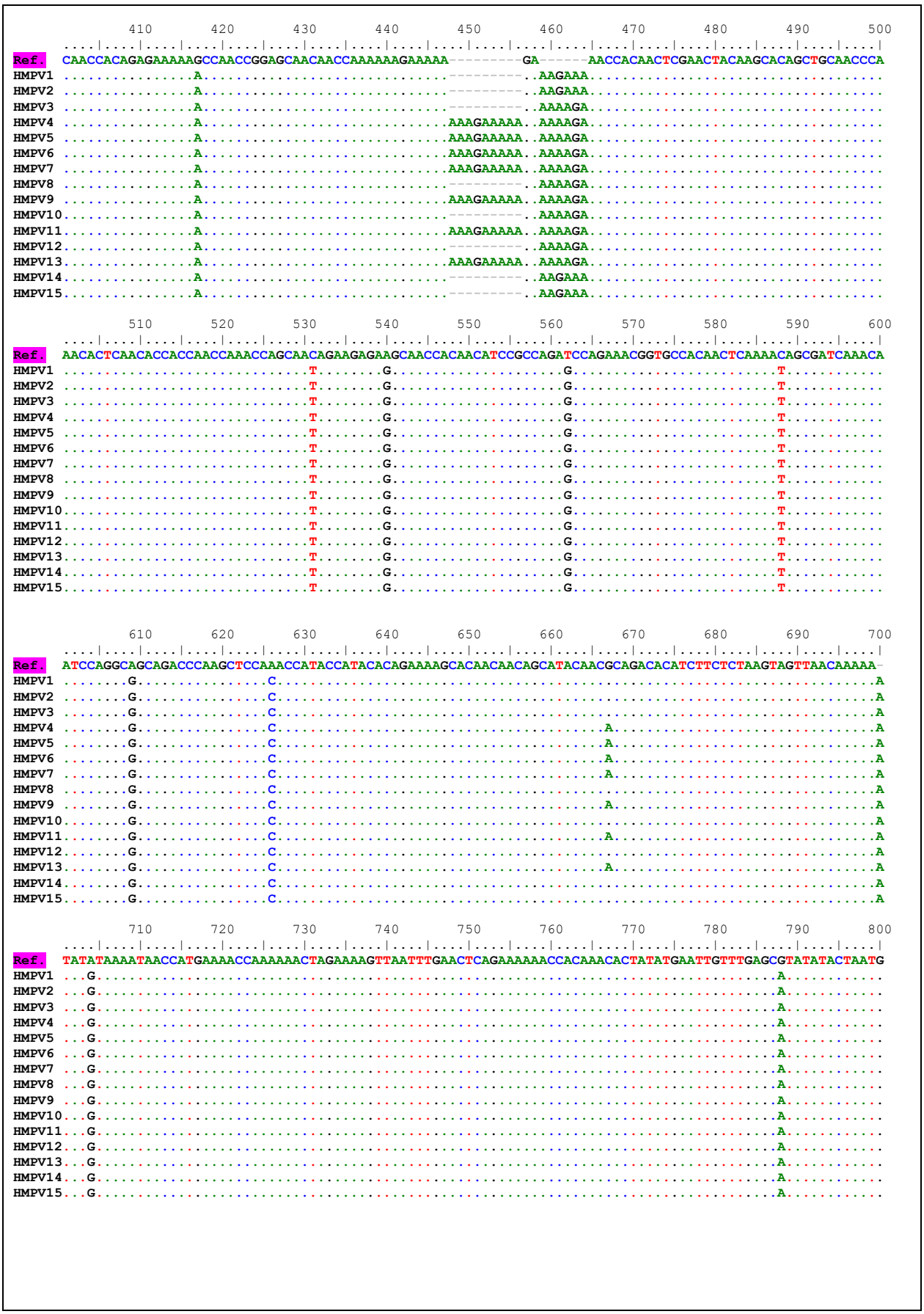
(HMPV1, MN178606.1; HMPV2, MN178607.1; HMPV3, MN178608.1; HMPV4, MN178609.1; HMPV5, MN178610.1; HMPV6, MN178611.1; HMPV7, MN178612.1; HMPV8, MN178613.1; HMPV9, MN178614.1; HMPV10, MN178615.1; HMPV11, MN178616.1; HMPV12, MN178617.1; HMPV13, MN178618.1; HMPV14, MN178619.1; HMPV15, MN178620.1).

4.10.3. Blast and Alignment of Partial HMPV (G) Nucleotide and Amino Acid Sequences:

In order to determine the genotypes of HMPV and the patterns of the observed variations, which including the positions of similarity and differences within this sequences, the nucleotide sequence of partial (G) gene of the HMPV1 – HMPV15 Iraqi local isolates were aligned with the reference sequences of the deposited surface attachment glycoprotein (acc. no. KU320936.1) using UniProtKB alignment suit (https://www.uniprot.org/align/).

It was noted that all the Iraqi strains clustered within the sub-lineage (B2) only. High homologies estimated by (98% and 97%) have been observed between Iraqi isolates (MN178608.1, MN178613.1, MN178615.1, MN178617.1, MN178606.1, MN178607.1, MN178619.1, MN178620.1) and HMPV genotype (B), sub-lineages (B2) prevalent in Spain (GenBank acc. KX829167.1) and Malaysia (GenBank acc. KU320936.1), in the other hand, pairwise sequence alignment showed homologies estimated by (96%) between Iraqi strains that have (AAAGAAAAA, KKE, insertion mutation in the table, 4.24), (HMPV4,5,6,7,9,11,13 in the figure, 4.11, 4.12), (MN178609.1, MN178610.1, MN178611.1, MN178612.1, MN178614.1, MN178616.1, MN178618.1) and Malaysian strains (GenBank acc. KU320936.1) Appendix (3). furthermore, by comparing the observed DNA sequences of these local isolates with the NCBI reference viral sequence (GenBank acc. KU320936.1), the amplified sequences were extended from 1 to 879 of this reference viral sequence (KU320936.1), (Chow *et al.*, 2016; Piñana *et al.*, 2017), (figure 4.11).


10 20 30 40 50 60 70 80 90 100
Ref. TTCAAAGCAAAGATGAAAAACCGTATAAGAAGTAGCAAGTGCTATAGAAATGCTACACTGATCCTTATTGGATTAACAGCATTAAGTATGGCACTTAATA
HMPV1 .....
HMPV2 .....
HMPV3 .....
HMPV4 .....
HMPV5 .....
HMPV6 .....
HMPV7 .....
HMPV8 .....
HMPV9 .....
HMPV10 .....
HMPV11 .....
HMPV12 .....
HMPV13 .....
HMPV14 .....
HMPV15 .....
110 120 130 140 150 160 170 180 190 200
Ref. TTTTTTTAATCATTGATTATGCAAAATTAAAAAACATGACCAAAGTGGAACTCTGTGTCAACATGCCACCGGTAGAACCAAGCAAGAAGACCCCAATGAC
HMPV1 .....A.....
HMPV2 .....A.....
HMPV3 .....A.....
HMPV4 .....A.....
HMPV5 .....A.....
HMPV6 .....A.....
HMPV7 .....A.....
HMPV8 .....A.....
HMPV9 .....A.....
HMPV10 .....A.....
HMPV11 .....A.....
HMPV12 .....A.....
HMPV13 .....A.....
HMPV14 .....A.....
HMPV15 .....A.....
210 220 230 240 250 260 270 280 290 300
Ref. CTCTGCAGTAGACTTAAACACTAAACTCAATCCACAGCAGGCAACACAGTTGACCACAGAGGATTCAACATCTCTAGCAGCAACCTCAGAGAATCATCTA
HMPV1 .....
HMPV2 .....
HMPV3 .....
HMPV4 .....
HMPV5 .....
HMPV6 .....
HMPV7 .....
HMPV8 .....
HMPV9 .....
HMPV10 .....
HMPV11 .....
HMPV12 .....
HMPV13 .....
HMPV14 .....
HMPV15 .....
310 320 330 340 350 360 370 380 390 400
Ref. CACACAGAGACAACTCCAACACCAGATGCAACAATCTCTCAGCAAGCCACAGACGAGCACACAACACTGCTGAGATCAATCAACAGACAGACCACCCAAA
HMPV1 .....A.....T.....A.....T.....
HMPV2 .....A.....T.....A.....T.....
HMPV3 .....A.....T.....A.....T.....
HMPV4 .....A.....T.....A.....T.....
HMPV5 .....A.....T.....A.....T.....
HMPV6 .....A.....T.....A.....T.....
HMPV7 .....A.....T.....A.....T.....
HMPV8 .....A.....T.....A.....T.....
HMPV9 .....A.....T.....A.....T.....
HMPV10 .....A.....T.....A.....T.....
HMPV11 .....A.....T.....A.....T.....
HMPV12 .....A.....T.....A.....T.....
HMPV13 .....A.....T.....A.....T.....
HMPV14 .....A.....T.....A.....T.....
HMPV15 .....A.....T.....A.....T.....


410 420 430 440 450 460 470 480 490 500
Ref. CAACCACAGAGAAAAAGCCAACCGGAGCAACAACCAAAAAAGAAAAA---------GA------AACCACAACTCGAACTACAAGCACAGCTGCAACCCA
HMPV1 .....A.....---------..AAGAAA.....
HMPV2 .....A.....---------..AAGAAA.....
HMPV3 .....A.....---------..AAAAGA.....
HMPV4 .....A.....AAAGAAAAA..AAAAGA.....
HMPV5 .....A.....AAAGAAAAA..AAAAGA.....
HMPV6 .....A.....AAAGAAAAA..AAAAGA.....
HMPV7 .....A.....AAAGAAAAA..AAAAGA.....
HMPV8 .....A.....---------..AAAAGA.....
HMPV9 .....A.....AAAGAAAAA..AAAAGA.....
HMPV10.....A.....---------..AAAAGA.....
HMPV11.....A.....AAAGAAAAA..AAAAGA.....
HMPV12.....A.....---------..AAAAGA.....
HMPV13.....A.....AAAGAAAAA..AAAAGA.....
HMPV14.....A.....---------..AAGAAA.....
HMPV15.....A.....---------..AAGAAA.....
510 520 530 540 550 560 570 580 590 600
Ref. AACACTCAACACCACCAACCAAACCAGCAACAGAAGAGAAGCAACCACAACATCCGCCAGATCCAGAAACGGTGCCACAACTCAAAACAGCGATCAAACA
HMPV1 .....T.....G.....G.....T.....
HMPV2 .....T.....G.....G.....T.....
HMPV3 .....T.....G.....G.....T.....
HMPV4 .....T.....G.....G.....T.....
HMPV5 .....T.....G.....G.....T.....
HMPV6 .....T.....G.....G.....T.....
HMPV7 .....T.....G.....G.....T.....
HMPV8 .....T.....G.....G.....T.....
HMPV9 .....T.....G.....G.....T.....
HMPV10.....T.....G.....G.....T.....
HMPV11.....T.....G.....G.....T.....
HMPV12.....T.....G.....G.....T.....
HMPV13.....T.....G.....G.....T.....
HMPV14.....T.....G.....G.....T.....
HMPV15.....T.....G.....G.....T.....
610 620 630 640 650 660 670 680 690 700
Ref. ATCCAGGCAGCAGACCCAAGCTCCAAACCATACCATACACAGAAAAGCACAACAACAGCATACAACGCAGACACATCTTCTCTAAGTAGTTAACAAAAA-
HMPV1 .....G.....C.....A.....A
HMPV2 .....G.....C.....A.....A
HMPV3 .....G.....C.....A.....A
HMPV4 .....G.....C.....A.....A
HMPV5 .....G.....C.....A.....A
HMPV6 .....G.....C.....A.....A
HMPV7 .....G.....C.....A.....A
HMPV8 .....G.....C.....A.....A
HMPV9 .....G.....C.....A.....A
HMPV10.....G.....C.....A.....A
HMPV11.....G.....C.....A.....A
HMPV12.....G.....C.....A.....A
HMPV13.....G.....C.....A.....A
HMPV14.....G.....C.....A.....A
HMPV15.....G.....C.....A.....A
710 720 730 740 750 760 770 780 790 800
Ref. TATATAAAATAACCATGAAAACCAAAAAACTAGAAAAGTTAATTTGAACTCAGAAAAAACCACAAACACTATATGAATTGTTTGAGCGTATATACTAATG
HMPV1 ...G.....A.....
HMPV2 ...G.....A.....
HMPV3 ...G.....A.....
HMPV4 ...G.....A.....
HMPV5 ...G.....A.....
HMPV6 ...G.....A.....
HMPV7 ...G.....A.....
HMPV8 ...G.....A.....
HMPV9 ...G.....A.....
HMPV10...G.....A.....
HMPV11...G.....A.....
HMPV12...G.....A.....
HMPV13...G.....A.....
HMPV14...G.....A.....
HMPV15...G.....A.....

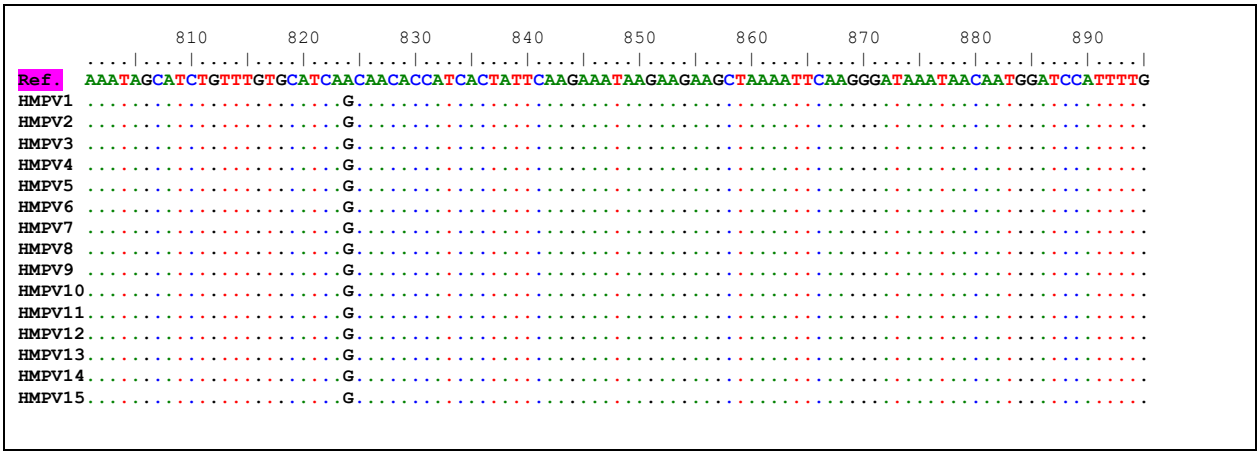
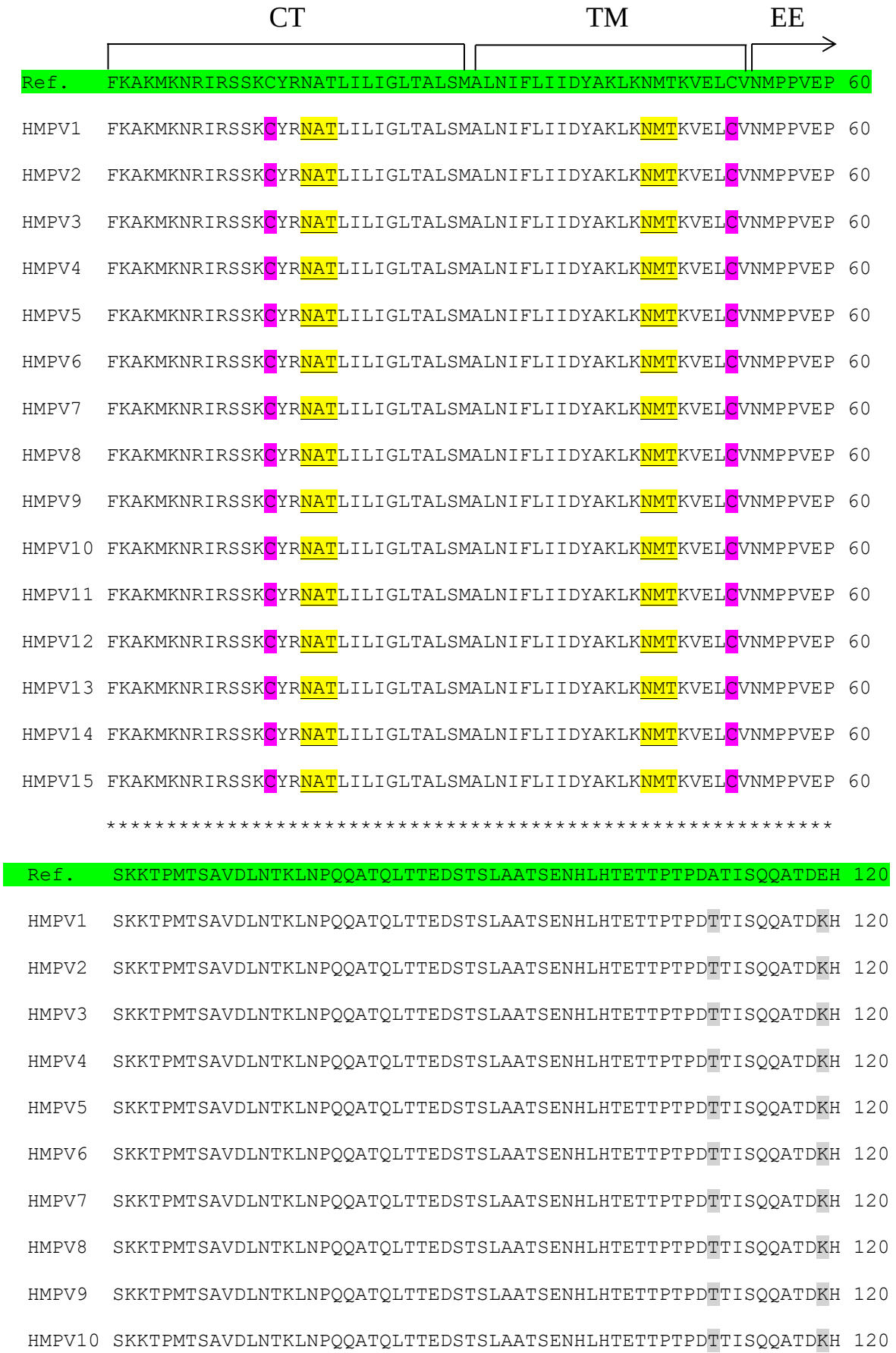


Figure (4.11): Multiple DNA sequences alignment of 15 local viral isolates the partial HMPV (G) gene sequences with its corresponding reference sequences of the (G) gene amplicon of the HMPV genomic sequences (acc. no. KU320936.1).

The alignment of the amino acid sequence of the HMPV (G) protein of Iraqi strains with their reference sequences prototype strains showed that both of cytoplasmic tail domain (CT) and transmembrane domain (TM) had no genetic changes at both levels of nucleotide and the amino acid sequences (mutations), indicating that these regions are highly conserved (figure 4. 12). Similarly, the sequences of amino acid of the HMPV (G) protein of the Indian strains showed that both intracellular and transmembrane domains were at a high degree of conserved across the strains (Agrawal *et al.*, 2011), similar observations were also noticed in other studies (Peret *et al.*, 2004; Yang *et al.*, 2013).


CT
TM
EE
Ref. FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV1 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV2 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV3 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV4 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV5 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV6 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV7 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV8 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV9 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV10 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV11 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV12 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV13 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV14 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
HMPV15 FKAKMKNRIRSSKCYRNATLILIGLTALSMALNIFLIIDYAKLKNMTKVELCVNMPPVEP 60
Ref. SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDATISQQATDEH 120
HMPV1 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV2 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV3 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV4 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV5 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV6 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV7 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV8 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV9 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV10 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120

```
HMPV11 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV12 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV13 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV14 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
HMPV15 SKKTPMTSAVDLNTKLNPQQATQLTTEDSTSLAATSENHLHTETTPTPDTTISQQATDKH 120
       **************************************************:********:*

Ref.   TTLLRSINRQTTQTTTEKKPTGATTKKE-----KETTTRTTSTAATQTLNTTNQTSNRRE 175
HMPV1  TTLLRSINRQTIQTTTEKKPTGATTKKEK---ERKTTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV2  TTLLRSINRQTIQTTTEKKPTGATTKKEK---ERKTTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV3  TTLLRSINRQTIQTTTEKKPTGATTKKEK---EKETTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV4  TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV5  TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV6  TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV7  TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV8  TTLLRSINRQTIQTTTEKKPTGATTKKEK---EKETTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV9  TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV10 TTLLRSINRQTIQTTTEKKPTGATTKKEK---EKETTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV11 TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV12 TTLLRSINRQTIQTTTEKKPTGATTKKEK---EKETTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV13 TTLLRSINRQTIQTTTEKKPTGATTKKEKKEKEKETTTRTTSTAATQTLNTTNQTSNRRE 180
HMPV14 TTLLRSINRQTIQTTTEKKPTGATTKKEK---ERKTTTRTTSTAATQTLNTTNQTSNRRE 177
HMPV15 TTLLRSINRQTIQTTTEKKPTGATTKKEK---ERKTTTRTTSTAATQTLNTTNQTSNRRE 177
       **********:***************:   :**************************

Ref.   ATTTSARSRNGATTQNSDQTIQAADPSSKPYHTQKSTTTAYNADTSSLSS 225
HMPV1  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV2  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV3  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV4  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV5  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
```

```
HMPV6  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV7  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV8  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV9  ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV10 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV11 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV12 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV13 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNTDTSSLSS 230
HMPV14 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
HMPV15 ATTTSARARNGATTQNSDQTIQAADPSSTPYHTQKSTTTAYNADTSSLSS 227
       *******:********************.*************:*******
```

Figure (4.12):Amino acid alignment of the observed amino acids variations of the surface attachment glycoprotein that found in the HMPV1 – HMPV15 local viral samples with the corresponding referring surface attachment glycoprotein (acc. no. KU320936.1), the grey color refers to the observed missense mutations, while the cyan brilliant color refers to the frameshift (insertion) mutation, purple refers to the cysteine residues and yellow to the N-glycosylation.

The greater number of amino acid changes were detected in the extracellular ectodomain EE which are located adjacent to the C-terminus, and this domain of the attachment viral protein is exposed to immune surveillance of the host by the antibody, due to mutations that occurred within the nucleotide sequence which include substitution and insertion mutations; nucleotide insertion causes differences in the length of polypeptide chains ranged from (227 to 230 aa) within the sub-lineage B2 local isolates. These observations are similarly

documented in other previous studies; Agrawal *et al.*, (2011), and, Yang *et al*., (2013), they founded that most changes in the amino acid sequences was noticed in the Ectodomain in contrast to remain regions of the attachment (G) protein that showed a high conservation status. The sequence of amino acid revealed that two cysteine residues were showed with a highly conservative position 14 aa in CT and 52 aa in TM among all strains, which corresponds to the initial reported previously by van den Hoogen *et al*., (2002), and Agrawal *et al*., (2011). On the other hand, high content of threonine and serine residues which are potential O-linked sugar acceptors (O-glycosylation sites) in detected sub-lineage B2 ranged from (70-71) aa and (21) aa respectively.

All Iraqi strains detected in this study have four N-glycosylation sites (Asn-X-Ser/Thr/Cys) at the position 17-19 aa in CT domain and the second located at the position (45-47 aa) in TM, third and fourth N-glycosylation located in the EE domain at the position (170-172, 173-175 aa) respectively which were conserved in all the strains (figure 4.12). In contrast to Agrawal *et al*., (2011), who found differences in polypeptides length (217-231 aa) as a result of changes in the stop codon position of (G) protein. Analysis of the current sequence of G protein in these local isolates did not show changes in stop codon position and the differences in polypeptides occurred due to insertion mutations.

As HRSV, both N- and O-linked glycosylation in the HMPV (G) protein is an important distinctive feature of antigenicity, perhaps because of its influential role in the expression of antigenic determinants (epitopes) by either capable of masking or facilitating the

recognition of antibodies and thus enable the virus to evade from the host immune system (Ahmed *et al*., 2016). It was scientifically known that the ectodomine of transmembrane surface antigens of viruses are responsible for both, attachment capability of the virus to susceptible target cells plus governing the type and magnitude of host immune responses. In this context, due to substitution and insertion mutations in nucleotide sequences, especially in the extracellular ectodomain, the HMPV (G) gene was undergoing to high level of variations (Peret *et al*., 2004).

Leyrat *et al*., (2014a), showed that the extracellular ectodomin of viral attachment (G) glycoprotein of this virus which is represent as a heavily glycosylated disordered polymer, conducts to modulates immune responses (innate and adaptive), this lead to incomplete immunity of the host and induces to re-infection. Different studies suggested that most changes in amino acid sequences due to substitution and insertion mutations is the result of the impact of immunological pressure that might play an important role in causing genetic diversity and antegenic drifts of HMPV (G) gene in the extracellular ctodomain region, these amino acid changes are useful in evasion and modulates the host immune responses (Peret *et al*., 2004; Ishiguro *et al*., 2004; van den Hoogen *et al*., 2004). In the same context, high rates of evolution were observed in (G) gene (2.13×10^{-3} substitution/site/year), in compared to (F) gene that was highly conserved, and other HMPV genes (Kim *et al*., 2016), multiple lineage of HMPV emerged as a result of genetic changes in this particular region, these greater genetic diversity that were observed in the (G)

gene of all HMPV lineages are located in the extracellular ectodomain region (Papenburg *et al*., 2013).

Positive selection is a strong impact factor on the genetic variability which aids the virus to acquire some evolutionary feature, which is reflected in its advantages in avoidance of antibody recognition of the host, and possibly facilitating recurrent infections with the same virus (Air *et al*., 1990) and effects on the continue circulating of the virus in the communities, these genetic variations pose future vaccine development challenges (Ludewick *et al*., 2005) Supporting that a leading study from India revealed that the (G) gene exhibited a high diversity at the nucleotide and amino acid sequences level among (77%) of HMPV strains, while less than (10%) of genetic variation was noted in the sequences of (F) gene, affirming that the HMPV G protein is continuously evolving (Agrawal *et al*., 2011).

Viruses containing the RNA genome are vulnerable to rapid changes in their viral nucleotide sequences due to high mutations, as a result to the lack of "RNA-based viral polymerase (RRP) polymerase" to proofreading activity (Saikusa *et al*., 2017; Duffy, 2018), From this perspective, the results of the present study showed changes in the sequence of nucleotides of HMPV (G) gene. By using Expasy translate server for translating the observed variations with in the nucleotide sequences, this study was found that the substitution mutations were taken three patterns of variations; silent mutations that are not able to change the resulting amino acid sequences which include KU320936.1; g.147G>A, g.348C>T, g.417G>A, g.516C>T, g.525A>G, g.573C>T, g.594A>G, g.688A>G, g.772G>A, and g.808A>G: missense mutations

that cause amino acid substitutions which include KU320936.1; g.328 G>A, g.355G>A, g.395C>T, g.547T>G, g.611A>C, and g.652G>A: while the third pattern of variations were found to take a frameshift form that was found in KU320936.1; g.447-448ins AAAGAAAAA, g.451-452ins AAGAAA, g.451-452ins AAAAGA, and g.684-685ins A (table 4. 24). These observed variations in the sequence of amino acid sequences of (G) protein due to substitution and insertion mutations may result in changes in antigenic characteristics, evasion of the host immune system, re-infection, and the emergence of new strains effectively spread, making it more difficult to develop effective vaccines against this virus and other respiratory viruses (Etemadi *et al*., 2013).

Table (4.24): Mutations apparent in all of the Studied Isolates of (G) gene Amplicons in the HMPV Sequences. The NCBI Reference Accession Number used is KU320936.1.

No.	Reference nucleotide	Mutant nucleotide	Position in the genome	Position in the protein	Type of mutation	Observed in	mutation summary
1	G	A	147	-	Silent	HMPV1– HMPV15	KU320936.1;g.147G>A
2	G	A	328	A110T	Missense	HMPV1– HMPV15	KU320936.1;g.328 G>A
3	C	T	348	-	Silent	HMPV1– HMPV15	KU320936.1;g.348C>T
4	G	A	355	E119K	Missense	HMPV1– HMPV15	KU320936.1;g.355G>A
5	C	T	395	T132I	Missense	HMPV1– HMPV15	KU320936.1;g.395C>T
6	G	A	417	-	Silent	HMPV1– HMPV15	KU320936.1;g.417G>A
7	-	AAAGA AAAA	447-448	150-151 KKE ins	frameshift	HMPV4, HMPV5, HMPV6, HMPV7, HMPV9, HMPV11, HMPV13	KU320936.1;g.447-448ins AAAGAAAAA
8	-	AAGAAA	451-452	150-151 KE ins	frameshift	HMPV1, HMPV2, HMPV14, HMPV15	KU320936.1;g.451-452ins AAGAAA
9		AAAAGA	451-452	150-151 KE ins	frameshift	HMPV3- HMPV4, HMPV5, HMPV6, HMPV7, HMPV8, HMPV9, HMPV10, HMPV11, HMPV12, HMPV13	KU320936.1;g.451-452ins AAAAGA
10	C	T	516	-	Silent	HMPV1– HMPV15	KU320936.1;g.516C>T
11	A	G	525	-	Silent	HMPV1– HMPV15	KU320936.1;g.525A>G
12	T	G	547	S183A	Missense	HMPV1– HMPV15	KU320936.1;g.547T>G
13	C	T	573	-	Silent	HMPV1– HMPV15	KU320936.1;g.573C>T
14	A	G	594	-	Silent	HMPV1– HMPV15	KU320936.1;g.594A>G
15	A	C	611	K212T	Missense	HMPV1– HMPV15	KU320936.1;g.61A>C
16	G	A	652	A218T	Missense	HMPV4, HMPV5, HMPV6, HMPV7, HMPV9, HMPV11, HMPV13	KU320936.1;g.652G>A
17	-	A	684-685	-	frameshift	HMPV1– HMPV15	KU320936.1;g.684-685insA
18	A	G	688	-	Silent	HMPV1– HMPV15	KU320936.1;g.688A>G
19	G	A	772	-	Silent	HMPV1– HMPV15	KU320936.1;g.772G>A
20	A	G	808	-	Silent	HMPV1	KU320936.1;g.808A>G

4.10.4. Analysis of Phylogenetic Tree:

For the best of our knowledge, this is the first study covers the genetic diversity of HMPV based on the sequence analysis of the (G) gene obtained in this study area. Phylogenetic analysis demonstrated that all the Iraqi strains clustered within the sub-lineage (B2) only based on the comparison of the sequence nucleotide of (G) gene with the retrieved sequences of different genotypes in the GenBank database. In a similar manner, in Saudi Arabia and Egypt, HMPV (B2) was most commonly reported in comparison to other sub-lineages (Mohamed *et al*., 2014; Amer, 2016).

Alignment of the (G) gene revealed high sequence diversity within members of the same (B2) sub-lineage, the frameshift variations (insertion mutations) which appeared in three forms in the studied HMPV isolates result in divided these sub-lineage (B2) viral variants into three subgroups, this current finding reveals three distinct HMPV variants, these viral variants were distributed into three main subgroups; the subgroup one, including and (HMPV3, MN178608.1; HMPV8, MN178613.1; HMPV10, MN178615.1, HMPV12, MN178617.1), the subgroup two, including HMPV1, MN178606.1; HMPV2, MN178607.1; HMPV14, MN178619.1, HMPV15, MN178620.1) and subgroup three, including (HMPV4, MN178609.1; HMPV5, MN178610.1; HMPV6, MN178611.1; HMPV7, MN178612.1; HMPV9, MN178614.1; HMPV11, MN178616.1, HMPV13, MN178618.1).

The current phylogenetic tree has revealed three close genetic identities of the HMPV isolates that were found to belong to the same (B2) sub-lineage, which were clearly different from other viral sequences deposited, indicating distinct phylogenetic sites occupied by these viral specimens in the international viral genetic database (figure 4.13). Abed and Boivin, (2008), reported two distinct variants in the same (B2) sub-lineage, and suggested that these distinct variants of HMPV may be represent viral drift as a results of immune pressure.

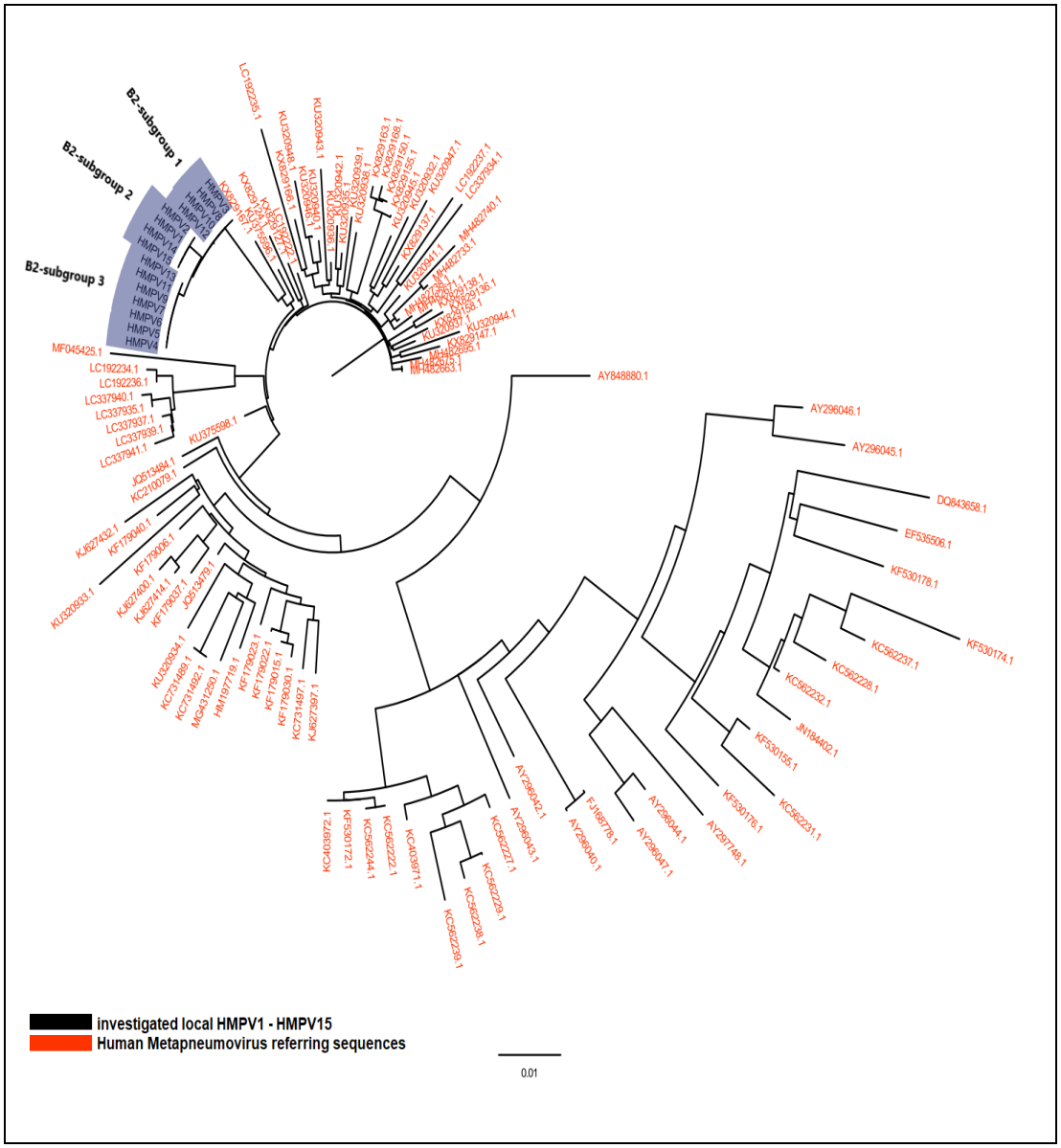


Figure (4.13): The comprehensive phylogenetic tree of variants of (G) gene of HMPV local isolates. The highlighted positions refer to the sequenced HMPV1 – HMPV15 variants, while the orange color refers to other referring NCBI HMPV deposited viruses. All the mentioned numbers referred to Genbank acc. no. of each referring virus. The number "0.01" at the bottom of the tree refers to the degree of scale range among the comprehensive tree categorized organisms.

4.11. HRSV Subgrouping:

Positive samples that were detected by the first step of diagnostic real-time PCR were subgrouped using sets of primers and TaqMan probe which targeting the F protein of the two HRSV subgroups A and B, according to Agoti *et al*. (2015). Fusion protein is preferred for subgrouping, because it is genetically highly stable compared to (G) protein which is commonly used subgrouping, out of 15 positive samples, only one sample in second season were subgrouped and showed circulate of the HRSV antigenic subgroup (B) in this study area, (figure 4. 14).

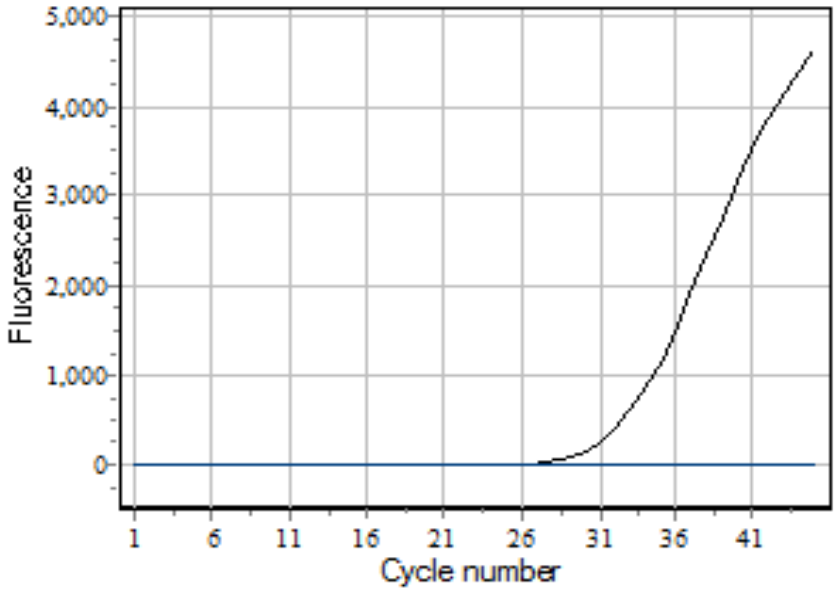


Figure (4.14): Real-Time PCR amplification log plot showed cycle of HRSV (B) subgroup positive results at CT: 33.

As a result of damage to other positive samples of HRSV that detected in January, February and March, and subgrouped one positive sample that detected in in December in this study; it is difficult to compare current results with those of other studies; However,

Abduljabbar *et al*. (2019) showed the dominance of the HRSV antigenic subgroup (B) in Baghdad. Other studies in Saudi Arabia and Japan have demonstrated the dominance of HRSV antigenic subgroup (A) as compared to the prevalence of the HRSV antigenic subgroup (B) (Ahmed *et al*., 2016; Hibino *et al*., 2018). The researcher was unable to sequence the nucleotide of the positive samples in order to determine the genotypes of HRSV, and thus the primers of HRSV (G) gene that mentioned in the table (3.4), Chapter Three were not used to determine the genotypes of HRSV despite the availability of the study.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

Chapter Five

5. Conclusions and Recommendations

5.1. Conclusions:

The outcomes of the present study can be concluded as follows:

1- Human Metapneumovirus showed a predominance of the prevalence as a causative agent of respiratory infection compared to other viruses under study in Diyala community.

2- Human Parainfluenza type-3 and Human Respiratory Syncytial viruses as single or co-viral pathogens with Human Metapneumovirus play the secondary role as respiratory viral pathogens.

3- Infections with Human Metapneumovirus, Human Respiratory Syncytial virus and Human Parainfluenza infections were detected among children less than 5 years old and cough, bronchiolitis and pneumonia are the most clinical presentations associated with these viruses.

4- Climatic changes have influenced the detection rate of respiratory viral pathogens.

5- Nasal and throat swabs have a great efficacy in detecting respiratory viruses.

6- Phylogenetic analysis of Human Metapneumovirus (G) gene sequences were documented the predominant circulation of Human Metapneumovirus (B) genotype, (B2) sub-lineage in among Diyala community.

7- New distinct Human Metapneumovirus variants within the (B2) sub-lineage were detected as a result of high genetic variations in the sequencing of nucleotides and amino acids in the (G) gene.
8- The phylogenetic subgrouping of Human Respiratory Syncytial virus documents the circulation of HRSV antigenic subgroup (B) in Diyala community.

5.2. Recommendations:

1. The outcomes of this study should be informed to the Diyala Directory of Health as they were the assisting partner and also to promote and strength future cooperation.

2. Further cooperative and comprehensive studies are needed to explore the epidemiology of respiratory viruses in all Iraqi provinces using multiplex real-time polymerase chain reaction technique.
3. As being the number one, future clinical and virological studies on Human Metapneumovirus are recommended particularly among children in Diyala province.
4. Further studies are needed to determine the relationship between HMPV viral load and disease severity concerning an oxygen supply, period of hospital stay, admission to ICU and mortality.
5. Additional studies are needed to assess the effect of changes caused by substitution and frameshift mutations in the extracellular ectodomain of Human Metapneumovirus (G) gene on clinical severity and host immune response of the virus.
6. Additional molecular studies are required targeting other genes of respiratory viruses for the purpose of adoption or the development of single or combined vaccines.
7. As being semi-neglected by scientific bodies, highlighting intensive clinical and epidemiological studies on the Human Parainfluenza viruses are recommended.

8. Cooperative educational activities to minimize the effect of risk factors and thus to reduce the rate or the severity of viral respiratory tract infection on the community.

REFERENES

References

A

Abduljabbar, H. L., Hussein, A. A., Al-Mayah, Q. S and Aufi, I. M. (2019). Phylogenetic Analysis of Respiratory Syncytial Virus Isolated from Children with Respiratory Tract Infections in Baghdad City, Iraq. Journal of Physics: Conference Series, 1234, pp.012082.

Abduljabbar, H. L., Hussein, A. A., Aufi, I. M. and Al-Mayah, Q. S. (2018). Detection of Respiratory Syncytial Virus and Human Metapneumovirus in Children with Respiratory Tract Infection by Use Real Time Polymerase Chain Reaction. Diyala Journal of Medicine, 15(2), pp.87-97.

Abed, Y., & Boivin, G. (2008). Human metapneumovirus infection in immunocompromised child. Emerging infectious diseases, 14(5), pp. 854–856. doi:10.3201/eid1405.071459

Abedi, G., Prill, M., Langley, G., Wikswo, M., Weinberg, G., Curns, A. and Schneider, E. (2014). Estimates of Parainfluenza Virus-Associated Hospitalizations and Cost Among Children Aged Less Than 5 Years in the United States, 1998–2010. Journal of the Pediatric Infectious Diseases Society, 5(1), pp.7-13.

Afonso, C.L., Amarasinghe, G.K., Bányai, K., Bào, Y., Basler, C.F., Bavari, S., Bejerman, N., Blasdell, K.R., Briand, F.X., Briese, T. and Bukreyev, A., (2016). Taxonomy of the order Mononegavirales: update 2016. Archives of virology, 161(8), pp.2351-2360.

Agoti, C., Otieno, J., Munywoki, P., Mwihuri, A., Cane, P., Nokes, D., Kellam, P. and Cotten, M. (2015). Local Evolutionary Patterns of Human Respiratory Syncytial Virus Derived from Whole-Genome Sequencing. Journal of Virology, 89(7), pp.3444-3454.

Agrawal, A., Roy, T., Ghosh, S. and Chawla-Sarkar, M. (2011). Genetic variability of attachment (G) and Fusion (F) protein genes of human metapneumovirus strains circulating during 2006-2009 in Kolkata, Eastern India. Virology Journal, 8(1), p.67.

Ahmed, A., Haider, S., Parveen, S., Arshad, M., Alsenaidy, H., Baaboud, A., Mobaireek, K., AlSaadi, M., Alsenaidy, A. and Sullender, W. (2016). Co-Circulation of 72bp Duplication Group A and 60bp Duplication Group B Respiratory Syncytial Virus (RSV) Strains in Riyadh, Saudi Arabia during 2014. PLOS ONE, 11(11), p.e0166145.

Air, G.M., Gibbs, A.J., Laver, W.G. and Webster, R.G., (1990). Evolutionary changes in influenza B are not primarily governed by antibody selection. Proceedings of the National Academy of Sciences, 87(10), pp.3884-3888.

AL-Bashar, S.H., Badawy, A.S. and Mohammed, B.A.R., (2017). Real time-PCR, ELISA, as an identification methods for Detection respiratory syncytial virus in children. Tikrit Journal of Pure Science, 22(2).

Albogami, S., Alotaibi, M., Alsahli, S., Masuadi, E. and Alshaalan, M. (2018). Seasonal variations of respiratory viruses detected from children with respiratory tract infections in Riyadh, Saudi Arabia. Journal of Infection and Public Health, 11(2), pp.183-186.

Ali, A., Alsuheel, A., Al-Hakami, A., Shati, A., Chandramoorthy, H. and Al-Qahtani, S. (2019). Human metapneumovirus in pediatric patients with acute respiratory tract infections in the aseer region of Saudi Arabia. Saudi Journal of Medicine and Medical Sciences, 7(2), p.80.

Ali, A., Khowaja, A., Bashir, M., Aziz, F., Mustafa, S. and Zaidi, A. (2013). Role of Human Metapneumovirus, Influenza A Virus and Respiratory Syncytial Virus in Causing WHO-Defined Severe Pneumonia in Children in a Developing Country. PLoS ONE, 8(9), p.e74756.

Ali, A., Yousafzai, M., Waris, R., Jafri, F., Aziz, F., Abbasi, I. and Zaidi, A. (2017). RSV associated hospitalizations in children in Karachi, Pakistan: Implications for vaccine prevention strategies. Journal of Medical Virology, 89(7), pp.1151-1157.

Ali, M., Han, S., Gunst, C. J., Lim, S., Luinstra, K., & Smieja, M. (2015). Throat and nasal swabs for molecular detection of respiratory viruses in acute pharyngitis. Virology journal, 12, 178. doi:10.1186/s12985-015-0408-z.

Ali, S.A., Williams, J.V., Chen, Q., Faori, S., Shehabi, A., Jundi, E.A., Khuri-Bulos, N. and Halasa, N., (2010). Human Metapneumovirus in hospitalized children in Amman, Jordan. Journal of medical virology, 82(6), pp.1012-1016.

Almajhdi, F., Alshaman, M. and Amer, H. (2012). Molecular characterization and phylogenetic analysis of Human Parainfluenza virus type 3 isolated from Saudi Arabia. Journal of Medical Virology, 84(8), pp.1304-1311.

Almasri, M., Papa, A., Souliou, E., Haidopoulou, K., & Eboriadou, M. (2013). Respiratory syncytial virus infection in hospitalized children older than 2 years with community-acquired pneumonia. Hippokratia, 17(2), 146–149.

Al-Shuhaib, M.B.S., Al-Kaaby, H.N., Alwan, S.L. (2019). A highly efficient electrophoretic method for discrimination between two Neoscytalidium species using a specific fungal internal transcribed spacer (ITS) fragment. Folia Microbiologica 64(2): 161-170. doi: 10.1007/s12223-018-0641-0.

Al-Turab, M., Chehadeh, W. and Al-Nakib, W. (2015). Phylogenetic analysis of human metapneumovirus detected in hospitalized patients in Kuwait during the years 2009–2011. Journal of Infection and Public Health, 8(5), pp.448-457.

Alvarez, R. and Tripp, R. (2005). The Immune Response to Human Metapneumovirus Is Associated with Aberrant Immunity and Impaired Virus Clearance in BALB/c Mice. Journal of Virology, 79(10), pp.5971-5978.

Amarasinghe, G.K., Ceballos, N. G. A., Banyard, A. C., Basler, C.F., Bavari, S., Bennett, A. J., Blasdell, K.R., Briese,T., Bukreyev, Caì, Y., Calisher, C.H., A. Beer, M., Bejerman, N., Blasdell, K.R., Bochnowski, A., Briese, T., and 2017. Taxonomy of the order Mononegavirales: update 2018. Archives of virology, (0123456789). doi: 10.1007/s00705-018-3814-x.

Amer, H. (2016). Molecular Epidemiology of Human Metapneumovirus in Riyadh Province, Saudi Arabia. Journal of Molecular Microbiology and Biotechnology, 26(6), pp.414-421.

Anderson, L., Dormitzer, P., Nokes, D., Rappuoli, R., Roca, A. and Graham, B. (2013). Strategic priorities for respiratory syncytial virus (RSV) vaccine development. Vaccine, 31, pp.B209-B215.

Arabpour, M., Samarbafzadeh, A., Makvandi, M., Shamsizadeh, A., Percivalle, E., Englud, J. and Latifi, S. (2008). The highest prevalence of human metapneumovirus in Ahwaz children accompanied by acute respiratory infections. Indian Journal of Medical Microbiology, 26(2), p.123.

Arbefeville, S. and Ferrieri, P. (2017). Epidemiologic Analysis of Respiratory Viral Infections Mainly in Hospitalized Children and Adults in a Midwest University Medical Center After the Implementation of a 14-Virus Multiplex Nucleic Acid Amplification Test. American Journal of Clinical Pathology, p.aqw185.

Arjeyni, Y., Faghihloo, E., Rad, K. S., Salimi, V., Mahmoudi, M., & Mokhtari-Azad, T. (2017). Molecular Epidemiology of Human Respiratory Syncytial Virus in Iranian≥ 60 Years old Hospitalized

Patients with Acute Respiratory Symptoms. Archives of Iranian Medicine (AIM), 20(6).

Arnott, A., Vong, S., Sek, M., Naughtin, M., Beauté, J., Rith, S., Guillard, B., Deubel, V. and Buchy, P. (2013). Genetic variability of human metapneumovirus amongst an all ages population in Cambodia between 2007 and 2009. Infection, Genetics and Evolution, 15, pp.43-52.

Aslanzadeh, J. and Tang, T. (2011). Human Metapneumovirus. In: *Molecular Detection of Human Viral Pathogens*. Liu, D. (ed). USA. Taylor and Francis Group, LLC. CRS. P: 481-2. 3: 978-1-4398-1237-2 (Ebook-PDF).

Atyah, N.S., Hula, Y.F., Faisal, G.A., Iman, M.A. and Manal, A.A., (2017). Molecular detection of Subfamily Pneumovirinae among Children with Flu-like illness by using RT-PCR. Current Research in Microbiology and Biotechnology, 5(5), pp.1239-1244.

Aziz, T. (2015). Detection of Human Metapneumovirus in Hospitalized Children with Acute Respiratory Tract Infections in Sulaimani Province, Iraq. Journal of Medical Microbiology and Diagnosis, 04(02). http://dx.doi.org/10.4172/2161-0703.1000185.

Aziz, T., Hamid, Z. and Bayati, A. (2016). Detection of Respiratory Syncytial Virus in Hospitalized Children with Acute Respiratory Tract Infections in Kurdistan Region of Iraq. Global Journal of Bio-scinece and Biotechnology, 5(13), pp.335-339.

B

Bachrach, V., Schwarz, E. and Bachrach, L. (2003). Breastfeeding and the Risk of Hospitalization for Respiratory Disease in Infancy. Archives of Pediatrics & Adolescent Medicine, 157(3), p.237.

Bao, X., Liu, T., Shan, Y., Li, K., Garofalo, R. and Casola, A. (2008). Human Metapneumovirus Glycoprotein G Inhibits Innate Immune Responses. PLoS Pathogens, 4(5), p.e1000077.

Barlam, T. (2005). Respiratory Diseases: Respiratory syncytial virus infection in elderly, high-risk, and hospitalized adults. Postgraduate Medicine, 118(3), pp.8-8.

Barr, J.; Chambers, P.; Pringle, C. R.; Easton, A. J. (1991). Sequence of the major nucleocapsid protein gene of pneumonia virus of mice: Sequence of comparisons suggest structural homology between nucleocapsid proteins of pneumovirus, paramyxoviruses, rhabdoviruses and filoviruses. J. Gen. Virol; 72, 677–685.

Bastien, N., Normand, S., Taylor, T., Ward, D., Peret, T.C., Boivin, G., Anderson, L.J. and Li, Y., (2003). Sequence analysis of the N, P, M and F genes of Canadian human metapneumovirus strains. Virus research, 93(1), pp.51-62.

Bayrakdar, F., Altaş, A. and Korukluoğlu, G. (2016). Phylogenetic Variability of Human Metapneumovirus Strains Circulating in Turkey During Two Consecutive Epidemic Seasons. Mikrobiyoloji Bulteni, 50(1), pp.63-72.

Beaty, S.M., Park, A., Won, S.T., Hong, P., Lyons, M., Vigant, F., Freiberg, A.N., Duprex, W.P. and Lee, B., (2017). Efficient and robust Paramyxoviridae reverse genetics systems. MSphere, 2(2).

Bernal, L.J., Velandia-Romero, M., Guevara, C. and Castellanos, J.E., (2019). Human Metapneumovirus: Laboratory Methods for Isolation, Propagation, and Plaque Titration. Intervirology, 61(6), pp.301-306.

Berry, M., Gamieldien, J. and Fielding, B. (2015). Identification of New Respiratory Viruses in the New Millennium. Viruses, 7(3), pp.996-1019.

Biacchesi, S., Murphy, B., Collins, P. and Buchholz, U. (2007). Frequent Frameshift and Point Mutations in the SH Gene of Human

Metapneumovirus Passaged In Vitro. Journal of Virology, 81(11), pp.6057-6067.

Biacchesi, S., Pham, Q., Skiadopoulos, M., Murphy, B., Collins, P. and Buchholz, U. (2005). Infection of Nonhuman Primates with Recombinant Human Metapneumovirus Lacking the SH, G, or M2-2 Protein Categorizes Each as a Nonessential Accessory Protein and Identifies Vaccine Candidates. Journal of Virology, 79(19), pp.12608-12613.

Biacchesi, S., Skiadopoulos, M., Yang, L., Lamirande, E., Tran, K., Murphy, B., Collins, P. and Buchholz, U. (2004). Recombinant Human Metapneumovirus Lacking the Small Hydrophobic SH and/or Attachment G Glycoprotein: Deletion of G Yields a Promising Vaccine Candidate. Journal of Virology, 78(23), pp.12877-12887.

Bimouhen, A., El Falaki, F., Ihazmad, H., Regragui, Z., Benkerroum, S. and Barakat, A. (2016). Circulation of Respiratory Syncytial Virus in Morocco during 2014-2016: Findings from a sentinel-based virological surveillance system for influenza. Eastern Mediterranean Health Journal, 22(7), pp.482-489.

Bjornson, C. and Johnson, D. (2008). Croup. The Lancet, 371(9609), pp.329-339.

Boivin, G., De Serres, G., Côté, S., Gilca, R., Abed, Y., Rochette, L., Bergeron, M. and Déry, P. (2003). Human Metapneumovirus Infections in Hospitalized Children1. Emerging Infectious Diseases, 9(6), pp.634-640.

Boivin, G., Serres, G., Hamelin, M., Cote, S., Argouin, M., Tremblay, G., Maranda-Aubut, R., Sauvageau, C., Ouakki, M., Boulianne, N. and Couture, C. (2007). An Outbreak of Severe Respiratory Tract Infection Due to Human Metapneumovirus in a Long-Term Care Facility. Clinical Infectious Diseases, 44(9), pp.1152-1158.

Borchers, A., Chang, C., Gershwin, M. and Gershwin, L. (2013). Respiratory Syncytial Virus—A Comprehensive Review. Clinical Reviews in Allergy & Immunology, 45(3), pp.331-379.

Bradley, B. and Bryan, A. (2019). Emerging respiratory infections: The infectious disease pathology of SARS, MERS, pandemic influenza, and Legionella. Seminars in Diagnostic Pathology.

Branche, A. and Falsey, A. (2016). Parainfluenza Virus Infection. Seminars in Respiratory and Critical Care Medicine, 37(04), pp.538-554.

Brini, I., Guerrero, A., Hannachi, N., Bouguila, J., Orth-Höller, D., Bouhlel, A., Boughamoura, L., Hetzer, B., Borena, W., Schiela, B., Von Laer, D., Boukadida, J. and Stoiber, H. (2017). Epidemiology and clinical profile of pathogens responsible for the hospitalization of children in Sousse area, Tunisia. PLOS ONE, 12(11), p.e0188325.

Brooks, G. F.; Morse, S. A.; Carroll, K. C.; Mietzner, T. A.; Butel, J. S.; Mietzner, T. A. (2013). Paramyxoviruses and Rubella Virus. In: *Medical Microbiology*. Brooks, G. F.; Morse, S. A.; Carroll, K. C.; Mietzner, T. A.; Butel, J. S.; Mietzner, T. A. (ed). 26th Ed. The McGraw-Hill Companies, Inc. p: 594-601.

Buchholz, U., Biacchesi, S., Pham, Q., Tran, K., Yang, L., Luongo, C., Skiadopoulos, M., Murphy, B. and Collins, P. (2005). Deletion of M2 Gene Open Reading Frames 1 and 2 of Human Metapneumovirus: Effects on RNA Synthesis, Attenuation, and Immunogenicity. Journal of Virology, 79(11), pp.6588-6597.

Burrell, C., Howard, C. and Murphy, F. (2017). Human Parainfluenza Virus. In: *Fenner and White's Medical Virology*. Burrell, C., Howard, C. and Murphy, F. (ed). 5th ed. Academic Press: Elsevier Inc. pp. 373.

C

Cai, H., Zhang, Y., Lu, M., Liang, X., Jennings, R., Niewiesk, S. and Li, J. (2016). Phosphorylation of Human Metapneumovirus M2-1 Protein Upregulates Viral Replication and Pathogenesis. Journal of Virology, 90(16), pp.7323-7338.

Cai, X., Wang, Q., Lin, G., Cai, Z., Lin, C., Chen, P., Zhou, X., Xie, J. and Lu, X. (2014). Respiratory virus infections among children in South China. Journal of Medical Virology, 86(7), pp.1249-1255.

Cangiano, G., Nenna, R., Frassanito, A., Evangelisti, M., Nicolai, A., Scagnolari, C., Pierangeli, A., Antonelli, G., Papoff, P., Petrarca, L. and Capocaccia, P., (2016). Bronchiolitis: analysis of 10 consecutive epidemic seasons. Pediatric pulmonology, 51(12), pp.1330-1335.

CDC. (2019). Common Colds, Protect yourself and others, National Center for Immunizations and Respiratory Diseases, Division of Viral Diseases. [online] Available at: https://www.cdc.gov/features/rhinoviruses/ [Accessed 14 Jul. 2019].

CDC. (2019). RSV | Symptoms and Care | Respiratory Syncytial Virus | CDC. [online] Available at: https://www.cdc.gov/rsv/about/symptoms.html [Accessed 5 Feb. 2019].

Céspedes, P., Palavecino, C., Kalergis, A. and Bueno, S. (2016). Modulation of Host Immunity by the Human Metapneumovirus. Clinical Microbiology Reviews, 29(4), pp.795-818.

Chan, P.C., Wang, C.Y.; Wu, P.S.; Chang, P.Y.; Yang, T.T.; Chiang, Y.P.; Kao, C.L.; Chang, L.Y.; Lu, C.Y.; Lee, P.I. and Chen, J.M. (2007). Detection of human metapneumovirus in hospitalized children with acute respiratory tract infection using real-time RT-PCR in a hospital in northern Taiwan. Journal of the Formosan Medical Association, 106(1), pp.16-24.

Chanock, R. (1956). Association of a new type of cytopathogenic myxovirus with infantile croup. Journal of Experimental Medicine, 104(4), pp.555-576.

Chanock, R. Roizman, B. and Myers, R. (1957). Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA), I. Isolation, properties and characterization. American Journal of Epidemiology, 66(3), pp.281-290.

Chanock, R., Parrott, R., Cook, K., Andrews, B., Bell, J., Reichelderfer, T., Kapikian, A., Mastrota, F. and Huebner, R. (1958). Newly Recognized Myxoviruses from Children with Respiratory Disease. New England Journal of Medicine, 258(5), pp.207-213.

Chemaly, R., Hanmod, S., Rathod, D., Ghantoji, S., Jiang, Y., Doshi, A., Vigil, K., Adachi, J., Khoury, A., Tarrand, J., Hosing, C. and Champlin, R. (2012). The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. Blood, 119(12), pp.2738-2745.

Chen, Y., Huang, Y., Ho, T., Huang, C., Tsao, K. and Lin, T. (2014). Viral etiology of bronchiolitis among pediatric inpatients in northern Taiwan with emphasis on newly identified respiratory viruses. Journal of Microbiology, Immunology and Infection, 47(2), pp.116-121.

Cho, H., Shim, S., Son, D., Sun, Y., Tchah, H. and Jeon, I. (2012). Respiratory viruses in neonates hospitalized with acute lower respiratory tract infections. Pediatrics International, 55(1), pp.49-53.

Choudhary, M., Anand, S., Sonawane, N. and Chadha, M. (2014). Development of real-time RT-PCR for detection of human metapneumovirus and genetic analysis of circulating strains (2009-2011) in Pune, India. Archives of Virology, 159(2), pp.217-225.

Chow, W., Chan, Y., Oong, X., Ng, L., Nor'E, S., Ng, K., Chan, K., Hanafi, N., Pang, Y., Kamarulzaman, A. and Tee, K. (2016). Genetic

diversity, seasonality and transmission network of human metapneumovirus: identification of a unique sub-lineage of the fusion and attachment genes. Scientific Reports, 6(1).

Chu, H., Renaud, C., Ficken, E., Thomson, B., Kuypers, J. and Englund, J. (2014). Respiratory Tract Infections Due to Human Metapneumovirus in Immunocompromised Children. Journal of the Pediatric Infectious Diseases Society, 3(4), pp.286-293.

Cody Meissner, H. (2018). Respiratory Syncytial Virus. In: *Principles and Practice of Pediatric Infectious Diseases*. Long, S. S., Prober, C. G., Fischer, M. (ed). 5th ed. Philadelphia, PA: Elsevier, Inc. pp. 1162-1165.e1, https://doi.org/10.1016/B978-0-323-40181-4.00225-5.

Collins, P. L. and Karron, R. A. (2013). Respiratory Syncytial Virus and Metapneumoviruses. In: *Fields virology*. Knipe, D. M., Howley, P. M., Cohen, J. I., Griffin, D. E., Lamb, R. A., Martin, M. A., Racaniello, V. R., Roizman, B., (ed). 6th ed. 2001 Market Street, Philadelphia, PA 19103 USA, Wolters Kluwer | Lippincott Williams & Wilkins. P: 1086-7.

Côté, S., Abed, Y. and Boivin, G., (2003). Comparative evaluation of real-time PCR assays for detection of the human metapneumovirus. Journal of clinical microbiology, 41(8), pp.3631-3635.

Cowton, V., McGivern, D. and Fearns, R. (2006). Unravelling the complexities of respiratory syncytial virus RNA synthesis. Journal of General Virology, 87(7), pp.1805-1821.

Cox, R., Livesay, S., Johnson, M., Ohi, M. and Williams, J. (2012). The Human Metapneumovirus Fusion Protein Mediates Entry via an Interaction with RGD-Binding Integrins. Journal of Virology, 86(22), pp.12148-12160.

Cseke, G., Maginnis, M., Cox, R., Tollefson, S., Podsiad, A., Wright, D., Dermody, T. and Williams, J. (2009). Integrin v 1 promotes infection by

human metapneumovirus. Proceedings of the National Academy of Sciences, 106(5), pp.1566-1571.

D

Dadashi, M., Goudarzi, H., Owlia, P. and Faghihloo, E. (2018). Prevalence of human respiratory syncytial virus in Iran: a systematic review and meta-analysis. Future Virology, 13(1), pp.61-72.

Daniel, W.W. and Cross, C.L., (2010). Biostatistics: basic concepts and methodology for the health sciences 9th ed. (pp. 410-412). New York: John Wiley & Sons.

Darniot, M., Petrella, T., Aho, S., Pothier, P. and Manoha, C. (2005). Immune response and alteration of pulmonary function after primary human metapneumovirus (hMPV) infection of BALB/c mice. Vaccine, 23(36), pp.4473-4480.

Das, S., Dunbar, S. and Tang, Y. (2018). Laboratory Diagnosis of Respiratory Tract Infections in Children – the State of the Art. Frontiers in Microbiology, 9.

Delgado, M.F., Coviello, S., Monsalvo, A.C., Melendi, G.A., Hernandez, J.Z., Batalle, J.P., Diaz, L., Trento, A., Chang, H.Y., Mitzner, W. and Ravetch, J., (2009). Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nature medicine, 15(1), p.34.

Denno, D.M., (2013). Child Health, Part 2: Interventions to Combat the Major Childhood Killers. University of Washington, Seattle, WA

de-Paris, F., Beck, C., de Souza Nunes, L., Pinheiro, A., Paiva, R., da Silva Menezes, D., Pires, M., dos Santos, R., de Souza Kuchenbecker, R. and Barth, A. (2014). Evaluation of respiratory syncytial virus group A and B genotypes among nosocomial and community-acquired pediatric infections in southern Brazil. Virology Journal, 11(1).

Dinwiddie, D. and Harrod, K. (2008). Human Metapneumovirus Inhibits IFN-α Signaling through Inhibition of STAT1 Phosphorylation. American Journal of Respiratory Cell and Molecular Biology, 38(6), pp.661-670.

do Carmo Debur, M., Raboni, S., Flizikowski, F., Chong, D., Persicote, A., Nogueira, M., Rosele, L., de Almeida, S. and de Noronha, L. (2010). Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal non-pandemic seasonal respiratory infections. Journal of Clinical Pathology, 63(10), pp.930-934.

Duffy, S. (2018). Why are RNA virus mutation rates so damn high?. PLoS biology, 16(8), p.e3000003.

E

Easton, A., Domachowske, J. and Rosenberg, H. (2004). Animal Pneumoviruses: Molecular Genetics and Pathogenesis. Clinical Microbiology Reviews, 17(2), pp.390-412.

Ebihara, T., Endo, R., Ishiguro, N., Nakayama, T., Sawada, H. and Kikuta, H. (2004). Early Reinfection with Human Metapneumovirus in an Infant. Journal of Clinical Microbiology, 42(12), pp.5944-5946.

Eccles, R. (2005). Understanding the symptoms of the common cold and influenza. The Lancet Infectious Diseases, 5(11), pp.718-725.

Edwards, K., Zhu, Y., Griffin, M., Weinberg, G., Hall, C., Szilagyi, P., Staat, M., Iwane, M., Prill, M. and Williams, J. (2013). Burden of Human Metapneumovirus Infection in Young Children. New England Journal of Medicine, 368(7), pp.633-643.

El Feghaly, R.E., McGann, L., Bonville, C.A., Branigan, P.J., Suryadevera, M., Rosenberg, H.F. and Domachowske, J.B., (2010). Local production of inflammatory mediators during childhood parainfluenza virus infection. The Pediatric infectious disease journal, 29(4), p.e26.

El-Sawaf, G.E.D., Eissa, M., Ghazal, A., Metwally, D., Abozahra, R. and Rafla, W. (2017). Molecular Diagnosis of Human Metapneumovirus Infection among Egyptian Infants with Acute Bronchiolitis. International Journal of Current Microbiology and Applied Sciences, 6(3), pp.2430-2437.

Englund, J. A. and Moscona, A. (2014). Paramyxoviruses: Parainfluenza Viruses. In: *Viral Infections of Humans: Epidemiology and Control*. Kaslow, R. A., Stanberry, L. R. and Le Duc, J. W. (ed). 5th (ed). New York: Springer. pp. 579-93.

Englund, J., Boeckh, M., Kuypers, J., Nichols, W., Hackman, R., Morrow, R., Fredricks, D. and Corey, L. (2006). Brief Communication: Fatal Human Metapneumovirus Infection in Stem-Cell Transplant Recipients. Annals of Internal Medicine, 144(5), p.344.

Escobar, K., Godinez, A., Merida, E.F. and Moscoso, F. (2015). Metapneumovirus infection vs. metapneumovirus and RSV coinfection. Is there a difference?. European Respiratory Journal. 46: PA3622; DOI: 10.1183/13993003.congress-2015.PA3622.

Essa, S., Al-Tawalah, H., AlShamali, S., & Al-Nakib, W. (2017). The potential influence of human parainfluenza viruses detected during hospitalization among critically ill patients in Kuwait, 2013-2015. Virology journal, 14(1), pp.19. doi:10.1186/s12985-017-0681-0

Etemadi, M., Sekawi, Z., Othman, N., Lye, M. and Moghaddam, F. (2013). Circulation of Human Respiratory Syncytial Virus Strains among Hospitalized Children with Acute Lower Respiratory Infection in Malaysia. Evolutionary Bioinformatics, 9, p.EBO.S10999.

F

Fadhil, H.Y., (2015). Real-Time PCR detection of respiratory syncytial virus (RSV) among Adults with Pneumonia. Iraqi Journal of Science, 56(2C), pp.1617-1621.

Faghihloo, E., Yavarian, J., Jandaghi, N., Shadab, A. and Azad, T. (2014). Genotype circulation pattern of human respiratory syncytial virus in Iran. Infection, Genetics and Evolution, 22, pp.130-133.

Falsey, A. (2008). Human Metapneumovirus Infection in Adults. The Pediatric Infectious Disease Journal, 27(Supplement), pp.S80-S83.

Falsey, A. (2015). Human Metapneumovirus. In: Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. Bennett, J. E., Dolin, R., and Blaser, M.J. (ed). 8th ed. Philadelphia, PA 19103-2899. Saunders, Elsevier Inc. P: 1964-5. ISBN: 978-1-4557-4801-3.

Feuillet, F., Lina, B., Rosa-Calatrava, M. and Boivin, G. (2012). Ten years of human metapneumovirus research. Journal of Clinical Virology, 53(2), pp.97-105.

Fieldhouse, J., Toh, T., Lim, W., Ting, J., Ha, S., Hii, K., Kong, C., Wong, T., Wong, S., Warkentien, T. and Gray, G. (2018). Surveillance for respiratory syncytial virus and parainfluenza virus among patients hospitalized with pneumonia in Sarawak, Malaysia. PLOS ONE, 13(8), p.e0202147.

Freymuth, F., Vabret, A., Legrand, L., Eterradossi, N., Lafay-Delaire, F., **Brouard**, J. and Guillois, B., (2003). Presence of the new human metapneumovirus in French children with bronchiolitis. The Pediatric infectious disease journal, 22(1), pp.92-94.

Fry, A., Curns, A., Harbour, K., Hutwagner, L., Holman, R. and Anderson, L. (2006). Seasonal Trends of Human Parainfluenza Viral Infections: United States, 1990–2004. Clinical Infectious Diseases, 43(8), pp.1016-1022.

G

Gamba-Sanchez, N., Rodriguez-Martinez, C. E. and Sossa-Briceno, M. P. (2016). Epidemic activity of respiratory syncytial virus is related to

temperature and rainfall in equatorial tropical countries. Epidemiology and Infection, 144(10), pp.2057-2063.

Gamiño-Arroyo, A., Moreno-Espinosa, S., Llamosas-Gallardo, B., Ortiz-Hernández, A., Guerrero, M., Galindo-Fraga, A., Galán-Herrera, J., Prado-Galbarro, F., Beigel, J., Ruiz-Palacios, G. and Noyola, D. (2016). Epidemiology and clinical characteristics of respiratory syncytial virus infections among children and adults in Mexico. Influenza and Other Respiratory Viruses, 11(1), pp.48-56.

Gan, S., Ng, L., Lin, X., Gong, X. and Torres, J. (2008). Structure and ion channel activity of the human respiratory syncytial virus (hRSV) small hydrophobic protein transmembrane domain. Protein Science, 17(5), pp.813-820.

Garcia-Garcia, M.L., Calvo, C., Martín, F., Perez-Brena, P., Acosta, B. and Casas, I. (2006). Human metapneumovirus infections in hospitalised infants in Spain. Archives of disease in childhood, 91(4), pp.290-295. Epub 2006/01/10.

Godoy, C., Peremiquel-Trillas, P., Andrés, C., Gimferrer, L., Uriona, S., Codina, M., Armadans, L., Martín, M., Fuentes, F., Esperalba, J., Campins, M., Pumarola, T. and Antón, A. (2016). A molecular epidemiological study of human parainfluenza virus type 3 at a tertiary university hospital during 2013–2015 in Catalonia, Spain. Diagnostic Microbiology and Infectious Disease, 86(2), pp.153-159.

Gomez, M., Mufson, M., Dubovsky, F., Knightly, C., Zeng, W. and Losonsky, G. (2009). Phase-I study MEDI-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. The Pediatric Infectious Disease Journal, 28(7), pp.655-658.

Gottlieb, J. (2019). Community-acquired respiratory viruses. Current Opinion in Organ Transplantation, 24(3), pp.311-317.

Goya, S., Mistchenko, A. and Viegas, M. (2016). Phylogenetic and molecular analyses of human parainfluenza type 3 virus in Buenos Aires, Argentina, between 2009 and 2013: The emergence of new genetic lineages. Infection, Genetics and Evolution, 39, pp.85-91.

Gralton, J., Tovey, E. R., McLaws, M.-L., and Rawlinson, W. D. (2013). Respiratory virus RNA is detectable in airborne and droplet particles. Journal of Medical Virology, 85(12), 2151–2159. doi:10.1002/jmv.23698

Greensill, J., McNamara, P., Dove, W., Flanagan, B., Smyth, R. and Hart, C. (2003). Human Metapneumovirus in Severe Respiratory Syncytial Virus Bronchiolitis. Emerging Infectious Diseases, 9(3), pp.372-375.

Gregianini, T., Seadi, C., Menegolla, I., Martins, L., Ikuta, N., Wolf, J. and Lunge, V. (2018). Human metapneumovirus in Southern Brazil. Revista da Sociedade Brasileira de Medicina Tropical, 51(1), pp.30-38.

Guerrero-Plata, A. (2013). Dendritic Cells in Human Pneumovirus and Metapneumovirus Infections. Viruses, 5(6), pp.1553-1570.

H

Hacimustafaoglu, M., Çelebi, S., Bozdemir, S.E., Özgür, T., Özcan, İ., Güray, A. and Çakir, D., (2013). RSV frequency in children below 2 years hospitalized for lower respiratory tract infections. The Turkish journal of pediatrics, 55(2), pp.130.

Hahn, A., Wang, W., Jaggi, P., Dvorchik, I., Ramilo, O., Koranyi, K. and Mejias, A. (2013). Human metapneumovirus infections are associated with severe morbidity in hospitalized children of all ages. Epidemiology and Infection, 141(10), pp.2213-2223.

Hall, C. (1983). The Nosocomial Spread of Respiratory Syncytial Viral Infections. Annual Review of Medicine, 34(1), pp.311-319.

Hall, C. (2000). Nosocomial Respiratory Syncytial Virus Infections: The "Cold War" Has Not Ended. Clinical Infectious Diseases, 31(2), pp.590-596.

Hall, C. B. (2001). Respiratory syncytial virus and parainfluenza virus. N. Engl. J. Med. (344). P: 1917-1928.

Hall, C.B., Weinberg, G.A., Iwane, M.K., Blumkin, A.K., Edwards, K.M., Staat, M.A., Auinger, P., Griffin, M.R., Poehling, K.A., Erdman, D. and Grijalva, C.G., (2009). The burden of respiratory syncytial virus infection in young children. New England Journal of Medicine, 360(6), pp.588-598.

Hamelin, M., Abed, Y. and Boivin, G. (2004). Human Metapneumovirus: A New Player among Respiratory Viruses. Clinical Infectious Diseases, 38(7), pp.983-990.

Hanada, S., Pirzadeh, M., Carver, K. Y., & Deng, J. C. (2018). Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia. Frontiers in immunology, 9, 2640. doi:10.3389/fimmu.2018.02640

Harada, Y., Kinoshita, F., Yoshida, L., Minh, L., Suzuki, M., Morimoto, K., Toku, Y., Tomimasu, K., Moriuchi, H. and Ariyoshi, K. (2013). Does Respiratory Virus Coinfection Increases the Clinical Severity of Acute Respiratory Infection Among Children Infected With Respiratory Syncytial Virus?. The Pediatric Infectious Disease Journal, 32(5), pp.441-445.

Hassan, D., Rachid, S. and Ziebuhr, J. (2018). A Single-Center Study of Viral Respiratory Tract Infections in Hospitalized Children from the Kurdistan Region of Iraq. Global Pediatric Health, 5, pp.2333794X1878499.

Hastings, A., Amato, K., Wen, S., Peterson, L. and Williams, J. (2016). Human metapneumovirus small hydrophobic (SH) protein downregulates

type I IFN pathway signaling by affecting STAT1 expression and phosphorylation. Virology, 494, pp.248-256.

Healy, C.M., Munoz, F.M., Rench, M.A., Halasa, N., Edwards, K.M. and Baker, C.J., (2004). Prevalence of pertussis antibodies in maternal delivery, cord, and infant serum. The Journal of infectious diseases, 190(2), pp.335-340.

Heikkinen, T., Österback, R., Peltola, V., Jartti, T. and Vainionpää, R. (2008). Human Metapneumovirus Infections in Children. Emerging Infectious Diseases, 14(1), pp.101-106.

Henrickson K. J. (2003). Parainfluenza viruses. Clinical microbiology reviews, 16(2), pp.242-64.

Henrickson, K.J. and Savatski, L.L., 1997. Antigenic structure, function, and evolution of the hemagglutinin-neuraminidase protein of human parainfluenza virus type 1. Journal of Infectious Diseases, 176(4), pp.867-875.

Herfst, S., Schrauwen, E.J., de Graaf, M., van Amerongen, G., van den Hoogen, B.G., de Swart, R.L., Osterhaus, A.D. and Fouchier, R.A., (2008). Immunogenicity and efficacy of two candidate human metapneumovirus vaccines in cynomolgus macaques. Vaccine, 26(33), pp.4224-4230.

Hibino, A., Saito, R., Taniguchi, K., Zaraket, H., Shobugawa, Y., Matsui, T. and Suzuki, H. (2018). Molecular epidemiology of human respiratory syncytial virus among children in Japan during three seasons and hospitalization risk of genotype ON1. PLOS ONE, 13(1), p.e0192085.

Hijano, D., Maron, G. and Hayden, R. (2018). Respiratory Viral Infections in Patients With Cancer or Undergoing Hematopoietic Cell Transplant. Frontiers in Microbiology, 9.

Hopkins, P., McNeil, K., Kermeen, F., Musk, M., McQueen, E., Mackay, I., Sloots, T. and Nissen, M. (2008). Human Metapneumovirus in Lung

Transplant Recipients and Comparison to Respiratory Syncytial Virus. American Journal of Respiratory and Critical Care Medicine, 178(8), pp.876-881.

Huck, B., Scharf, G., Neumann-Haefelin, D., Puppe, W., Weigl, J. and Falcone, V. (2006). Novel Human Metapneumovirus Sublineage. Emerging Infectious Diseases, 12(1), pp.147-150.

Humphries, J., Byron, A. and Humphries, M. (2006). Integrin ligands at a glance. Journal of Cell Science, 119(19), pp.3901-3903.

I

Ipp, M., Carson, S., Petric, M. and Parkin, P. (2002). Rapid painless diagnosis of viral respiratory infection. Archives of Disease in Childhood, 86(5), pp.372-373.

Ishiguro, N., Ebihara, T., Endo, R., Ma, X., Kikuta, H., Ishiko, H. and Kobayashi, K., (2004). High genetic diversity of the attachment (G) protein of human metapneumovirus. Journal of clinical microbiology, 42(8), pp.3406-3414.

J

Jain, S., Williams, D.J., Arnold, S.R., Ampofo, K., Bramley, A.M., Reed, C., Stockmann, C., Anderson, E.J., Grijalva, C.G., Self, W.H. and Zhu, Y., (2015). Community-acquired pneumonia requiring hospitalization among US children. New England Journal of Medicine, 372(9), pp.835-845.

James, E and Crowe, J. (2016). Human Metapneumovirus. In: Nelson Textbook of Pediatrics. Kliegman, R., Stanton, B., Schor, N., St Geme, J. and Behrman, R (ed). 20th ed. 1600 John F. Kennedy Blvd. Ste. 1800 Philadelphia, PA 19103-2899: Elsevier, Inc., p.1609.

Janahi, I., Abdulkayoum, A., Almeshwesh, F., Alkuwari, M., Al hammadi, A. and Alameri, M. (2017). Viral aetiology of bronchiolitis in hospitalised children in Qatar. BMC Infectious Diseases, 17(1).

Jenkins, G., Rambaut, A., Pybus, O. and Holmes, E. (2002). Rates of Molecular Evolution in RNA Viruses: A Quantitative Phylogenetic Analysis. Journal of Molecular Evolution, 54(2), pp.156-165.

Johnson, J., Gonzales, R., Olson, S., Wright, P. and Graham, B. (2006). The histopathology of fatal untreated human respiratory syncytial virus infection. Modern Pathology, 20(1), pp.108-119.

Junge, S., Nokes, D. J., Simoes, E. A. F., Weber, M. W. (2010). Respiratory Syncytial Virus. In: Public Health and Infectious Diseases. Hamer, D. H., Griffiths, J. K., Maguire, J. H., Heggenhougen, H. K., Quah, S. R. (ed). USA. Academic Press, Elsevier Inc. P: 433-42.

K

Kadim, A. A. (2016). Molecular Detection of Parainfluenza virus in Infants and Young Children in AL-Muthanna Governorate. (Publish thesis). College of Science, Muthanna University. AL-Muthanna Governorate, Iraq.

Karron, R. A. and Collins, P. L. (2013). Parainfluenza Viruses. In: *Fields virology*. Knipe, D. M., Howley, P. M., Cohen, J. I., Griffin, D. E., Lamb, R. A., Martin, M. A., Racaniello, V. R., Roizman, B., (ed). 6th ed. 2001 Market Street, Philadelphia, PA 19103 USA, Wolters Kluwer | Lippincott Williams & Wilkins. P: 996-1020.

Kaveri, K., Indumathi, C., Gunanasekaran, P., Arunagiri, K., Mohana, S., Sheriff, A., SureshBabu, B., Padmapriya, P., Senthilraja, R. and Fathima, G. (2015). Isolation & molecular characterization of human parainfluenza virus in Chennai, India. Indian Journal of Medical Research, 142(5), p.583.

Kenmoe, S., Bigna, J. J., Well, E. A., Simo, F., Penlap, V. B., Vabret, A., & Njouom, R. (2018). Prevalence of human respiratory syncytial virus infection in people with acute respiratory tract infections in Africa: A systematic review and meta-analysis. Influenza and other respiratory viruses, 12(6), pp.793-803.

Kessler, H.H., 2007. Design and work-up of a new molecular diagnostic assay based on real-time PCR. In Protocols for Nucleic Acid Analysis by Nonradioactive Probes (pp. 227-236). Humana Press.

Khan, J. (2006). Epidemiology of Human Metapneumovirus. Clinical Microbiology Reviews, 19(3), pp. 546-557.

Kim, J.I., Park, S., Lee, I., Park, K.S., Kwak, E.J., Moon, K.M., Lee, C.K., Bae, J.Y., Park, M.S. and Song, K.J., 2016. Genome-wide analysis of human metapneumovirus evolution. PloS one, 11(4), p.e0152962.

Kini, S., Kalal, B. S., Chandy, S., Shamsundar, R., & Shet, A. (2019). Prevalence of respiratory syncytial virus infection among children hospitalized with acute lower respiratory tract infections in Southern India. World journal of clinical pediatrics, 8(2), 33–42. doi:10.5409/wjcp.v8.i2.33

Kolli, D., Bataki, E., Spetch, L., Guerrero-Plata, A., Jewell, A., Piedra, P., Milligan, G., Garofalo, R. and Casola, A. (2008). T Lymphocytes Contribute to Antiviral Immunity and Pathogenesis in Experimental Human Metapneumovirus Infection. Journal of Virology, 82(17), pp.8560-8569.

Korsman, S., van Zyl, G., Nutt, L., Andersson, M. and Preiser, W. (2012). Respiratory viruses. Virology, pp.108-109.

Kouni, S., Karakitsos, P., Chranioti, A., Theodoridou, M., Chrousos, G. and Michos, A. (2013). Evaluation of viral co-infections in hospitalized and non-hospitalized children with respiratory infections using microarrays. Clinical Microbiology and Infection, 19(8), pp.772-777.

L

Laham, F. R., V. Israele, J. M. Casellas, A. M. Garcia, C. M. Lac Prugent, S. J. Hoffman, D. Hauer, B. Thumar, M. I. Name, A. Pascual, N. Taratutto, M. T. Ishida, M. Balduzzi, M. Maccarone, S. Jackli, R. **Passarino**, R. A. Gaivironsky, R. A. Karron, N. R. Polack, and F. P. Polack. (2004). Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. J. Infect. Dis. 189:2047-2056.

Lamarão, L., Ramos, F., Mello, W., Santos, M., Barbagelata, L., Justino, M., da Silva, A., Quaresma, A., da Silva, V., Burbano, R. and Linhares, A. (2012). Prevalence and clinical features of respiratory syncytial virus in children hospitalized for community-acquired pneumonia in northern Brazil. BMC Infectious Diseases, 12(1).

Lamb, R. and Parks, G., (2013). Paramyxoviridae. In: *Fields virology*. Knipe, D. M., Howley, P. M., Cohen, J. I., Griffin, D. E., Lamb, R. A., Martin, M. A., Racaniello, V. R., Roizman, B., (ed). 6th ed. 2001 Market Street, Philadelphia, PA 19103 USA, Wolters Kluwer | Lippincott Williams & Wilkins. P: 1449–1496.

Lamb, R. and Parks, G., (2013). Paramyxoviridae. *In: Fields virology*. Knipe, D. M., Howley, P. M., Cohen, J. I., Griffin, D. E., Lamb, R. A., Martin, M. A., Racaniello, V. R., Roizman, B., (ed). 6th ed. 2001 Market Street, Philadelphia, PA 19103 USA, Wolters Kluwer | Lippincott Williams & Wilkins. P: 957–995.

Landry, M., Cohen, S. and Ferguson, D. (2008). Prospective Study of Human Metapneumovirus Detection in Clinical Samples by Use of Light Diagnostics Direct Immunofluorescence Reagent and Real-Time PCR. Journal of Clinical Microbiology, 46(3), pp.1098-1100.

Lee, A., Bibby, D., Oakervee, H., Rohatiner, A., Ushiro-Lumb, I., Clark, D. and Mattes, F. (2011). Nosocomial transmission of parainfluenza 3

virus in hematological patients characterized by molecular epidemiology. Transplant Infectious Disease, 13(4), pp.433-437.

Leung, J., Esper, F., Weibel, C. and Kahn, J.S., (2005). Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. Journal of clinical microbiology, 43(3), pp.1213-1219.

Leyrat, C., Paesen, G.C., Charleston, J., Renner, M. and Grimes, J.M., (2014a). Structural insights into the human metapneumovirus glycoprotein ectodomain. Journal of virology, 88(19), pp.11611-11616.

Leyrat, C., Renner, M., Harlos, K., Huiskonen, J. and Grimes, J. (2014b). Structure and Self-Assembly of the Calcium Binding Matrix Protein of Human Metapneumovirus. Structure, 22(1), pp.136-148.

Li, H., McCormac, M. A., Estes, R. W., Sefers, S. E., Dare, R. K., Chappell, J. D., Erdman, D. D., Wright, P. F. and Tang, Y. W. (2007). Simultaneous detection and high-throughput identification of a panel of RNA viruses causing respiratory tract infections. Journal of clinical microbiology, 45(7), pp.2105-2109.

Li, Y., Han, G.Y., Liu, Y.F., Liu, L.F., Li, Q. and Qi, S.X., (2014). Detection of respiratory viruses in influenza-like illness in Shijiazhuang, China in 2011. Bing du xue bao= Chinese journal of virology, 30(4), pp.391-395.

Li, Y., Liang, Y., Ling, Y., Duan, M., Pan, L. and Chen, Z. (2019). The spectrum of viral pathogens in children with severe acute lower respiratory tract infection: A 3-year prospective study in the pediatric intensive care unit. Journal of Medical Virology, 91(9) pp.1633-1642. https://doi.org/10.1002/jmv.25502.

Lim, F., de Klerk, N., Blyth, C., Fathima, P. and Moore, H. (2016). Systematic review and meta-analysis of respiratory viral coinfections in children. Respirology, 21(4), pp.648-655.

Linden, D., Guo-Parke, H., Coyle, P., Fairley, D., McAuley, D., Taggart, C. and Kidney, J. (2019). Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD. European Respiratory Review, 28(151), p.180063.

Linster, M., Do, L., Minh, N., Chen, Y., Zhe, Z., Tuan, T., Tuan, H., Su, Y., van Doorn, H., Moorthy, M. and Smith, G. (2018). Clinical and Molecular Epidemiology of Human Parainfluenza Viruses 1–4 in Children from Viet Nam. Scientific Reports, 8(1).

Liu, W., Liu, Q., Chen, D., Liang, H., Chen, X., Huang, W., Qin, S., Yang, Z. and Zhou, R. (2013). Epidemiology and clinical presentation of the four human parainfluenza virus types. BMC Infectious Diseases, 13(1).

Lo, M.S., Brazas, R.M. and Holtzman, M.J., (2005). Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness. Journal of virology, 79(14), pp.9315-9319.

Ludewick, H.P., Abed, Y., Van Niekerk, N., Boivin, G., Klugman, K.P. and Madhi, S.A., (2005). Human metapneumovirus genetic variability, South Africa. Emerging infectious diseases, 11(7), p.1074.

M

Madi, N., Chehadeh, W., Asadzadeh, M., Al-Turab, M. and Al-Adwani, A. (2018). Analysis of genetic variability of respiratory syncytial virus groups A and B in Kuwait. Archives of Virology, 163(9), pp.2405-2413.

Maertzdorf, J., Wang, C., Brown, J., Quinto, J., Chu, M., de Graaf, M., van den Hoogen, B., Spaete, R., Osterhaus, A. and Fouchier, R. (2004).

Real-Time Reverse Transcriptase PCR Assay for Detection of Human Metapneumoviruses from All Known Genetic Lineages. Journal of Clinical Microbiology, 42(3), pp.981-986.

Mahdy El-Wakil, D., Ahmed El-Seidi, E., Aly Dwedar, R., Abd El-Fattah Madkour, L. and Ibrahim Rady, H. (2017). Human Metapneumovirus Pediatric Respiratory Infections: Comparing Direct Immunofluorescence versus Polymerase Chain Reaction. Archives of Pediatric Infectious Diseases, 5(2).

Malekshahi, S.S., Azad, T.M., Shahmahmoodi, S., Yavarian, J., Rezaei, F. and Naseri, M., (2010). First report of respiratory syncytial virus and human metapneumovirus co-infection in a 2-year-old kawasaki patient in iran. Iranian journal of public health, 39(4), 140–142.

Malhotra, B., Swamy, M. A., Reddy, P. V., Kumar, N., & Tiwari, J. K. (2016). Evaluation of custom multiplex real - time RT - PCR in comparison to fast - track diagnostics respiratory 21 pathogens kit for detection of multiple respiratory viruses. Virology journal, 13, 91. doi:10.1186/s12985-016-0549-8.

Mao, N., Ji, Y., Xie, Z., Wang, H., Wang, H., An, J., Zhang, X., Zhang, Y., Zhu, Z., Cui, A., Xu, S., Shen, K., Liu, C., Yang, W. and Xu, W. (2012). Human Parainfluenza Virus-Associated Respiratory Tract Infection among Children and Genetic Analysis of HPIV-3 Strains in Beijing, China. PLoS ONE, 7(8), p.e43893.

Marx, A., Gary, Jr., H., Marston, B., Erdman, D., Breiman, R., Török, T., Plouffe, J., File, Jr., T. and Anderson, L. (1999). Parainfluenza Virus Infection Among Adults Hospitalized for Lower Respiratory Tract Infection. Clinical Infectious Diseases, 29(1), pp.134-140.

Masante, C., El Najjar, F., Chang, A., Jones, A., Moncman, C. and Dutch, R. (2014). The Human Metapneumovirus Small Hydrophobic Protein Has Properties Consistent with Those of a Viroporin and Can

Modulate Viral Fusogenic Activity. Journal of Virology, 88(11), pp.6423-6433.

Matsumiya, T. and Stafforini, D. (2010). Function and Regulation of Retinoic Acid-Inducible Gene-I. Critical Reviews™ in Immunology, 30(6), pp.489-513.

Matsuzaki, Y., Itagaki, T., Ikeda, T., Aoki, Y., Abiko, C. and Mizuta, K. (2012). Human metapneumovirus infection among family members. Epidemiology and Infection, 141(04), pp.827-832.

Matu, M. N. (2015). Risk factors and cost of illness for acute respiratory infections in children under five years of age attending selected health facilities in Nakuru (Publish Philosophiae Doctoral Dissertation). Jomo Kenyatta University of Agriculture and Technology, Department of Public Health. County, Kenya.

McCracken, J., Arvelo, W., Ortíz, J., Reyes, L., Gray, J., Estevez, A., Castañeda, O., Langley, G. and Lindblade, K. (2014). Comparative epidemiology of human metapneumovirus- and respiratory syncytial virus-associated hospitalizations in Guatemala. Influenza and Other Respiratory Viruses, 8(4), pp.414-421.

Mitra, R., Baviskar, P., Duncan-Decocq, R., Patel, D. and Oomens, A. (2012). The Human Respiratory Syncytial Virus Matrix Protein Is Required for Maturation of Viral Filaments. Journal of Virology, 86(8), pp.4432-4443.

Mizuta, K., Abiko, C., Aoki, Y., Ikeda, T., Matsuzaki, Y., Itagaki, T., Katsushima, F., Katsushima, Y., Noda, M., Kimura, H. and Ahiko, T. (2013). Seasonal Patterns of Respiratory Syncytial Virus, Influenza A Virus, Human Metapneumovirus, and Parainfluenza Virus Type 3 Infections on the Basis of Virus Isolation Data between 2004 and 2011 in Yamagata, Japan. Japanese Journal of Infectious Diseases, 66(2), pp.140-145.

Moattari, A., Aleyasin, S., Arabpour, M. and Sadeghi, S., (2010). Prevalence of human Metapneumovirus (hMPV) in children with wheezing in Shiraz-Iran. Iranian Journal of Allergy, Asthma and Immunology, pp.251-254.

Moe, N. (2017). Human metapneumovirus: Clinical and virological aspects in Norwegian children. (Publish Philosophiae Doctoral Dissertation). Norwegian University of Science and Technology, Faculty of Medicine and Health Sciences, Department of Laboratory Medicine, Children's and Women's Health. Trondheim, Norway.

Moe, N., Krokstad, S., Stenseng, I., Christensen, A., Skanke, L., Risnes, K., Nordbø, S. and Døllner, H. (2017). Comparing Human Metapneumovirus and Respiratory Syncytial Virus: Viral Co-Detections, Genotypes and Risk Factors for Severe Disease. PLOS ONE, 12(1), p.e0170200.

Mohamed, M, S, E., Reiche, J., Jacobsen, S., Thabit, A.G., Badary, M.S., Brune, W., Schweiger, B. and Osmann, A.H., 2014. Molecular analysis of human metapneumovirus detected in patients with lower respiratory tract infection in upper Egypt. International journal of microbiology, 2014.

Monto, A.S., (2002). Epidemiology of viral respiratory infections. The American journal of medicine, 112(6), pp.4-12.

Morris, J., Blount, R. and Savage, R. (1956). Recovery of Cytopathogenic Agent from Chimpanzees with Goryza. Experimental Biology and Medicine, 92(3), pp.544-549.

Moscona, A. (2005). Entry of parainfluenza virus into cells as a target for interrupting childhood respiratory disease. Journal of Clinical Investigation, 115(7), pp.1688-1698.

Mullins, J., Erdman, D., Weinberg, G., Edwards, K., Hall, C., Walker, F., Iwane, M. and Anderson, L. (2004). Human Metapneumovirus Infection

among Children Hospitalized with Acute Respiratory Illness. Emerging Infectious Diseases, 10(4), pp.700-705.

N

Nair, H., Simões, E., Rudan, I., Gessner, B., Azziz-Baumgartner, E., Zhang, J., Feikin, D., Mackenzie, G., Moiïsi, J., Roca, A., Baggett, H., Zaman, S., Singleton, R., Lucero, M., Chandran, A., Gentile, A., Cohen, C., Krishnan, A., Bhutta, Z., Arguedas, A., Clara, A., Andrade, A., Ope, M., Ruvinsky, R., Hortal, M., McCracken, J., Madhi, S., Bruce, N., Qazi, S., Morris, S., El Arifeen, S., Weber, M., Scott, J., Brooks, W., Breiman, R. and Campbell, H. (2013). Global and regional burden of hospital admissions for severe acute lower respiratory infections in young children in 2010: a systematic analysis. The Lancet, 381(9875), pp.1380-1390.

Nidaira, M., Taira, K., Hamabata, H., Kawaki, T., Gushi, K., Mahoe, Y., Maeshiro, N., Azama, Y., Okano, S., Kyan, H., Kudaka, J., Tsukagoshi, H., Noda, M. and Kimura, H. (2012). Molecular Epidemiology of Human Metapneumovirus from 2009 to 2011 in Okinawa, Japan. Japanese Journal of Infectious Diseases, 65(4), pp.337-340.

Nokso-Koivisto, J., Pyles, R., Miller, A., Patel, J., Loeffelholz, M. and Chonmaitree, T. (2012). Viral Load and Acute Otitis Media Development After Human Metapneumovirus Upper Respiratory Tract Infection. The Pediatric Infectious Disease Journal, 31(7), pp.763-766.

Nunes, M.C., Cutland, C.L., Jones, S., Hugo, A., Madimabe, R., Simoes, E.A., Weinberg, A. and Madhi, S.A., (2016). Duration of infant protection against influenza illness conferred by maternal immunization: secondary analysis of a randomized clinical trial. JAMA pediatrics, 170(9), pp.840-847.

O

Obodai, E., Odoom, J., Adiku, T., Goka, B., Wolff, T., Biere, B., Schweiger, B. and Reiche, J. (2018). The significance of human

respiratory syncytial virus (HRSV) in children from Ghana with acute lower respiratory tract infection: A molecular epidemiological analysis, 2006 and 2013-2014. PLOS ONE, 13(9), p.e0203788.

Ogonczyk Makowska, D., Hamelin, M. È., & Boivin, G. (2020). Engineering of Live Chimeric Vaccines against Human Metapneumovirus. Pathogens (Basel, Switzerland), 9(2), 135. https://doi.org/10.3390/pathogens9020135

Oliveira, D., Durigon, E., Carvalho, A., Leal, A., Souza, T., Thomazelli, L., Moraes, C., Vieira, S., Gilio, A. and Stewien, K. (2009). Epidemiology and genetic variability of human metapneumovirus during a 4-year-long study in Southeastern Brazil. Journal of Medical Virology, 81(5), pp.915-921.

Ortiz de la Tabla, V., Masia, M., Antequera, P., Martin, C., Gazquez, G., Bunuel, F. and Gutierrez, F. (2010). Comparison of Combined Nose-Throat Swabs with Nasopharyngeal Aspirates for Detection of Pandemic Influenza A/H1N1 2009 Virus by Real-Time Reverse Transcriptase PCR. Journal of Clinical Microbiology, 48(10), pp.3492-3495.

Oumei, H., Xuefeng, W., Jianping, L., kunling, S., Rong, M., Zhenze, C., Li, D., Huimin, Y., Lining, W., Zhaolan, L., Xinmin, L., Hua, X., Zhiyan, J., Yanning, L., Yan, H., Baoqing, Z., Xiaochun, F., Chunhui, H., Yonghong, J., Xue, Z., Wei, W. and Zi, W. (2018). Etiology of community-acquired pneumonia in 1500 hospitalized children. Journal of Medical Virology, 90(3), pp.421-428.

Owor, B., Masankwa, G., Mwango, L., Njeru, R., Agoti, C. and Nokes, D. (2016). Human metapneumovirus epidemiological and evolutionary patterns in Coastal Kenya, 2007-11. BMC Infectious Diseases, 16(1).

P

Palmer, S., DeVito, I., Jenkins, S., Niewiesk, S., Porotto, M. and Moscona, A. (2014). Circulating Clinical Strains of Human Parainfluenza

Virus Reveal Viral Entry Requirements for In Vivo Infection. Journal of Virology, 88(22), pp.13495-13502.

Pan, Y., Zhang, Y., Shi, W., Peng, X., Cui, S., Zhang, D., Lu, G., Liu, Y., Wu, S., Yang, P. and Wang, Q. (2017). Human parainfluenza virus infection in severe acute respiratory infection cases in Beijing, 2014-2016: A molecular epidemiological study. Influenza and Other Respiratory Viruses, 11(6), pp.564-568.

Panasik, A. and Pancer, K., (2009). Human metapneumovirus (HMPV) infections in young children. Przeglad epidemiologiczny, 63(3), pp.369-374.

Pancham, K., Sami, I., Perez, G., Huseni, S., Kurdi, B., Rose, M., Rodriguez-Martinez, C. and Nino, G. (2016). Human Metapneumovirus Infection is Associated with Severe Respiratory Disease in Preschool Children with History of Prematurity. Pediatrics & Neonatology, 57(1), pp.27-34.

Panda, S., Mohakud, N., Pena, L. and Kumar, S. (2014). Human metapneumovirus: review of an important respiratory pathogen. International Journal of Infectious Diseases, 25, pp.45-52.

Papenburg, J., Carbonneau, J., Isabel, S., Bergeron, M., Williams, J., De Serres, G., Hamelin, M. and Boivin, G. (2013). Genetic diversity and molecular evolution of the major human metapneumovirus surface glycoproteins over a decade. Journal of Clinical Virology, 58(3), pp.541-547.

Papenburg, J., Hamelin, M., Ouhoummane, N., Carbonneau, J., Ouakki, M., Raymond, F., Robitaille, L., Corbeil, J., Caouette, G., Frenette, L., De Serres, G. and Boivin, G. (2012). Comparison of Risk Factors for Human Metapneumovirus and Respiratory Syncytial Virus Disease Severity in Young Children. The Journal of Infectious Diseases, 206(2), pp.178-189.

Park, G., Lee, J.Y., Lee, S.Y., Kim, J.H., Kang, J.H. and Choi, U.Y., (2018). Seroprevalence of Human Parainfluenza Virus Types 1–4 among Healthy Children Under 5 Years of Age in Korea. Viral immunology, 31(5), pp.352-357.

Parsania, M., Poopak, B., Pouriayevali, M., Haghighi, S., Amirkhani, A. and Nateghian, A. (2016). Detection of Human Metapneumovirus and Respiratory Syncytial Virus by Real-Time Polymerase Chain Reaction Among Hospitalized Young Children in Iran. Jundishapur Journal of Microbiology, 9(3).

Pavlin, J. A., Hickey, A. C., Ulbrandt, N., Chan, Y. P., Endy, T. P., Boukhvalova, M. S., Chunsuttiwat, S., Nisalak, A., Libraty, D. H., Green, S., Rothman, A. L., Ennis, F. A., Jarman, R., Gibbons, R. V., Broder, C. C. (2008). Human metapneumovirus reinfection among children in Thailand determined by ELISA using purified soluble fusion protein. The Journal of infectious diseases, 198(6), 836-42.

Peiris, J., Tang, W., Chan, K., Khong, P., Guan, Y., Lau, Y. and Chiu, S. (2003). Children with Respiratory Disease Associated with Metapneumovirus in Hong Kong. Emerging Infectious Diseases, 9(6), pp.628-633.

Peña, S.A., Davis, S.S., Lu, X., Sakthivel, S.K.K., Peret, T.C., Rose, E.B., Smelser, C., Schneider, E., Stone, N.D. and Watson, J., (2019). Severe Respiratory Illness Associated with Human Metapneumovirus in Nursing Home, New Mexico, USA. Emerging infectious diseases, 25(2), p.383.. https://dx.doi.org/10.3201/eid2502.181298.

Peret, T., Boivin, G., Li, Y., Couillard, M., Humphrey, C., Osterhaus, A., Erdman, D. and Anderson, L. (2002). Characterization of Human Metapneumoviruses Isolated from Patients in North America. The Journal of Infectious Diseases, 185(11), pp.1660-1663.

Peret, T.C., Abed, Y., Anderson, L.J., Erdman, D.D. and Boivin, G., (2004). Sequence polymorphism of the predicted human

metapneumovirus G glycoprotein. Journal of general virology, 85(3), pp.679-686.

Perk, Y. and Ozdil, M. (2018). Respiratory syncytial virüs infections in neonates and infants. Türk Pediatri Arşivi, 53(2), pp.63-70.

Pilger, D., Cantarelli, V., Amantea, S. and Leistner-Segal, S. (2011). Detection of human bocavirus and human metapneumovirus by real-time PCR from patients with respiratory symptoms in Southern Brazil. Memórias do Instituto Oswaldo Cruz, 106(1), pp.56-60.

Piñana, M., Vila, J., Gimferrer, L., Valls, M., Andrés, C., Codina, M.G., Ramón, J., Martín, M.C., Fuentes, F., Saiz, R. and Alcubilla, P., (2017). Novel human metapneumovirus with a 180-nucleotide duplication in the G gene. Future microbiology, 12(7), pp.565-571.

Pinky, L. and Dobrovolny, H. (2016). Coinfections of the Respiratory Tract: Viral Competition for Resources. PloS one, 11(5), p.e0155589.

Piyaratna, R., Tollefson, S.J. and Williams, J.V., (2011). Genomic analysis of four human metapneumovirus prototypes. Virus research, 160(1-2), pp.200-205.

R

Rabarison, J. H., Tempia, S., Harimanana, A., Guillebaud, J., Razanajatovo, N. H., Ratsitorahina, M. and Heraud, J. M., (2018). Burden and Epidemiology of Influenza-and Respiratory Syncytial Virus-Associated Severe Acute Respiratory Illness Hospitalization in Madagascar, 2011-2016. Influenza and other respiratory viruses. doi: 10.1111/irv.12557.

Rafiefard, F., Yun, Z. and Örvell, C. (2008). Epidemiologic characteristics and seasonal distribution of human metapneumovirus infections in five epidemic seasons in Stockholm, Sweden, 2002-2006. Journal of Medical Virology, 80(9), pp.1631-1638.

Rahman, M.M., Wong, K.K., Hanafiah, A. and Isahak, I., (2014). Influenza and respiratory syncytial viral infections in Malaysia: Demographic and clinical perspective. Pakistan journal of medical sciences, 30(1), p.161.

Rahman, M.Z., Sumiya, M., Sahabuddin, M., Pell, L.G., Gubbay, J.B., Rahman, R., Momtaz, F., Azmuda, N., Shanta, S.S., Jahan, I. and Rahman, M., (2019). Genetic characterization of human metapneumovirus identified through community and facility-based surveillance of infants in Dhaka, Bangladesh. Journal of medical virology, 91(4), pp.549-554.

Ralston, S. and Hill, V. (2009). Incidence of Apnea in Infants Hospitalized with Respiratory Syncytial Virus Bronchiolitis: A Systematic Review. The Journal of Pediatrics, 155(5), pp.728-733.

Rebuffo-Scheer, C., Bose, M., He, J., Khaja, S., Ulatowski, M., Beck, E., Fan, J., Kumar, S., Nelson, M. and Henrickson, K. (2011). Whole Genome Sequencing and Evolutionary Analysis of Human Respiratory Syncytial Virus A and B from Milwaukee, WI 1998-2010. PLoS ONE, 6(10), p.e25468.

Reijans, M., Dingemans, G., Klaassen, C.H., Meis, J.F., Keijdener, J., Mulders, B., Eadie, K., van Leeuwen, W., van Belkum, A., Horrevorts, A.M. and Simons, G., (2008). RespiFinder: a new multiparameter test to differentially identify fifteen respiratory viruses. Journal of clinical microbiology, 46(4), pp.1232-1240.

Reina, J., Ferres, F., Alcoceba, E., Mena, A., de Gopegui, E.R. and Figuerola, J., (2007). Comparison of different cell lines and incubation times in the isolation by the shell vial culture of human metapneumovirus from pediatric respiratory samples. Journal of Clinical Virology, 40(1), pp.46-49.

Ren, J., Liu, G., Go, J., Kolli, D., Zhang, G. and Bao, X. (2014). Human Metapneumovirus M2-2 Protein Inhibits Innate Immune Response in Monocyte-Derived Dendritic Cells. PLoS ONE, 9(3), p.e91865.

Ren, J., Phan, T. and Bao, X., (2015). Recent vaccine development for human metapneumovirus. The Journal of general virology, 96(Pt 7), p.1515.

Ren, J., Wang, Q., Kolli, D., Prusak, D., Tseng, C., Chen, Z., Li, K., Wood, T. and Bao, X. (2012). Human Metapneumovirus M2-2 Protein Inhibits Innate Cellular Signaling by Targeting MAVS. Journal of Virology, 86(23), pp.13049-13061.

Ren, L., Xia, Q., Xiao, Q., Zhou, L., Zang, N., Long, X., Xie, X., Deng, Y., Wang, L., Fu, Z., Tian, D., Zhao, Y., Zhao, X., Li, T., Huang, A. and Liu, E. (2014). The genetic variability of glycoproteins among respiratory syncytial virus subtypes A in China between 2009 and 2013. Infection, Genetics and Evolution, 27, pp.339-347.

Rima, B., Collins, P., Easton, A., Fouchier, R., Kurath, G., Lamb, R., Lee, B., Maisner, A., Rota, P., Wang, L., and ICTV Report Consortium. (2017). ICTV Virus Taxonomy Profile: Pneumoviridae, Journal of General Virology, 98, 2912–2913.

Robinson, J., Lee, B., Kothapalli, S., Craig, W. and Fox, J. (2008). Use of Throat Swab or Saliva Specimens for Detection of Respiratory Viruses in Children. Clinical Infectious Diseases, 46(7), pp.e61-e64.

S

Safamanesh, S., Azimian, A., Shakeri, A., Ghazvini, K., Amel Jamehdar, S., Nojoumi, S., Khosrojerdi, M. and Youssefi, M. (2018). Frequency and Molecular Typing of Human Metapneumovirus in Children with Acute Respiratory Tract Infection in Bojnurd, Northeastern Iran. Archives of Pediatric Infectious Diseases, 6(3).

Saikusa, M., Kawakami, C., Nao, N., Takeda, M., Usuku, S., Sasao, T., Nishimoto, K. and Toyozawa, T., (2017). 180-nucleotide duplication in the G gene of human metapneumovirus A2b subgroup strains circulating in Yokohama City, Japan, since 2014. Frontiers in microbiology, 8, p.402.

Salimi, V., Tavakoli-Yaraki, M., Yavarian, J., Bont, L. and Mokhtari-Azad, T., (2016). Prevalence of human respiratory syncytial virus circulating in Iran. Journal of infection and public health, 9(2), pp.125-135.

Schildgen, O., Geikowski, T., Glatzel, T., Schuster, J. and Simon, A. (2005). Frequency of human metapneumovirus in the upper respiratory tract of children with symptoms of an acute otitis media. European Journal of Pediatrics, 164(6), pp.400-401.

Schildgen, V., van den Hoogen, B., Fouchier, R., Tripp, R., Alvarez, R., Manoha, C., Williams, J. and Schildgen, O. (2011). Human Metapneumovirus: Lessons Learned over the First Decade. Clinical Microbiology Reviews, 24(4), pp.734-754.

Schomacker, H., Schaap-Nutt, A., Collins, P. L., & Schmidt, A. C. (2012). Pathogenesis of acute respiratory illness caused by human parainfluenza viruses. Current opinion in virology, 2(3), 294–299. doi:10.1016/j.coviro.2012.02.001

Schowalter, R., Smith, S. and Dutch, R. (2006). Characterization of Human Metapneumovirus F Protein-Promoted Membrane Fusion: Critical Roles for Proteolytic Processing and Low pH. Journal of Virology, 80(22), pp.10931-10941.

Schuster, J. and Williams, J. (2018). Human Metapneumovirus. In: *Principles and Practice of Pediatric Infectious Diseases*. Long, S. S., Prober, C.G., Ficher, M. (ed). 5th ed. Philadelphia, PA 19103-2899. Philadelphia, PA: Elsevier, Inc. pp.1165-1169.e3. https://doi.org/10.1016/B978-0-323-40181-4.00225-5.

Schuster, J. E. and Williams, J. V. (2019). Human Metapneumovirus. In: *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. Cherry, J.D., Steinbach, W. J., Harrison, G. J., Hotez, P. J., Kaplan, S. L. (ed). 8th ed. Philadelphia, PA 19103-2899. Elsevier, Inc. P: 1797-1803. ISBN: 978-0-323-37692-1

Schuster, J. E., and Williams, J. V. (2013). Human metapneumovirus. Pediatrics in review, 34(12), 558-65.

Schuster, J., Khuri-Bulos, N., Faouri, S., Shehabi, A., Johnson, M., Wang, L., Fonnesbeck, C., Williams, J. and Halasa, N. (2015). Human Metapneumovirus Infection in Jordanian Children. The Pediatric Infectious Disease Journal, 34(12), pp.1335-1341.

Semple, M., Cowell, A., Dove, W., Greensill, J., McNamara, P., Halfhide, C., Shears, P., Smyth, R. and Hart, C. (2005). Dual Infection of Infants by Human Metapneumovirus and Human Respiratory Syncytial Virus Is Strongly Associated with Severe Bronchiolitis. The Journal of Infectious Diseases, 191(3), pp.382-386.

Shafagati, N. and Williams, J. (2018). Human metapneumovirus - what we know now. F1000Research, 7, p.135. doi: 10.12688/f1000research.12625.1

Shatizadeh Malekshahi, S., Mokhtari Azad, T., Yavarian, J., Shahmahmoodi, S., Naseri, M. and Rezaei, F. (2010). Molecular Detection of Respiratory Viruses in Clinical Specimens From Children With Acute Respiratory Disease in Iran. The Pediatric Infectious Disease Journal, 29(10), pp.931-933.

Shi, T., McAllister, D.A., O'Brien, K.L., Simoes, E.A., Madhi, S.A., Gessner, B.D., Polack, F.P., Balsells, E., Acacio, S., Aguayo, C. and Alassani, I. (2017). Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. The Lancet, 390(10098), pp.946-958.

Shi, W., Cui, S., Gong, C., Zhang, T., Yu, X., Li, A., Chen, M., Luo, M. and Huang, F., (2015). Prevalence of human parainfluenza virus in patients with acute respiratory tract infections in Beijing, 2011–2014. Influenza and other respiratory viruses, 9(6), p.305.

Souza, J., Watanabe, A., Carraro, E., Granato, C. and Bellei, N. (2013). Severe metapneumovirus infections among immunocompetent and immunocompromised patients admitted to hospital with respiratory infection. Journal of Medical Virology, 85(3), pp.530-536.

Spann, K., Tran, K., Chi, B., Rabin, R. and Collins, P. (2004). Suppression of the Induction of Alpha, Beta, and Gamma Interferons by the NS1 and NS2 Proteins of Human Respiratory Syncytial Virus in Human Epithelial Cells and Macrophages. Journal of Virology, 78(8), pp.4363-4369.

Stover, C. and Litwin, C. (2014). The Epidemiology of Upper Respiratory Infections at a Tertiary Care Center: Prevalence, Seasonality, and Clinical Symptoms. Journal of Respiratory Medicine, 2014, pp.1-8.

Sun, H., Sun, J., Ji, W., Hao, C., Yan, Y., Chen, Z. and Wang, Y. (2019). Impact of RSV coinfection on human bocavirus in children with acute respiratory infections. Journal of tropical pediatrics.

T

Tabatabai, J., Prifert, C., Pfeil, J., Grulich-Henn, J. and Schnitzler, P. (2014). Novel Respiratory Syncytial Virus (RSV) Genotype ON1 Predominates in Germany during Winter Season 2012–13. PLoS ONE, 9(10), p.e109191.

Talaat, K., Karron, R., Thumar, B., McMahon, B., Schmidt, A., Collins, P. and Buchholz, U. (2013). Experimental Infection of Adults With Recombinant Wild-Type Human Metapneumovirus. Journal of Infectious Diseases, 208(10), pp.1669-1678.

Tamaki, K., Kinjo, T., Aoyama, H., Tomoyose, T., Nakachi, S., Hanashiro, T., Shimabukuro, N., Tedokon, I., Morichika, K., Nishi, Y., Taira, N., Fujita, J., Yoshimi, N., Fukushima, T. and Masuzaki, H. (2015). Fatal pneumonia and viremia due to human parainfluenza virus type 1 in a patient with adult T-cell leukemia–lymphoma treated with mogamulizumab. Journal of Infection and Chemotherapy, 21(11), pp.820-823.

Tambyah, P., Isa, M. S., Tan, C.X.-T. (2019). New and Emerging Infections of the Lung. In: *Kendig's Disorders of the Respiratory Tract in Children*. Wilmott, R. W., Li, A., Sly, P., Bush, A., Deterding, R., Ratjen, F., Zar, H. J. (ed). 9th ed. Elsevier Inc. pp. 466-474.e2.

Taniguchi, A., Kawada, J., Go, K., Fujishiro, N., Hosokawa, Y., Maki, Y., Sugiyama, Y., Suzuki, M., Tsuji, T., Hoshino, S., Muramatsu, H., Kidokoro, H., Kinoshita, F., Hirakawa, A., Takahashi, Y., Sato, Y. and Natsume, J. (2019). Comparison of clinical characteristics of human metapneumovirus and respiratory syncytial virus infection in hospitalized young children. Japanese Journal of Infectious Diseases.

Thammawat, S., Sadlon, T., Hallsworth, P. and Gordon, D. (2008). Role of Cellular Glycosaminoglycans and Charged Regions of Viral G Protein in Human Metapneumovirus Infection. Journal of Virology, 82(23), pp.11767-11774.

Thomazelli, L., Oliveira, D., Durigon, G., Whitaker, B., Kamili, S., Berezin, E. and Durigon, E. (2018). Human parainfluenza virus surveillance in pediatric patients with lower respiratory tract infections: a special view of parainfluenza type 4. Jornal de Pediatria (Versão em Português), 94(5), pp.554-558.

Thongpan, I., Suntronwong, N., Vichaiwattana, P., Wanlapakorn, N., Vongpunsawad, S., & Poovorawan, Y. (2019). Respiratory syncytial virus, human metapneumovirus, and influenza virus infection in Bangkok, 2016-2017. PeerJ, 7, e6748. doi:10.7717/peerj.6748.

Tregoning, J. and Schwarze, J. (2010). Respiratory Viral Infections in Infants: Causes, Clinical Symptoms, Virology, and Immunology. Clinical Microbiology Reviews, 23(1), pp.74-98.

Tsagarakis, N. J., Sideri, A., Makridis, P., Triantafyllou, A., Stamoulakatou, A., & Papadogeorgaki, E. (2018). Age-related prevalence of common upper respiratory pathogens, based on the application of the FilmArray Respiratory panel in a tertiary hospital in Greece. Medicine, 97(22), e10903. doi:10.1097/MD.0000000000010903.

V

van den Hoogen, B. (2007). Respiratory Tract Infection Due to Human Metapneumovirus among Elderly Patients. Clinical Infectious Diseases, 44(9), pp.1159-1160.

van den Hoogen, B. G., T. M. Bestebroer, A. D. Osterhaus, and R. A. Fouchier. (2002). Analysis of the genomic sequence of a human metapneumovirus. Virology 295:119–132.

van den Hoogen, B., Herfst, S., Sprong, L., Cane, P., Forleo-Neto, E., de Swart, R., Osterhaus, A. and Fouchier, R. (2004). Antigenic and Genetic Variability of Human Metapneumoviruses. Emerging Infectious Diseases, 10(4), pp.658-666.

van den Hoogen, B.G., de Jong, J.C., Groen, J., Kuiken, T., de Groot, R., Fouchier, R.A. and Osterhaus, A.D., (2001). A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nature medicine, 7(6), p.719

van Den Hoogen, B.G., Osterhaus, D.M.E. and Fouchier, R.A., (2004). Clinical impact and diagnosis of human metapneumovirus infection. The Pediatric Infectious Disease Journal, 23(Supplement), pp.S25-S32.

Vargas, S., Kozakewich, H., Perez-Atayde, A. and McAdam, A. (2004). Pathology of Human Metapneumovirus Infection: Insights into the

Pathogenesis of a Newly Identified Respiratory Virus. Pediatric and Developmental Pathology, 7(5), pp.478-486.

Villaran, M., García, J., Gomez, J., Arango, A., Gonzales, M., Chicaiza, W., Alemán, W., Lorenzana de Rivera, I., Sanchez, F., Aguayo, N., Kochel, T. and Halsey, E. (2013). Human parainfluenza virus in patients with influenza-like illness from Central and South America during 2006-2010. Influenza and Other Respiratory Viruses, 8(2), pp.217-227.

Vinci, A., Lee, P. and Krilov, L. (2018). Human Metapneumovirus Infection. Pediatrics in Review, 39(12), pp.623-624.

Vinh, D.C., Newby, D., Charest, H. and McDonald, J., (2008). Evaluation of a commercial direct fluorescent-antibody assay for human metapneumovirus in respiratory specimens. Journal of clinical microbiology, 46(5), pp.1840-1841.

W

Walsh, E .E.; Peterson, D. R. and Falsey, A. R., (2008). Another piece of the puzzle: human metapneumovirus infections in adults. Archives of internal medicine, 168(22), p.2489.

Walsh, E. E. and Hall, C. B. (2015). Respiratory Syncytial Virus (RSV). In: *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Bennett, J. E., Dolin, R., and Blaser, M.J. (ed). 8th ed. Philadelphia, PA 19103-2899. Saunders, Elsevier Inc. P: 1949. ISBN: 978-1-4557-4801-3.

Wang, F., Zhao, L., Zhu, R., Deng, J., Sun, Y., Ding, Y., Tian, R. and Qian, Y. (2015). Parainfluenza Virus Types 1, 2, and 3 in Pediatric Patients with Acute Respiratory Infections in Beijing During 2004 to 2012. Chinese Medical Journal, 128(20), pp.2726-2730.

Wang, W., Chen, S., Liu, I., Chen, Y., Chen, H., Yang, C., Chen, P., Yeh, S., Kao, C., Huang, L., Hsueh, P., Wang, J., Sheng, W., Fang, C., Hung,

C., Hsieh, S., Su, C., Chiang, W., Yang, J., Lin, J., Hsieh, S., Hu, H., Chiang, Y., Wang, J., Yang, P. and Chang, S. (2004). Detection of SARS-associated Coronavirus in Throat Wash and Saliva in Early Diagnosis. Emerging Infectious Diseases, 10(7), pp.1213-1219.

Wei, H., Tsao, K., Huang, C., Huang, Y. and Lin, T. (2013). Clinical features of different genotypes/genogroups of human metapneumovirus in hospitalized children. Journal of Microbiology, Immunology and Infection, 46(5), pp.352-357.

Weinberg, G., Hall, C., Iwane, M., Poehling, K., Edwards, K., Griffin, M., Staat, M., Curns, A., Erdman, D. and Szilagyi, P. (2009). Parainfluenza Virus Infection of Young Children: Estimates of the Population-Based Burden of Hospitalization. The Journal of Pediatrics, 154(5), pp.694-699.e1.

Welliver Sr, R. C. (2019). Parainfluenza Viruses. In: *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. Cherry, J.D., Steinbach, W. J., Harrison, G. J., Hotez, P. J., Kaplan, S. L. (ed). 8th ed. Philadelphia, PA 19103-2899. Elsevier, Inc. P: 1745-53. ISBN: 978-0-323-37692-1.

Welliver Sr, R. C. and Hall, C. B. (2019). Respiratory Syncytial Virus. In: *Feigin and Cherry's Textbook of Pediatric Infectious Diseases*. Cherry, J.D., Steinbach, W. J., Harrison, G. J., Hotez, P. J., Kaplan, S. L. (ed). 8th ed. Philadelphia, PA 19103-2899. Elsevier, Inc. P: 1780. ISBN: 978-0-323-37692-1.

Wertz, G. W. and Moudy, R. M. (2004). Antigenic and genetic variation in human respiratory syncytial virus. The Pediatric Infectious Disease Journal, 23(Supplement), pp.S19-S24.

WHO. (2003). WHO Communicable Disease Profile for Iraq. Communicable Disease Working Group on Emergencies, HQ Division of Communicable Disease Control, EMROWHO Office, Baghdad.

WHO. (2013). Global Health Estimates Summary Tables: Deaths by Cause, Age and Sex, by WHO Region.

WHO. (2015). Iraq's neglected health and humanitarian crisis. Lancet. 385:232-4.

Wiegand, S. B., Jaroszewicz, J., Potthoff, A., Höner Zu Siederdissen, C., Maasoumy, B., Deterding, K., Manns, M. P., Wedemeyer, H., & Cornberg, M. (2015). Dominance of hepatitis C virus (HCV) is associated with lower quantitative hepatitis B surface antigen and higher serum interferon-γ-induced protein 10 levels in HBV/HCV-coinfected patients. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 21(7), 710.e1–710.e7109. https://doi.org/10.1016/j.cmi.2015.03.003.

Wiertsema, S., Chidlow, G., Kirkham, L., Corscadden, K., Mowe, E., Vijayasekaran, S., Coates, H., Harnett, G. and Richmond, P. (2011). High detection rates of nucleic acids of a wide range of respiratory viruses in the nasopharynx and the middle ear of children with a history of recurrent acute otitis media. Journal of Medical Virology, 83(11), pp.2008-2017.

Williams, J. V., Piedra, P. A. and Englund, J. A. (2017). Respiratory Syncytial, Human Metapneumovirus and Parainfluenza Viruses. In: *Clinical Virology*. Richman, D. D., Whitley, R. J. and Hayden, F. G. (ed). 4th ed. Washington, DC: ASM Press. USA. P: 885-886. LCCN 2016020817| ISBN 9781555819422 (hard cover) | ISBN 9781555819439 (e-ISBN)

Williams, J., Edwards, K., Weinberg, G., Griffin, M., Hall, C., Zhu, Y., Szilagyi, P., Wang, C., Yang, C., Silva, D., Ye, D., Spaete, R. and Crowe Jr, J. (2010). Population-Based Incidence of Human Metapneumovirus Infection among Hospitalized Children. The Journal of Infectious Diseases, 201(12), pp.1890-1898.

Williams, J., Harris, P., Tollefson, S., Halburnt-Rush, L., Pingsterhaus, J., Edwards, K., Wright, P. and Crowe, J. (2004). Human Metapneumovirus

and Lower Respiratory Tract Disease in Otherwise Healthy Infants and Children. New England Journal of Medicine, 350(5), pp.443-450.

Williams, J.V., Wang, C.K., Yang, C.F., Tollefson, S.J., House, F.S., Heck, J.M., Chu, M., Brown, J.B., Lintao, L.D., Quinto, J.D. and Chu, D., (2006). The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. The Journal of infectious diseases, 193(3), pp.387-395. Epub 2006/01/03

Wu, K., Wang, S., Shen, C., Ho, T., Wang, J. and Liu, C. (2018). Clinical and epidemiological characteristics of human parainfluenza virus infections of children in southern Taiwan. Journal of Microbiology, Immunology and Infection, 51(6), pp.749-755.

X

Xiao, N., Duan, Z., Xie, Z., Zhong, L., Zeng, S., Huang, H., Gao, H. and Zhang, B. (2016). Human parainfluenza virus types 1-4 in hospitalized children with acute lower respiratory infections in China. Journal of Medical Virology, 88(12), pp.2085-2091.

Y

Yang, C., Wang, C., Tollefson, S., Lintao, L., Liem, A., Chu, M. and Williams, J. (2013). Human Metapneumovirus G protein is highly conserved within but not between genetic lineages. Archives of Virology, 158(6), pp.1245-1252.

Yang, C., Wang, C., Tollefson, S., Piyaratna, R., Lintao, L., Chu, M., Liem, A., Mark, M., Spaete, R., Crowe, J. and Williams, J. (2009). Genetic diversity and evolution of human metapneumovirus fusion protein over twenty years. Virology Journal, 6(1), p.138.

Yousafzai, M., Ibrahim, R., Thobani, R., Aziz, F. and Ali, A. (2018). Human metapneumovirus in hospitalized children less than 5 years of age in Pakistan. Journal of Medical Virology, 90(6), pp.1027-1032.

Yu, Q., Hardy, R.W. and Wertz, G.W., (1995). Functional cDNA clones of the human respiratory syncytial (RS) virus N, P, and L proteins support replication of RS virus genomic RNA analogs and define minimal trans-acting requirements for RNA replication. Journal of Virology, 69(4), pp.2412-2419.

Z

Zaki, S. R. and Keating, M. K. (2018). Viral Diseases. In: *In Foundations in Diagnostic Pathology*. Zander, D. S. and Farver, C. F. (ed.). 2nd Ed. P: 244-288. ISBN: 9780323393089. https://doi.org/10.1016/B978-0-323-39308-9.00013-3. (http://www.sciencedirect.com/science/article/pii/B9780323393089000133).

Zaki, S. R. and Keating, M. K., (2018). Human Parainfluenza Virus. In: *Pulmonary Pathology*. Zander, D. S., and Farver, C. F. (ed). 2nd ed. Philadelphia, PA: Elsevier Inc. pp. 244-288, https://doi.org/10.1016/B978-0-323-39308-9.00013-3.

Zeng, S., Xiao, N., Zhong, L., Yu, T., Zhang, B. and Duan, Z. (2015). Clinical features of human metapneumovirus genotypes in children with acute lower respiratory tract infection in Changsha, China. Journal of Medical Virology, 87(11), pp.1839-1845.

Zhang, C., Du, L., Zhang, Z., Qin, X., Yang, X., Liu, P., Chen, X., Zhao, Y., Liu, E. and Zhao, X. (2012). Detection and Genetic Diversity of Human Metapneumovirus in Hospitalized Children with Acute Respiratory Infections in Southwest China. Journal of Clinical Microbiology, 50(8), pp.2714-2719.

Zhang, L., Liu, W., Liu, D., Chen, D., Tan, W., Qiu, S., Xu, D., Li, X., Liu, T. and Zhou, R. (2018). Epidemiological and clinical features of human metapneumovirus in hospitalised paediatric patients with acute respiratory illness: a cross-sectional study in Southern China, from 2013 to 2016. BMJ Open, 8(2), p.e019308.

Zhang, S., Tellier, R., Zafar, R., Cheung, R., Adachi, D. and Richardson, S. (2009). Comparison of human metapneumovirus infection with respiratory syncytial virus infection in children. The Pediatric Infectious Disease Journal, 28(11), pp.1022-1024.

Zhang, Y., Wei, Y., Zhang, X., Cai, H., Niewiesk, S., & Li, J. (2014). Rational design of human metapneumovirus live attenuated vaccine candidates by inhibiting viral mRNA cap methyltransferase. Journal of virology, 88(19), 11411–11429. doi:10.1128/JVI.00876-14

Zhang, Y., Yuan, L., Zhang, Y., Zhang, X., Zheng, M. and Kyaw, M. (2015). Burden of respiratory syncytial virus infections in China: Systematic review and meta–analysis. Journal of Global Health, 5(2).

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. (2000). A greedy algorithm for aligning DNA sequences. J Comput Biol, 7(1-2):203-14. doi: 10.1089/10665270050081478

Zheng, Y., Liu, L., Wang, S., Li, Z., Hou, M., Li, J., Yu, X., Zhang, W. and Hua, S. (2017). Prevailing genotype distribution and characteristics of human respiratory syncytial virus in northeastern China. Journal of Medical Virology, 89(2), pp.222-233.

APPENDIX

APPENDIX (1)

Number

Outpatient **Inpatient**

Month

Age

Male **Female**

Resident

Children **Adults** **Elderly** **Immunocompromised**

Signs and symptoms		Complications	
1.Cough		8. Otitis media	
2.Feverishness/chills		9. Pharyngitis	
3.Tachypnea/dyspnea		10. Bronchiolitis/bronchitis	
4. Wheezing		11. Pneumonitis	
5. Rhinorrhea/Rhinitis		12. Sinusitis	
6. Sore throat		13. Tonalities	
7. Laryngitis			

Other	
14. Asthma exacerbation	
15. chronic obstructive pulmonary disease	
16. prematurity	
17. congenital heart disease	
18. cancer	
19. hematopoietic stem cell transplants	
20. bone marrow transplant recipients	
21. hypertension in elderly subjects	
22. lung transplantation	

Signature

APPENDIX (2)

Sequencing

Human metapneumovirus isolate Sub-GRO-2-1D attachment glycoprotein (G) gene, partial cds

GenBank: MN178606.1

FASTA Graphics PopSet

Go to:

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  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
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Human metapneumovirus isolate Sub-GRO-2-2D attachment glycoprotein (G) gene, partial cds

GenBank: MN178607.1

FASTA Graphics PopSet

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REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
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Human metapneumovirus isolate Sub-GRO-1-3D attachment glycoprotein (G) gene, partial cds

GenBank: MN178608.1

FASTA Graphics PopSet

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  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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Human metapneumovirus isolate Sub-GRO-3-4D attachment glycoprotein (G) gene, partial cds

GenBank: MN178609.1

FASTA Graphics PopSet

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ACCESSION   MN178609
VERSION     MN178609.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
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      841 caagaaataa gaagaagcta aaattcaagg gataaataac aatggatcca ttttg
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Human metapneumovirus isolate Sub-GRO-3-5D attachment glycoprotein (G) gene, partial cds

GenBank: MN178610.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-3-5D attachment glycoprotein
            (G) gene, partial cds.
ACCESSION   MN178610
VERSION     MN178610.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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      841 caagaaataa gaagaagcta aaattcaagg gataaataac aatggatcca ttttg
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Human metapneumovirus isolate Sub-GRO-3-6D attachment glycoprotein (G) gene, partial cds

GenBank: MN178611.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-3-6D attachment glycoprotein
            (G) gene, partial cds.
ACCESSION   MN178611
VERSION     MN178611.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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                     /country="Iraq"
                     /collection_date="Jan-2018"
                     /collected_by="Asmaa Haseeb Hwaid"
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      841 caagaaataa gaagaagcta aaattcaagg gataaataac aatggatcca ttttg
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Human metapneumovirus isolate Sub-GRO-3-7D attachment glycoprotein (G) gene, partial cds

GenBank: MN178612.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-3-7D attachment glycoprotein
            (G) gene, partial cds.
ACCESSION   MN178612
VERSION     MN178612.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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      841 caagaaataa gaagaagcta aaattcaagg gataaataac aatggatcca ttttg
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Human metapneumovirus isolate Sub-GRO-1-8D attachment glycoprotein (G) gene, partial cds

GenBank: MN178613.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-1-8D attachment glycoprotein
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VERSION     MN178613.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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Human metapneumovirus isolate Sub-GRO-3-9D attachment glycoprotein (G) gene, partial cds

GenBank: MN178614.1

FASTA Graphics PopSet

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DEFINITION  Human metapneumovirus isolate Sub-GRO-3-9D attachment glycoprotein
            (G) gene, partial cds.
ACCESSION   MN178614
VERSION     MN178614.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
            Sequencing Technology :: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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Human metapneumovirus isolate Sub-GRO-1-10D attachment glycoprotein (G) gene, partial cds

GenBank: MN178615.1

FASTA Graphics PopSet

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SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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      661 gacacatctt ctctaagtag ttaacaaaaa atatgtaaaa taaccatgaa aaccaaaaaa
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Human metapneumovirus isolate Sub-GRO-3-11D attachment glycoprotein (G) gene, partial cds

GenBank: MN178616.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-3-11D attachment glycoprotein
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ACCESSION   MN178616
VERSION     MN178616.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
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                     /db_xref="taxon:162145"
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                     /collected_by="Asmaa Haseeb Hwaid"
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                     TAYNTDTSSLSS"
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      781 tttgagcata tatactaatg aaatagcatc tgtttgtgca tcagcaacac catcactatt
      841 caagaaataa gaagaagcta aaattcaagg gataaataac aatggatcca ttttg
//
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Human metapneumovirus isolate Sub-GRO-1-12D attachment glycoprotein (G) gene, partial cds

GenBank: MN178617.1

FASTA Graphics PopSet

Go to:

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DEFINITION  Human metapneumovirus isolate Sub-GRO-1-12D attachment glycoprotein
            (G) gene, partial cds.
ACCESSION   MN178617
VERSION     MN178617.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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                     /country="Iraq"
                     /collection_date="Jan-2018"
                     /collected_by="Asmaa Haseeb Hwaid"
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Human metapneumovirus isolate Sub-GRO-3-13D attachment glycoprotein (G) gene, partial cds

GenBank: MN178618.1

FASTA Graphics PopSet

Go to:

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VERSION     MN178618.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 895)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
COMMENT     ##Assembly-Data-START##
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Human metapneumovirus isolate Sub-GRO-2-14D attachment glycoprotein (G) gene, partial cds

GenBank: MN178619.1

FASTA Graphics PopSet

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VERSION     MN178619.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
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            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
            Baquba, Diyala 32001, Iraq
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Human metapneumovirus isolate Sub-GRO-2-15D attachment glycoprotein (G) gene, partial cds

GenBank: MN178620.1

FASTA Graphics PopSet

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VERSION     MN178620.1
KEYWORDS    .
SOURCE      Human metapneumovirus (HMPV)
  ORGANISM  Human metapneumovirus
            Viruses; Riboviria; Negarnaviricota; Haploviricotina;
            Monjiviricetes; Mononegavirales; Pneumoviridae; Metapneumovirus.
REFERENCE   1  (bases 1 to 886)
  AUTHORS   Hwaid,A.H., Hasan,A.S. and Abdulla,H.N.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JUL-2019) Biology, University of Diyala, Al-Gadria,
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Sequencing

https://www.ncbi.nlm.nih.gov/popset/?term=MN178607&utm_source=gquery&utm_medium=search

Human metapneumovirus attachment glycoprotein (G) gene, partial cds.

PopSet: 1812756318

GenBank FASTA

Sequences in this data set

- MN178620.1Human metapneumovirus isolate Sub-GRO-2-15D attachment glycoprotein (G) gene, partial cds
- MN178619.1Human metapneumovirus isolate Sub-GRO-2-14D attachment glycoprotein (G) gene, partial cds
- MN178618.1Human metapneumovirus isolate Sub-GRO-3-13D attachment glycoprotein (G) gene, partial cds
- MN178617.1Human metapneumovirus isolate Sub-GRO-1-12D attachment glycoprotein (G) gene, partial cds
- MN178616.1Human metapneumovirus isolate Sub-GRO-3-11D attachment glycoprotein (G) gene, partial cds
- MN178615.1Human metapneumovirus isolate Sub-GRO-1-10D attachment glycoprotein (G) gene, partial cds
- MN178614.1Human metapneumovirus isolate Sub-GRO-3-9D attachment glycoprotein (G) gene, partial cds
- MN178613.1Human metapneumovirus isolate Sub-GRO-1-8D attachment glycoprotein (G) gene, partial cds
- MN178612.1Human metapneumovirus isolate Sub-GRO-3-7D attachment glycoprotein (G) gene, partial cds
- MN178611.1Human metapneumovirus isolate Sub-GRO-3-6D attachment glycoprotein (G) gene, partial cds
- MN178610.1Human metapneumovirus isolate Sub-GRO-3-5D attachment glycoprotein (G) gene, partial cds
- MN178609.1Human metapneumovirus isolate Sub-GRO-3-4D attachment glycoprotein (G) gene, partial cds
- MN178608.1Human metapneumovirus isolate Sub-GRO-1-3D attachment glycoprotein (G) gene, partial cds
- MN178607.1Human metapneumovirus isolate Sub-GRO-2-2D attachment glycoprotein (G) gene, partial cds
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-

APPENDIX (3)

Pairwise Sequence Alignment of Iraqi strain that have (AAAGAAAAA, KKE, insertion mutation in the table, 4.24), (HMPV4, 5, 6, 7, 9, 11, 13 in the figure, 4.11, 4.12), (MN178609.1, MN178610.1, MN178611.1, MN178612.1, MN178614.1, MN178616.1, MN178618.1) and Malaysian strains (GenBank acc. KU320936.1)

Human metapneumovirus isolate MY/U1784/2013 attachment surface glycoprotein (G) gene, partial cds
Sequence ID: KU320936.1 Length: 879 Number of Matches: 1
Range 1: 1 to 879 GenBankGraphics

96% identity

Score	Expect	Identities	Gaps	Strand
1476 bits(1636)	0.0	863/895(96%)	16/895(1%)	Plus/Plus

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Query  1    TTCAAAGCAAAGATGAAAAACCGTATAAGAAGTAGCAAGTGCTATAGAAATGCTACACTG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTCAAAGCAAAGATGAAAAACCGTATAAGAAGTAGCAAGTGCTATAGAAATGCTACACTG  60

Query  61   ATCCTTATTGGATTAACAGCATTAAGTATGGCACTTAATAttttttAATCATTGATTAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATCCTTATTGGATTAACAGCATTAAGTATGGCACTTAATATTTTTTTAATCATTGATTAT  120

Query  121  GCAAAATTAAAAAACATGACCAAAGTAGAACTCTGTGTCAACATGCCACCGGTAGAACCA  180
            |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  121  GCAAAATTAAAAAACATGACCAAAGTGGAACTCTGTGTCAACATGCCACCGGTAGAACCA  180

Query  181  AGCAAGAAGACCCCAATGACCTCTGCAGTAGACTTAAACACTAAACTCAATCCACAGCAG  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  AGCAAGAAGACCCCAATGACCTCTGCAGTAGACTTAAACACTAAACTCAATCCACAGCAG  240

Query  241  GCAACACAGTTGACCACAGAGGATTCAACATCTCTAGCAGCAACCTCAGAGAATCATCTA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GCAACACAGTTGACCACAGAGGATTCAACATCTCTAGCAGCAACCTCAGAGAATCATCTA  300
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Query  301  CACACAGAGACAACTCCAACACCAGATACAACAATCTCTCAGCAAGCTACAGACAAGCAC  360
            |||||||||||||||||||||||||| |||||||||||||||||| |||||| |||||
Sbjct  301  CACACAGAGACAACTCCAACACCAGATGCAACAATCTCTCAGCAAGCCACAGACGAGCAC  360

Query  361  ACAACACTGCTGAGATCAATcaacagacagaccatccaaacaaccacagagaaaaaacca  420
            |||||||||||||||||||||||||||||||||| |||||||||||||||||||| |||
Sbjct  361  ACAACACTGCTGAGATCAATCAACAGACAGACCACCCAAACAACCACAGAGAAAAAGCCA  420

Query  421  accggagcaacaaccaaaaaagaaaaaaaagaaaaagaaaaagaaaCCACAACTCGAACT  480
            ||||||||||||||||               ||||||||||||||||||||||||||||||
Sbjct  421  ACCGGAGCAACAACCA---------------AAAAAGAAAAAGAAACCACAACTCGAACT  465

Query  481  ACAAGCACAGCTGCAACCCAAACACTCAACACCACCAACCAAACCAGCAATAGAAGAGAG  540
            |||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  466  ACAAGCACAGCTGCAACCCAAACACTCAACACCACCAACCAAACCAGCAACAGAAGAGAA  525

Query  541  GCAACCACAACATCCGCCAGAGCCAGAAACGGTGCCACAACTCAAAATAGCGATCAAACA  600
            |||||||||||||||||||| |||||||||||||||||||| ||||||||||||
Sbjct  526  GCAACCACAACATCCGCCAGATCCAGAAACGGTGCCACAACTCAAAACAGCGATCAAACA  585

Query  601  ATCCAGGCGGCAGACCCAAGCTCCACACCATACCATACACAGAAAAGCACAACAACAGCA  660
            |||||||| ||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  586  ATCCAGGCAGCAGACCCAAGCTCCAAACCATACCATACACAGAAAAGCACAACAACAGCA  645

Query  661  TACAACACAGACACATCTTCTCTAAGTAGTTAACaaaaaatatgtaaaataaccatgaaa  720
            |||||| |||||||||||||||||||||||| |||||||| ||||||||||||||||
Sbjct  646  TACAACGCAGACACATCTTCTCTAAGTAGTTAAC-AAAAATATATAAAATAACCATGAAA  704

Query  721  accaaaaaactagaaaagttaatttgaactcagaaaaaaccacaaacACTATATGAATTG  780
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  705  ACCAAAAAACTAGAAAAGTTAATTTGAACTCAGAAAAAACCACAAACACTATATGAATTG  764

Query  781  TTTGAGCATATATACTAATGAAATAGCATCTGTTTGTGCATCAGCAACACCATCACTATT  840
            ||||||| |||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  765  TTTGAGCGTATATACTAATGAAATAGCATCTGTTTGTGCATCAACAACACCATCACTATT  824
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Query  841  CAAGAAATAAGAAGAAGCTAAAATTCAAGGGATAAATAACAATGGATCCATTTTG  895
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  825  CAAGAAATAAGAAGAAGCTAAAATTCAAGGGATAAATAACAATGGATCCATTTTG  879
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الخلاصة

عالميا، تعد التهابات الجهاز التنفسي الفيروسية سببًا مهمًا للاصابات والوفيات وتمثل تهديدًا خطيرًا لصحة الإنسان، من بين هذه الفيروسات Human Metapneumovirus (HMPV)، وهو أحد مسببات الأمراض التنفسية الناشئة التي تم وصفها لأول مرة في عام 2001، الذي يشبه إلى حد كبير فيروس Human Respiratory Syncytial Virus (HRSV) فيما يتعلق بالأعراض والعلامات السريرية، فيروسات Human Parainfluenza Viruses النوع 1 و 3 (HPIV-3 and HPIV-1) من مسببات الأمراض التنفسية الأخرى. هذه الفيروسات مسؤولة عن مجموعة واسعة من التهابات القناة التنفسي العلوية والسفلية.

الدراسة الحالية هي دراسة مقطعية أجريت في مستشفى بعقوبة التعليمي ومستشفى البتول التعليمي للأمومة والطفل بالاضافة للعيادات الخارجية في محافظة ديالى، العراق، للفترة من 16/ كانون الثاني/ 2018 إلى 30/ تشرين الاول/ 2019. تضمنت اهداف الدراسة الحالية الكشف عن معدلات انتشار الإصابة بفيروس HMPV، HRSV، HPIV نوع 1 و 3 بين المرضى المصابين سريريًا بالتهابات القناة التنفسية، تحديد الإصابة المشتركة الناتجة عن الإصابة بفيروسين او اكثر من الفيروسات التنفسية المدرجة في الدراسة، معرفة عوامل الخطر (الاجتماعية والديموغرافية) المرتبطة بها، علاوة على ذلك، تحديد التوزيع الموسمي والتنوع الجيني (الانماط الوراثية السائدة) لسلالات HMPV و HRSV المنتشرة محليا من خلال تحليل التتابعات او التسلسلات النيوكليوتيدية والشجرة الوراثية التطورية لجين (G) و (F) لفيروس HMPV والتنميط الجيني لجين (F) لـفيروس HRSV، واخيرا المقارنة الجينية لعزلات HMPV مع عزلات اخرى من دول مختلفة من خلال التراصف المتعدد والمزدوج multiple and pairwise alignment لهذه العزلات مع العزلات الدولية المتاحة في قاعدة بيانات GeneBank .

شملت هذه الدراسة ثلاثمائة وثلاثة وعشرين مريضا من أولئك المشتبه سريريا لديهم التهابات القناة التنفسية RTIs (الأطفال والكبار والمسنين). امتدت الدراسة على مدار موسمين. تم شمول 185 مريضا في الموسم الأول (كانون الثاني، شباط، اذار، نيسان وايار) و 138 في الموسم الثاني (تشرين الثاني وكانون الاول). تم جمع ثلاثة أنواع مختلفة من العينات التنفسية بما في ذلك المسحات البلعومية الأنفية، مسحات الأنف ومسحات الحلق. تم احترام خصوصية الإنسان من خلال الحصول على موافقة كتابية رسمية من لجنة أخلاقيات البحوث في دليل ديالى للصحة ، وتم جمع العينات تحت إشراف مباشر من الأطباء والممارسين المتخصصين بعد موافقة المشاركين في الدراسة أو أولياء أمورهم. تم تحليل العينات باستخدام تقنية تفاعل البلمرة المتسلسل بالزمن الحقيقي أو اللحظي real-time PCR (RT-PCR/qPCR) لفيروس (HMPV، HRSV وHPIV-3/HPIV-1) وتفاعل البلمرة المتسلسل التقليدي PCR لفيروس (HMPV وHRSV). تم إجراء التنميط الجيني لكل من العينات الإيجابية لـ HMPV والتنميط الفرعي الجيني genetic subgrouping لفيروس HRSV.

اظهرت النتائج ان الاصابات المنفردة بفيروس HMPV كُشفت في 30 (9.3%) وان الاصابة المشتركة مع فيروس HPIV-3 كانت (n = 1) والذي كان له نفس المظاهر السريرية وتم اكتشاف إصابة منفردة بفيروس HRSV في 15 (4.6%) و الاصابة المشتركة مع فيروس HPIV-3 كانت (n = 1)، بينما ظهر فيروس HPIV-3 منفردا في 17(5.3%) في حين لم يتم اكتشاف أي إصابات بفيروس HPIV-1. ظهرت أعلى معدلات إيجابية لـ HMPV في العينات التي تم جمعها خلال شهري نوفمبر وديسمبر (الموسم الثاني) مع اختلاف كبير مقارنة مع الأشهر الأخرى (50% ، P = 0.0001). من الواضح أن أعلى المعدلات الإيجابية لـ HMPV قد ظهرت في مسحات الجيوب الأنفية مع وجود فرق ذي دلالة إحصائية مقارنة بمسحات البلعوم والحنجرة (96.7% ، P = 0.0001). معدل إيجابية فيروس HRSV في الموسم الأول كان أعلى بفارق احصائي معنوي مقارنة بالموسم الثاني (93.3 % مقابل 6.7 % ،

P = 0.004)، اظهر شهر شباط مقارنة مع الأشهر الأخرى من السنة أعلى معدل ظهور للفيروس (P = 0.012)، ظهر فيروس HRSV في (53.3 %) من مسحات الحلق التي كانت أعلى بالمقارنة مع مسحات البلعومية الأنفية (40.0 %) ومسحات الجيوب الأنفية (6.7 %) (P = 0.003). معدل إيجابية ظهور HPIV-3 خلال الموسم الأول كان أعلى مقارنةً بالموسم الثاني (64.7% مقابل 35.3%)، ومع ذلك، فشل الفرق للوصول إلى دلالة إحصائية لكليهما (P = 0.525)، بالإضافة إلى ذلك، أثبتت مسحات الحلق والأنف فعالية في إظهار أعلى معدل إيجابية لفيروس HPIV-3 (35.3%، 64.7%) على التوالي. فيما يتعلق بايجابية ظهورHMPV، HRSV و HPIV-3 المرتبط بالعمر، كانت الإصابات أعلى بين الأطفال دون سن 5 سنوات (86.7 % ، 53.3 % ، 82.4 %) على التوالي. الصور السريرية الأكثر ارتباطًا بارتفاع معدل الإصابة بفيروس HMPV و HPIV-3 كانت التهاب القصيبات/ التهاب الشعب الهوائية (86.7% ، 88.2%) مع وجود اختلاف ذي دلالة إحصائية (P=0.001, 0.003)

أظهر التحليل الوراثي لجين attachment glycoprotein (G) لفيروس HMPV أنه من بين 28 عزلة عراقية تم جمعها خلال الموسم الثاني، 15 من هذه العزلات تنتمي وتتجمع ضمن النمط الوراثي (B)، السلالات الفرعية (B2) في هذه المنطقة. تم تسجيل تلك العزلات في بنك الجينات (GeneBank) في المركز الوطني لمعلومات التكنولوجيا الاحيائية (NCBI) وتم الحصول على رقم انضمام عالمي (accession NO). اظهر التراصف المتعدد multiple sequence alignment تماثلات عالية تقدر بنسبة (98% و 97%) بين السلالات العراقية (MN178608.1، MN178613.1، MN178615.1، MN178617.1، MN178606.1، MN178607.1، MN178619.1،MN178620.1) والنمط الوراثي (B) لفيروس HMPV، السلالات الفرعية B2 المنتشرة في اسبانيا (GenBank acc. KX829167.1) وماليزيا (GenBank acc. KU320936.1) ، من جانب اخر، اظهر التراصف المزدوج pairwise sequence alignment تماثلا يقدر ب (96%) بين السلالات

العراقية (MN178609.1، MN178610.1، MN178611.1، MN178612.1 ، MN178614.1، MN178616.1، MN178618.1) والسلالات الماليزية (GenBank acc. KU320936.1). تباين وراثي كبير في تسلسل النيوكليوتيدات والأحماض الأمينية لوحظ في جين (G) ، أظهر رصف التتابعات النيوكليوتيدية والاحماض الامينية لجين (G) وتحليل الشجرة التطورية الوراثية أن ثلاثة متغايرات لفيروس HMPV متميزة في نفس السلالة الفرعية B2 تم اكتشافها كنتيجة لطفرات الازاحة (طفرات الحشر)، المجموعة الفرعية الأولى، تشمل (MN178608.1، MN178613.1، MN178615.1، MN178617.1)؛ المجموعة الفرعية الثانية، وتشمل (MN178606.1، MN178607.1، MN178619.1، MN178620.1)؛ والمجموعة الفرعية الثالثة، وتضم (MN178609.1، MN178610.1، MN178611.1، MN178612.1، MN178614.1، MN178616.1، MN178618.1).

وخلصت الدراسة إلى أن فيروس HMPV له دور مهم كسبب فيروسي لارتفاع معدل التهابات القناة التنفسية RTIs في مجتمع ديالى، وعلى وجه الخصوص بين الأطفال يليه أهمية فيروس HRSV وHPIV-3 كمسببات مرضية للجهاز التنفسي. يمكن ملاحظة الاختلاف الموسمي لـ HMPV في هذه الدراسة وسيادة انتشار النمط الوراثي (B)، السلالة الفرعية (B2) لفيروس HMPV مقارنة بالفيروسات الأخرى قيد الدراسة. السلالات الناشئة لفيروس HMPV تتطور باستمرار.

وزارة التعليم العالي والبحث العلمي
جامعة ديالى
كلية التربية للعلوم الصرفة
قسم علوم الحياة

دراسة جزيئية لفيروس

Human Respiratory ،Human Metapneumovirus Syncytial Virus و Human Parainfluenza Virus type 1, 3 بين المرضى المصابين بالتهابات القناة التنفسية في محافظة ديالى، العراق

أطروحة

مقدمة إلى مجلس كلية التربية للعلوم الصرفة / جامعة ديالى وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في علوم الحياة

من قبل

أسماء حسيب هويد الجوراني

بكلوريوس علوم حياة/ كلية التربية للعلوم الصرفة/جامعة ديالى 2005م
ماجستير علوم حياة/ أحياء مجهرية/كلية التربية للعلوم الصرفة/جامعة ديالى 2009م

بإشراف

الاستاذ الدكتور
عبد الرزاق شفيق حسن
دكتوراه احياء مجهرية طبية/ فيروسات

الاستاذ الدكتور
هناء ناجي عبدالله
دكتوراه مناعة وراثية

١٤٤١ هـ

٢٠٢٠ م