SEROPREVALENCE OF HUMAN *PARAINFLUENZA VIRUS* TYPE 3 INFECTION AMONG CHILDREN 1-5 YEARS IN ZARIA, KADUNA STATE, NIGERIA

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MARCH, 2009

DECLARATION

I declare that the work in the thesis entitled 'Seroprevalence of *Parainfluenza Virus* Type 3 among Children (1-5 years) In Zaria' has been performed by me in the department of microbiology under the supervision of Prof. A. A. Ahmad and Dr H. W. Idris. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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Dahiru Lawal Rogo Signature Date

CERTIFICATION

This thesis entitled "SEROPREVALENCE OF PARAINFLUENZA VIRUS TYPE 3 INFECTION AMONG CHILDREN 1-5 YEARS IN ZARIA" by Dahiru Lawal Rogo meet the regulations governing the award of the degree of masters of Science in the Department of Microbiology, Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late parent Dahiru Bala and Rabi'atu Dahiru Danbaba, my beloved wife Rabi'atu Sani Yunusa and my late daughter Hamidah Lawal Rogo.

ACKNOWLEDGEMENT

All praise is to Almighty God for His guidance and for giving me the health and perseverance to carryout the work.

First and foremost my unreserved gratitude to my supervisor Prof. A. A. Ahmad for having the patience to accommodate me as his student, condoned and tolerated me, and above all inspired me to achieve this lofty standard. May Allah reward you, protect you for us and the future students coming, and for the development of humanity in general. May He reward you with heavenly blessing.

My regard to Dr H.W. Idris for being attentive, supportive and accommodative all the time. May Allah reward you abundantly.

I shall for ever remain grateful to my parents may their sole rest in perfect peace, amen.

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I am grateful to all the laboratory staff of Microbiology Department, my well wishers and all those who have contributed in some way to the completion of this research, whose names may not have been mentioned, may Allah reward you all with the goodies of this life and the hereafter.

To God Almighty be the glory and may the peace and blessing of God be on his noble prophet Muhammad (SAWA) and his household.

ABSTRACT

Human *Parainfuenza virus* type 3 (HPIV-3), the causative agent of upper and lower respiratory tract infection can cause severe lower respiratory tract infection in immunocompetent individuals as well as immunocompromised patients. The aim of this study was to assess the seroprevalence of HPIV-3 IgG antibody in children 1-5 years in Zaria. Blood samples were obtained from 379 children whose parents consented. The sera obtained were analyzed using HPIV-3 IgG SERION ELISA classic GmbH Germany to determine the HPIV-3 IgG level. Information about the children's demographic factors and other variables were obtained from their caregivers using a designed questionnaire. Of the 379 serum samples analyzed 323 were positive giving overall seroprevalence for HPIV-3 as 85.2%. Prevalence of HPIV-3 antibodies was 43.0% in males and 42.2% in females. Seropositivity was found to increase with age, cough and parental smoking/ toxin ($P< 0.05$). On the other hand there is no association between the HPIV-3 infection with catarrh/running nose and sickle cell disease. Children aged 1year had the lowest prevalence of 7.4%, while those aged between 1-2 years had the highest prevalence of 26.1%. In view of the high prevalence rate of HPIV-3 infection in this study, it is suggested that further epidemiological studies should be conducted to establish the exact role of HPIV-3 in respiratory tract infections among children in Nigeria.

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CHAPTER ONE

1.0 INTRODUCTION

Parainfluenza viruses (PIVs) are responsible for 30-40% of all acute respiratory tract infections in infants and children worldwide (Santanu *et al.*, 2004). These conditions include common cold, croup, bronchitis, and pneumonia. It contributes to community acquired respiratory tract infections of variable severity in adults (Glezen *et al.*, 1984; Welliver *et al.*, 1986; Santanu *et al.*, 2004).

Parainfluenza viruses are pleomorphic viruses whose envelope is derived from the host cell they last infected (Collins, 1996; Puppe *et al.*, 2004; Porotto *et al.*, 2005). These viruses are 150-300nm in diameter and posses a single-stranded, non-segmented, negative-sense RNA genome with nucleoproteins P and L, and a lipid bilayer covered with glycoprotein spikes which surround a helical nucleocapsid that measures 12-17nm in diameter (Jose *et al.*, 2000). These glycoproteins are hemagglutinin–neuraminidase (HN) and fusion (F) proteins, which play a role in the pathogenesis of the disease (Jawetz *et al.*, 2004). The viruses are included in the order *Mononegavirales*, the family *Paramyxoviridae* and the subfamily *Paramyxovirinae*. They belong to 2 different genera: HPIV-1 and HPIV-3 belong to the Respirovirus genus, and HPIV-2 and HPIV-4a and 4b belong to the Rubulavirus genus (Jawetz *et al.*, 2004; Porotto *et al.*, 2005).

Human Parainfleunza virus serotype 3 (HPIV-3), is a member of the *Respirovirus* genus, of the *Paramyxoviridae* family. The virus is one of the most virulent among the group, frequently causing bronchiolitis and pneumonia during the first months of life (Micheal

and Amiya, 1997). It is a common community–acquired respiratory pathogen without ethnic, socioeconomic, gender, age or geographic boundaries (Gulen *et al.*, 2007). It is an airborne pathogen that infects human lung epithelial cells from the apical (laminal) plasma membrane domain (Chanock *et al.*, 2001; Lamb and Kolakofsky, 2001; Santanu *et al.*, 2004). Airborne infection by HPIV-3 not only manifests in disease states in infants but also causes high morbidity among immunocompromised adults (Chanock *et al.*, 2001). Respiratory epithelium appears to be the major site of virus binding and subsequent infection. Human Parainfluenza virus Type 3 initiates infection following the engagement of its two envelope proteins, the hemagglutinin-neuraminidase (HN) and fusion (F) proteins with the cell surface receptor (s) present on the plasma membrane of airway epithelia. It is evident that HN promotes the attachment function following its interaction with cell surface sialic acid-containing (SAR) receptors (s). These initial interactions promote F-mediated fusion of the viral membrane with the cellular plasma membrane, leading to the penetration of the virus into the cell (Ah-Type *et al.*, 1999; Porotto *et al.*, 2006). Subsequently, the viruses enter the cell via fusion with the cell membrane mediated by F_1 and F_2 receptors (Laura *et al.*, 2007; Porotto *et al.*, 2007).

Viral transmission could also occur via direct inoculation of contagious secretions from the hands or via large particle aerosols into the eyes and nose (Laura *et al.*, 2007). Prolonged survival of HPIV-3 on skin, cloth and other objects emphasizes the importance of fomites in nosocomial spread. The main pathological features include airway inflammation, necrosis and sloughing of respiratory epithelium, edema, excessive mucus production, and interstitial infiltration of the lungs. These inflammatory features cause

swelling of the vocal cords, larynx, trachea, airway inflow and subsequent stridor, which is characteristic of croup (Gulen *et al.,* 2007). After the primary infection, the virus continues to be excreted in respiratory exudates for 3-16 days following primary infection and 1-4 days following infection (Zambon *et al.,* 1998; Hohenthal *et al.,* 2001; Jawetz *et al.,* 2004). The inoculating dose is very small (Ah-Type *et al.,* 1999). The predisposing factors found to predispose individuals to these infections, including malnutrition, overcrowding, vitamin A deficiency, lack of breast feeding, and environmental smoke or toxins (Laura *et al.,* 2007).

Human Parainfluenza virus type 3 has worldwide distribution. Mortality associated with HPIV-3 is unusual in developed countries and occurs almost exclusively in young infants, people who are immunocompromised and the elderly. In developing countries however, children in the preschool age were found to be more at risk for HPIV-3 associated death (Gulen *et al.,* 2007).

The socioeconomic conditions within a country are the biggest determinants of the ultimate cost of HPIV-3 infection. In undeveloped regions, the cost is in human lives. In developed countries example United State of America, mortality is unusual and the cost of these virus is seen mostly in health care utilization, hospitalization, lost productivity for parents, and perhaps, long-term respiratory tract sequelae in children (Kelly, 2003). A parent's lost time both from work and home secondary to HPIV-3 infections has not been quantitated but is considerable. Human Parainfluenza virus type 3 causes longer and more costly stays than other HPIVs or the economic burden of infections in adults and immunocompromised parent (Elizaga *et al.,* 2001).

1.1 STATEMENT OF THE PROBLEM

Respiratory infection caused by Parainfluenza virus type 3 is one of the major causes of upper and lower respiratory tract disease in infants and young children, causing croup, bronchoilitis, and pneumonia (Hall, 2001). Additionally, this virus has been associated with significant morbidity, and mortality, especially in immunocomporomised patients and the elderly (Whimbey *et al.*, 1996; Ljunman *et al.*, 2001; Greenberg, 2002).

Parainfluenza virus type 3 accounts for unscheduled visits to a health care provider for respiratory illness and was associated with unnecessary antibiotic prescriptions that may contribute to antimicrobial resistance (Hall, 2001).

1.2 JUSTIFICATION

Improving the etiological diagnosis of viral infections like HPIVs might avoid unnecessary therapy with antibiotics in particular, and will allow for preventive isolation of infected patients when necessary (Gulen *et al.*, 2007).

1.3 AIM

The aim of this research is to determine the seroprevalence of Human Parainfluenza virus type 3 IgG antibody in children 12-60 months attending the out patients clinics of the ABUTH Zaria, Kaduna State.

1.4 OBJECTIVES

Specific objectives of the study are:

1. To determine the level of Parainfluenza virus type 3 IgG antibodies in children 12-60 months attending the out patients clinics of ABUTH Zaria, Kaduna State.
2. To determine the relationship if any between serum IgG level among children with respiratory symptoms and those without respiratory symptoms.

3. To determine the possible association between Parainfluenza virus type 3 serum IgG level in children and the variables of age, sex, cough, parental smoking/toxin, catarrh/running nose and sickle cell disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Background

The first Human Parainfluenza virus discovered was the Sendai virus in 1952 in Japan. In 1955, Human Parainfluenza virus type 2 (HPIV-2) was isolated from children with acute laryngotracheobronchitis (croup). In 1960, HPIV type 4 (HPIV-4) was isolated from children with mild respiratory infections. In 1985, HPIV type 3 (HPIV-3) was isolated from children with respiratory infection. Human Parainfluenza virus type 4 was further divided into subtypes 4a and 4b (Canchola *et al.*, 1964; Lau *et al.*, 2005; Templeton *et al.*, 2005; Arden *et al.*, 2006; Fry *et al.*, 2006; Subhash and Thomas, 2008). Subgroups/genotypes of HPIV-1 (Henrickson, 1991; Henrickson and Savatski, 1992; Henrickson and Savatski, 1996; Emmalere *et al.*, 2007) and HPIV-3 (Jawetz *et al.*, 2007) have been described. These viruses are included in the order *Mononegavirales*, the family *Paramyxoviridae* and the sub family *Paramyxovirinae*. They belong to 2 different genera: HPIV-1 and HPIV-3 belong to the *Respirovirus* genus, and HPIV-2and HPIV-4a and 4b belong to the *Rubulla* virus genus (Jawetz *et al.*, 2004).

2.2 VIROLOGY OF HUMAN PARAINFLUENZA VIRUS TYPE 3 VIRION

Human Parainfluenza virus serotype 3 (HPIV-3) is a member of the *Respirovirus* genus, of the *Paramyxoviridae* family (Wendt *et al.*, 1992; Whimbey *et al.*, 1996; Michael and Amiya, 1997; Hohenthal *et al.*, 2001). It is a pleomorphic envelope, single strand, non-segmented RNA virus with negative sense polarity. Its envelop is derived from the host cell it last infected.

Human *Parainfluenza virus* type 3 appears to be the most virulent. The viral envelop contains two virus-specific glycoproteins: the hemagglutinin-neuraminidase (HN) and the fusion (F) proteins. It initiates infection following the engagement of its two envelop proteins, the hemagglutinin- neuraminidase (HN) and fusion (F) proteins, with the cell surface receptor (s) present on the plasma membrane of airway epithelia. It HN promotes the attachment function of HPIV-3 following its interaction with a cell surface sialic acid-containing receptor (s) (SAR). This initial interaction promotes F-mediated fusion of the viral membrane with the cellular plasma membrane, leading to the penetration of the virus into the cell (Ah-Type *et al.,* 1999; Porotto *et al.,* 2006), allowing release of the viral nucleocapsid directly into the cell. Thus, HPIV-3 is able to bypass internalization through endosomes (Jawetz *et al., 2007).*

2.2.1 STRUCTURE OF PARAINFLUENZA VIRUS TYPE 3

The virion structure of HPIV-3 consists of a filamentous, herringbone-like nucleocapsid core surrounded by a lipid envelop with virus-specific glycoprotein spikes. The nucleocapsid is composed of the genome, (a single-stranded RNA molecule of negative sense polarity) which is tightly coated with the nucleocapsid protein (NP). The nucleocapsid structure also contains two other proteins, the phosphoprotein (P) and the large protein (L), which occur discontinuously as clusters (Hu *et al.,* 2005; Yoo *et al.,* 2007; Juazapaitis *et al.,* 2008). The NP is the most abundant protein in the virion, and it is believed to be responsible, together with P and L proteins, for RNA-dependent RNA polymerase activity (Garlinghouse *et al.,* 1987; Juozapaitis *et al*., 2008).

The F protein is synthesized as a biologically inactive precursor form (F_0), which is cleaved posttranslationally with cellular protease to produce an active form of two

disulfide-linked subunits, F_1 and F_2 (Hamid *et al.,* 2007). The fatty acid composition of the envelope is identical to that of the host cell (Jawetz *et al.,* 2004). The matrix (M) protein is highly hydrophobic in character, and its function in the glycoproteins and the nucleocapsid (Raija and Timo, 1994; Mariana *et al.,* 2008).

2.2.2 GENOME ORGANISATION

Human Parainfluenza virus type 3 is a linear, nonsegmented, negative- sense RNA molecule containing 15,463 nucleotide and it consist of six or seven genes that encode eight or nine Proteins, some of which are not detected in the virion but participate in the virus replication cycle in the cell (Mao *et al.,* 2008). The genomic RNA is always tightly bound to NP making it undetectable in naked form within the cells (Plotnick- Gilquin *et al.,* 2001).

The organization of the genome is 3′-NP-P/C/V-M-F-HN-L-5′ (Raija and Timo, 1994; Kelly, 2003). Extensive sequence analysis of HPIV-3 genome (Raija and Timo, 1994; Subhash and Thomas, 2008) has shown that the 3′ and 5′ ends of this genome contain the short, extra-cistronic noncoding regions, approximately 50 and 40 nucleotide (nt) long respectively. The first 20nt at the 3′ end are highly conserved suggesting their biological significance in initiation of mRNA transcription and genome replication. The adjacent genes in the genome are separated by intercistronic regions, which contain a transcriptional control sequence found at the beginning of every gene (gene start), a stop sequence (gene end) at the end of every gene, and a short sequence between the genes (Gallinski *et al.,* 1988). The control regions are conserved (Jambou *et al.,* 1986) and are transcribed to mRNAs, whereas the short intergenic sequences are copied.

The length of the monocistronic NP gene in HPIV-3 is in the range of 1,641 to 1,850 nt, and it encodes a 515- amino acid protein (Jambou *et al.,* 1986; Sanchezetal, 1986).

The P gene is a multicistronic genome region with a unique coding strategy. The P genes have continuous, discontinuous, and overlapping cistrons (Galinski *et al.,* 1992), and *Human* Parainfluenza virus types 3 encode their 500 to 600 – amino acid P proteins from continuous P cistrons. The second start codon in the P gene region initiates a shorter overlapping open reading frame coding for a smaller, nonstructural C protein (Luk *et al.,* 1986). The proportional quantities of the P and C proteins produced are evidently controlled at the level of translation. The C protein (about 204 amino acids) is not found within the virion but is detected in infected cells (Fry *et al.,* 2006). The mechanism responsible for addition of the nontemplate residue is unknown, nor is it known whether it occurs co-transcriptionally or posttranscriptionally, but virus- encoded proteins are probably needed for this process (Galinski *et al.,* 1992). The P and V proteins are amino coterminal and share 164 amino acids before the sequences diverge. The V proteins have a cysteine rich region, which is similar to the metal-binding domains present in proteins known to bind to DNA or RNA. This cysteine-rich region has been found in all Paramyxoviruses P genes, suggesting it is biologically important (Paterson *et al.,* 1989).

The M gene is the most highly conserved gene in HPIV-3 (Spriggs and Collins, 1986), and the homology is most extensive at the carboxy-terminal third of the protein sequences. Most conserved regions represent hydrophobic regions, which may be structurally involved in membrane interactions (Luk *et al.,* 1987).

The HPIV-3 F gene is 1,845 to 1,851 nt long and encodes a 539- amino acid protein (Cote *et al.,* 1987). The F gene sequence reported for a number of clinical HPIV-3

isolates show a very high degree of conservation at the nucleotide (>94%) and amino acid (>97%) level (Prinoski *et al.,* 1991). The F protein contains two hydrophobic regions and a single sequence, which is later removed through a proteolytic cleavage (Morrison and Porltner, 1991).

The HN genes of HPIV-3 encode a 565-572- amino acid protein containing one major hydrophobic region and no signal sequence (Elango *et al.,* 1986). The highest homology, 62% at the amino acid level, occurs in HPIV-3 (Morrison and Porltner, 1991).

The gene is located at the 5′ end of the genome and is the largest HPIV-3 gene (6,799nt). It represents about 40% of the coding capacity of the entire genome (Shioda *et al.,* 1986). Because of the length of the gene and its location, the number of L mRNA is the smallest among the transcripts.

2.2.3 VIRAL PROTEINS

The HPIV-3 proteins can be structural or non structural.

Structural

Human Parainfluenza virus type 3 consists of six structural proteins in its genome.

The HPIV-3 protein, “L” otherwise referred to as the “Large” is the largest nucleocapsid protein (polymerase, molecular weight 175,000 to 251,000), followed by phosphor protein (P protein) (Molecular weight (MW) 83,000 to 90,000) (Storey *et al.*, 1984; Sanchez and Banerje, 1985; Wecchsler *et al*., 1985; Spriggs and Collins, 1986). These two viral proteins and the nucleocapsid protein (N or NP) (MW66, 000 to 70,000) are closely associated with viral RNA (vRNA) (Raija and Timo, 1994). Two surface glycoproteins are found in HPIV-3; the hemagglutinin-neuraminidase (HN) (MW69, 000

to 82,000) and the fusion protein (F_o) (MW60, 000 to 66,000). The third protein is the membrane protein (M) (MW 28,000 to 40,000) this is found just beneath the viral membrane and is strongly associated with this membrane (Hohenthel *et al.*, 2001; Kelly, 2003).

Non- Structural Protein

Human Parainfluenza virus *type* 3 produces 2 nonstructural proteins. The P gene of the virus produce two non-structural proteins from multiple overlapping reading frames. These proteins include "C" and "V" (Spriggs and Collins, 1986). The production of these proteins required additional editing by the mRNA. These proteins appear to have several actions. They bind nucleoprotein and may play a role in regulating viral replication (Watanabe *et al.,* 1996). The second non structural protein is the V protein. However, the V protein distribution (nuclei and cytoplasm) does not appear to be similar to that of the N or P protein (cytoplasmic granules) within the virus infected cells (Nishio *et al.,* 1999). Studies suggested that slowing the cell cycle might be one way the V protein favors viral replication (Lin and Lamb, 2000).

The virus also has been shown to contain cellular actin as a structural component; however, its function in virus structure and replication is not fully understood (Giuffre *et al.,* 1982; Stallcup and Field, 1983; Storey *et al.,* 1984; Hamid *et al*., 2007)

2.2.4 HUMAN PARAINFLUENZA VIRUS TYPE-3 REPLICATION

Replication of the HPIV-3 RNA genome is similar to that of the other members of the *Paramyxoviridae* family and the closely related *Rhabdoviridae* family. Upon infection of a cell, transcription is the major RNA synthesis event, resulting in the production of viral mRNAs from the negative-sense genome (Jawetz *et al.,* 2004). Later in infection, a

transition to RNA replication occurs, resulting in synthesis of antigenomic, positive-sense RNA, which serves as the template for synthesis of additional negative-sense genomic RNA. Transcription and replication of the genomic RNA is dependent on formation of ribonucleo-protein complex (RNP) consisting of the 15,462 nucleotide genomic RNA encapsidated by the the nucleocapsid proetein (NP), the closely associated phosphoprotein (P), and the large (L) polymerase protein (Hamid et al., 2007).

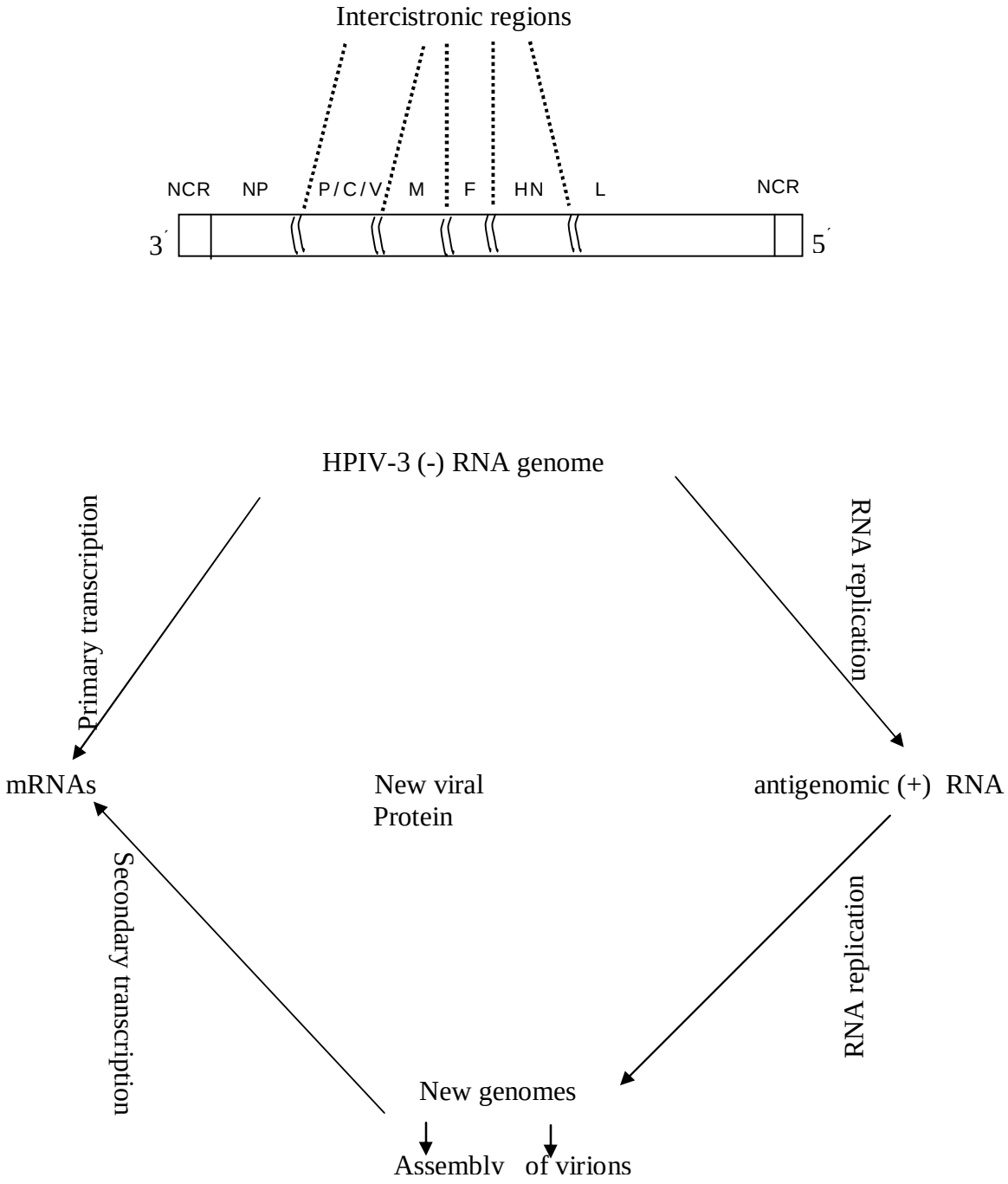


Figure1. Schematic representation of the HPIV-3 genome and the replication cycle.

2.3 EPIDEMIOLOGY OF HPIV-3 INFECTION

Parainfluenza viruses (PIVs) are responsible for 30-40% of all acute respiratory tract infections in infants and children worldwide (Santanu *et al.*, 2004). Human Parainfluenza virus type 3 has worldwide distribution. There is no age, sex, ethnic, socioeconomic or geographical predilection in the distribution of the disease. Many factors have been found to predispose individuals to HPIV-3 infections. These factors include malnutrition, over crowding, vitamin A deficiency, lack of breastfeeding, and exposure to environmental smoke or toxins (Reed *et al.,* 1997; Shek and Lee, 2003). Respiratory secretions from infected humans are the source of infection. Transmission is via respiratory droplets or via direct person–to–person contact with infected secretions or contact with contaminated surfaces or objects. The inoculating dose is very small (Ah-Type *et al.,* 1999). Human Parainfluenza virus type 3 infection occurs throughout the year, but the peak prevalence rate occurs in winter with outbreaks of respiratory tract infections. The infection also occurs earlier in life or in the elderly (Hamid *et al*., 2007).

2.4 PATHOLOGY AND PATHOGENESIS OF HPIV-3

Doctor's offices, hospitals, and chronic care facilities are institutions where respiratory viruses are frequently transmitted between patients. The populations at greatest risk for HPIV-3 infection are young children, the immunocompromised, and the elderly (Zambon *et al.*, 1998).

Children infected with HPIV-3 will transmit this virus to a minimum of 20% of uninfected control children residing on the same word (Mufson *et al.,* 1973; Kelly, 2003). About one–third of these will develop mild respiratory symptoms, but some will

experience serious LRI and even death (Downham *et al.,* 1974; Kelly, 2003). Serous sequelae are most common in patients with underlying medical problems.

Respiratory epithelium appears to be the major site of virus binding and subsequent infection. Viral transmission occurs via droplet, direct and indirect inoculation of contagious secretions from the hands or via large-particle aerosols into the eyes and nose. Prolonged survival of HPIV-3 on skin, cloth, and other objects emphasizes the importance of formites in nosocomial spread. The virus attaches to the host cells through hemagglutinin, which specifically combines with the cell membrane mediated by F_1 and F_2 receptors (Moscona, 2005). Infection of nervous tissue (olfactory) during intranasal challenge with Sendai virus has been suggested by studies in mice. Tissue tropism for HPIV-3 is known to be directed by the HN protein. *Human* Parainfluenza virus type 3 appears to be absorbed and released more extensively from the apical then the basolateral domains of AS49 cells. Viral antigen has been localized to the apical portion of epithelial cells in infected cotton rats (Porter et al., 1991). Actin and the cytoskeleton have been reported to play roles in transcription, maturation, and the movement of viral glycoproteins to the surface of the infected cells (Kelly, 2003) human Parainfluenza virus type3 needs functioning microtubules for efficient release (Bose *et al.,* 2001). When HPIV-3 infects a cell, the first observable morphologic changes may include focal rounding and growing of the cytoplasm and nucleus and decreased host- cell mitotic activity. Other observable changes include single or multilocular cytoplasmic vacuoles, basophilic or eosinophilic inclusions, and formation of multinucleated giant cells. These giant cells (fusion cells) usually develop late in the infection, and each giant cell contains

between 2 and 7 nuclei (Craighead and Brennan, 1968; Elizaga *et al.,* 2001; Hohenthal *et al.,* 2001; Laura *et al.,* 2007).

Persistent infection with active viral replication has been demonstrated both in vitro (tissue culture) and in Vivo (human infection) (Murphy *et al.,* 1991; Moscona and Peluso, 1991; Ah-Type *et al.,* 1999). The immune response in an infected patient clearly affects the clearance of HPIV-3 and the development of persistent infection (Defaritus *et al.,* 1979; Weintrub *et al.,* 1987). Both HN and F proteins have been shown to be involved with cell membrane fusion, but this appears to be virus (type) specific (Hu *et al.,* 1992; Moscona and Peluso, 1992). Furthermore, fusion may demonstrate different Kinetics depending on whether it takes place virus to cell or cell to cell (Moscona and Peluso, 1992). Human Parainfluenza virus type-3 host range and virulence are determined in part by the ability of host epithelial cells to enzymatically activate the F protein. Also, the neuraminidase activity of the HN protein may play role in altering muscarinic receptors, leading to vagus-induced bronchospasm, and can cause HPIV-3 infected cells to resist infection when challenged with HPIV-3 (viral interference) (Horg *et al.,* 2000).

The precise immune mechanisms and pathways involved in local cell injury and tissue damage are largely unclear. Human Parainfluenza virus type 3 specific immunoglobulin E (IgE) and histamine may contribute to disease severity in infants (Welliver *et al.,* 1982). During acute infection in guinea pigs, increased number of inflammatory cells can be found and airway hyperreactivity and decreased bronchoalveolar cellular superoxide production can be demonstrated (Folkerts *et al.,* 1990; Riedel *et al.,* 1996).

2.4.1 MECHANISMS OF AIRWAY INFLAMMATION

Human Parainfluenza virus type 3 infection in the respiratory tract leads to secretion of high levels of inflammatory cytokines such as interferon (IFN) - alpha, interleukin (IL)-2, IL-6, and tumor necrosis factor (TNF) alpha. The peak duration of secretion is 7-10 days after initial exposure. Increasing of certain chemokines such as regulated upon activation, normal T- cell expressed and secreted (RANTES), Macrophage inflammatory proteins (MIP)-K are detected in the nasal secretion of pediatric patients. These are responsible for pathological changes in the respiratory tract and clinical manifestations of this condition (Arden *et al.,* 2006). The chief pathological features include airway inflammation, necrosis and sloughing of respiratory epithelium, edema, excessive mucus production, and interstitial infiltration of lung. Edema of the mucus layer causes swelling in the vocal cords, larynx, trachea, and bronchi. These changes lead to obstruction of the airway inflow and subsequent strider, which is characteristic of croup (Gulen *et al.*, 2007). ,Human Parainfluenza virus type 3 infection in humans is known to induce expression of intercellular adhesion molecule-1 (ICAM-1) in the trachea and other cells with receptors for rhinoviruses, thus paving the way for rhinoviral superinfecfion (Arden *et al.*, 2006).

2.5 IMMUNITY

Host defense against HPIV-3 is mediated largely by humoral immunity to the two surface glycoproteins HN and F (Springgs *et al.,* 1987). Most children are born with neutralizing antibody to HPIV-3, but these titers quickly fall during the first 6 months of life because of the antibody decay. About two-third of children however, became expose to the virus in the first year of life, and by the age of 3-years, virtually all children will have serological evidence of infection with the virus (Durbin *et al.,* 2000). The antibody

response in most cases appears to involve serum immunoglobulin G_1 (IgG_1). Other immunoglobulin like serum immunoglobulin G_3 (IgG_3), immunoglobulin G_4 (IgG_4), Serum immunoglobulin A (IgA), and immunoglobulin M (IgM) had been reported to be high in about 30% of adults. Secretary IgA is said to plays an important role in the protection against natural HPIV-3 infections (Fry *et al.*, 2006) but the specific role in that protection has not been fully defined. Immunity to infection with HPIV-3 usually occurs after most children and adults develop measurable levels of serum antibodies; these antibodies have been shown to be correlated with disease prevention and amelioration in adults. Local interferon production has been noted in about 30% of children with HPIV-3 infection.

Although immunity to HPIV-3 infection is long-lasting, reinfection may occur many times throughout life and at variable intervals even in the presence of neutralizing antibodies. This can not be explained merely based on the relatively stable antigenic determinants of HPIV-3; thus, some workers advocated for further research (Jawetz, *et al.*, 2007).

In recent years, interesting facts regarding cell-mediated immunity have emerged (Plotnicky-Gilquin *et al.*, 2001) as infections tend to be more severe in individuals with defective cell-mediated immunity; thus indicating that T cells may have a greater role in containing the disease (Kimmel *et al.*, 1982). The human cytotoxic T-lymphocyte responses also appear to be important in the clearance of virus from the lower respiratory tract during infections with HPIV-3 (Henderson, 1979; Kimmel *et al.*, 1982). Alpha and beta T cells appear to play a larger role in cell-mediated viral clearance than do gamma,

delta or natural Killer cells, although all may be involved in the process (Hogan *et al.*, 2001; Mandelboim *et al.,* 2001). The human cytotoxic T-lymphocyte response has been shown to be directed to determinants on the HN, P, and NP proteins of HPIV-3 (Cole *et al*., 1994). However, the viral epitopes important in stimulating this line of defense are still largely unknown. Cytokines produce by CD4 and CD8 T cells have been found during acute lower respiratory tract infection caused by Sendai virus in a mouse model (Hogan *et al.,* 2001). Interleukin-2 (IL-2), gamma interferon, tumor necrosis factor, IL-6 and IL-10 were all detected in the lungs of the mouse (Mo *et al.,* 1995). Human peripheral blood leukocytes stimulated by Sendai virus have similarly been shown to produce macrophage inflammatory protein-1 alpha, monocyte chemotactic protein 1, tumor necrosis factor alpha, IL-6, and IL-8 (Hua *et al.,* 1996). Interferon may inhibit HPIV-3 primary transcription (Choudhary *et al.,* 2000). Human Parainfluenza virus type 3 may also inhibit interferon-mediated pathways in T cells, including major histocompatibility complex class II antigen expression (Gao *et al.*, 2001).

One of the many inflammatory mediators produced by HPIV-3 that stimulate T-cells is IL-10. This cytokine causes T cells to decrease their proliferation response and help protect them from virus-mediated apoptosis (Sieg *et al.,* 1996). Children with upper respiratory tract infection caused by HPIV-3 produce IL-11, which correlates with clinical bronchospasm (Einarson *et al.,* 1996). There is no permanent immunity to upper respiratory tract infection caused by HPIV-3 ever develops, and infections continue to occur throughout life (Kelly, 2003).

2.6 CLINICAL MANIFESTATION OF HPIV-3 INFECTION

Clinical manifestations of HPIV are variable ranging from asymptomatic infection to those with severe presentation. In the symptomatic cases, they usually manifest with respiratory symptoms (pneumonia and bronchiolitis). In few cases some children however may present with extrapulmunary manifestation. The incubation period following infection with HPIV-3 is 3-7 days. After the Signs and symptoms similar to those of other respiratory diseases: includes fever, nasal discharge, reddened throat with little or no exudates, chills and muscle pain developed (Elizaga *et al.,* 2001; Specter *et al.,* 2001; Matsuse *et al.,* 2005; Mao *et al.,* 2008). Other clinical presentation includes noisy breathing/wheezing, hoarse cry,change in phonation, barking cough, rattling felt over the chest or back, decreased appetite, irritability, vomiting and diarrhea are usually observed (Subhash and Thomas, 2007). Cough and nasal discharge occur in 80% for up to 3 days before the onset of the infection. Inspiratory rhonchi are heard frequently throughout the lung fields in those with bronchiolitis. Radiological examination however may be usually normal. In uncomplicated cases occasionally the epiglottis is grossly swollen and reddened (Henrickson, 2003; Iwane *et al.,* 2004; Weinbera, 2006; Mariana *et al.,* 2008). Laryngotracheobronchitis, pneumonia, and/or bronchiolitis in infants and young children may later manifest in severe cases. *Human Parainfluenza virus* type 3 has been shown in few cases to manifest with parotitis, aseptic meningitis, and encephalitis (Vreede *et al.,* 1992). Infection with HPIV-3 can also exacerbate the symptoms of chronic lung disease in children and adults (Jawetz *et al.,*2007). Infection can be particularly severe and persistent in immunodeficient children (Winther *et al.,* 2002;

Tsung-ven *et al.,* 2003; Meissner and Rennels, 2004; Segal *et al.,* 2005; Bourgeois *et al.*, 2006).

Bronchiolitis

The predominant symptom of brochiolitis in children includes fever, expiratory wheezing tacchypnea, chest wall retractions, rales and features in the lung air trapping. The peak incidence of bronchiolitis is in the first year of life (81%) of cases occurs during this period) and then dramatically declines until it virtually disappears by school age. Human Parainfluenza virus type 3 has been reported to be the most common cause of bronchiolitis with 10-15% of all cases in nonhospitalized children (Subhash and Thomas, 2007),with higher incidence in hospitalized patients (Counihan *et al.*,2001).

Croup (acute laryngotrcheobronchitis)

Croup is a generic term that encompasses a heterogeneous group of illnesses that affect the larynx, the trachea, and the bronchi. Children present with fever, a hoarse barking cough, laryngeal obstruction, and inspiratory stridor (Fry *et al.*, 2006). *Human* Parainfluenza virus type 3 accounts for about 10% of all cases of croup in children (Laurichesse *et al.,* 1999). It has also been demonstrated to cause severe croup in adults (Woo *et al.,* 2000).

Pneumonia

Human Parainfluenza virus type 3 causes about 10% of outpatient pneumonia cases, but with higher percentage in hospitalized patients. The symptoms of pneumonia include

fever, rales and clinical evidence of lung consolidation on physical examination or chest X-ray (Marx *et al.,* 1999).

Tracheobronchitis

Human Parainfluenza viruses causes more than 25% of cases of tracheobronchitis with HPIV-3 been more commonly associated with tracheobronchitis than other HIPVs (Hu *et al.,* 2005).

2.6.1 HUMAN PARAINFLUENZA VIRUS TYPE 3 COMPLICATION

Infection with HPIV-3 may lead to the following complication depending on the immune status of the individual.

Immunocompromised Host

Immunocompromised children and adults appear to be particularly susceptible to developing severe and fatal lower respiratory tract infection with HPIV-3.

Complication associated with HPIV-3 infection in immunocompromised patients has been noted which include the following:

Giant-Cell Pneumonia and Persistent of HPIVs Infection and Excretiom

It is found to be associated with giant-cell pneumonia in severe combined immunodeficiency syndrome (SCIDS), and acute myelomonocytic leukemia. Giant cell pneumonia was also reported in patient who had bone marrow transplantation who developed HPIVs infection (Little *et al.,* 1981; Weintrub *et al.,* 1987; Henrickson, 1999; Hohenthel *et al.,* 2001). Persistent respiratory tract infection and excretion of HPIV-3 have been described in patients with SCIDS (Gross *et al*.,1973; Joseph *et al*., 1988;

Henrickson, 1999), DiGeorge syndrome after a thymic transplant, and in children infected with human immunodeficiency virus- infected children (Beard *et al.,* 1993; King, 1994).

Interstitial Pneumonia

Between 5% and 7% of adult with BMT and leukemia patients become infected with HPIV-3 (Nicholos *et al.,* 2001). Interstitial Pneumonia was reported to occur commonly in patient who had solid organ transplant and/or BMT who became infected with HPIV-3 (Laughlin *et al.,* 1979; Ljungman *et al.,* 1989; Apalsch *et al.,* 1995; Lewis *et al.,* 1996). *Human Parainfluenza virus* type 3 has been the most frequent HPIVs. Those immunocompromised patients usually presented features of lower respiratory tract infection, fever and neutropenia especially in children with cancer (Sorkness *et al.,*1991; *Marx et al.,* 1999; Nicholas *et al.,* 2001). Approximately 24% to 50% develop pneumonia and 22% to 75% of this will die (Matsuse *et al.,* 2005).

A retrospective study found 41% of pediatric BMT patients with HPIV-3 infection (47% of viral respiratory infections) developed pneumonia and 6% died (Lujan-Zubermann *et al.,* 2001). It has been shown to cause serious lower respiratory tract infection and death (8%) in lung transplant recipients up to 5years after transplantation (Vilchez *et al.,* 2001).

Disseminated Disease

Immunocompromised patients have been found to have HPIV-3 infection in other nonrespiratory sites including cerebrospinal fluid (CSF), pericardial fluid, white blood cells, and liver. Postmortem cultures of the myocardium had also revealed features

inkeeping with myocardial involment by the virus (Baxter *et al.*, 1977; Fishaut *et al.*, 1989; Jose *et al.*, 2000; Santanu *et al.*, 2004).

Human Parainfluenza virus type 3 has been reported to cause parotitis in children with SCIDS and “common variable” hypogammaglobulenemia (Cullen and Baublis, 1980).

Central Nervous System

Human *Parainfluenza virus* type 3 has been associated with neurological diseases which include the following:

Guilliain-Barre syndrome

Human Parainfluenza virus type 3 has been isolated from the CSF of a patient with Guilliain-Barre syndrome (GBS) and adults with demyelinization syndromes (Roman *et al.*, 1978; Vreede *et al.*, 1992). The virus has occasionally been shown to cause aseptic meningitis in children (Wong *et al.*, 1988; Arguedas *et al.*, 1990) and adults.

Acute Encephalitis and Ventriculitis

Children hospitalized with HPIV have had significant problems with febrile seizures which about 17% are caused by HPIV -3 (Chiu *et al.*, 2001; Hamid, *et al.*, 2007).

Human Parainfluenza virus type 3 has been also isolated from the respiratory tract of children with acute encephalitis (Bitnun *et al.*, 2001).

In addition, several diseases have been linked to HPIV-3 by serology, including ventriculitis, and cluster headaches in an adult (Seidman *et al.*, 1996; Blanchord, 1998).

Otitis media

Human Parainfluenza virus types 3 routinely cause Otitis media, pharyngitis, and conjunctivitis, and these can occur singly or in combination with a lower respiratory tract infection (Heikkinen *et al.,* 1999; Winther *et al.,* 2002). High detection rate of HPIV-3 in the turbinate epithelial cells of post viral olfactory dysfunction (PVOD) patients suggest that HPIV-3 may be the causative virus of post viral olfactory dysfunction (Wang *et al.,* 2007).

Other Complications

Cardiovascular

Full-term and premature infants have developed apnea and bradycardia during HPIV -3 infection (Seidman *et al.,* 1996). Human Parainfluenza *virus* type 3 has been cultured from a 37-years old man with myopericarditis (Wilks and Bume, 1998).

Supraglottitis

Human *Parainfluenza virus* type 3 has been implicated in supraglottitis (Grattan-Smith *et al.,* 1998; Peramaki *et al.,* 1991).

Bronchiolitis Obliterans

Bronchiolitis obliterans has been observed in patients infected with HPIV-3 (Peramaki *et al.,* 1991).

Parotitis has been diagnosed in otherwise healthy children infected with HPIV-3 (Zollar and Mufson, 1970), protitis resulting from HPIV-3 infection was common with SCIDS and common variable hypogammaglobulinaemia in immunocompromised children (Wimbey *et al*., 1997), and in cystic fibrosis (Buckner *et al.*, 1972).

Bacterial Tracheitis

Croup caused by HPIV-3 has been associated with bacterial tracheitis (Edward *et al.*, 1983; Liston *et al.*, 1983; Cressman and Myer, 1994; Rittichier and Ledwith, 2000; Segal *et al.*, 2005).

Exacerbations of Nephritic Syndrome

Exacerbations of nephritic syndrome in children have been linked to viral respiratory infection in general (MacDonald *et al.*, 1986). Similarly children and adults with acute non-A, B, C hepatitis have had HPIV-3 like nucleocapsids found in their liver, with giant cells, and syncytia on tissue examination (Phillips *et al.*, 1999). It has also been cultured from a child who died with rhabdomyolysis (Ueda *et al.*, 1978). *Human Parainfluenza virus* type 3 has been cultured from throat and rectal cultures in an adult patient with bloody diarrhea, but other now common gaetrointetinal pathogens were not sought (Kelly, 2003).

Rejection episode

Human Parainfluenza virus type 3 has been associated with acute rejection episodes in renal and liver transplant recipients (Defabritus *et al.,* 1979; Herzog *et al.,* 1989; Hohenthal *et al.,* 2001).

2.7 DIAGNOSIS OF HUMAN PARAINFLUENZA VIRUS TYPE 3

Diagnosis of HPIV-3 infection depend on both clinical and laboratory diagnosis.

Clinical Diagnosis

Some clinical signs and symptoms (example croup, running nose and cough) are frequent in infection cause by HPIV-3 than in other respiratory infections but these are unreliable as many other agent with similar features. Specific etiological diagnosis always requires detection of infectious virus or virus components in clinical samples or serological response.

Laboratory Diagnosis

Proper diagnosis of etiological agent of an infection always requires adequate collection and preparation of clinical specimen. The nasopharynx and oropharynx are primary locations of initial HPIV-3 replication. Virus is shed at high titer early in an infection and then sharply declines. However, HPIV-3 has been isolated in children from as early as 6 days before up to 6 weeks past the first symptom. Throat swabs, nasopharyngeal swabs, nasal washings, and nasal aspiration have all been used successfully to recover HPIV-3 (Glezen *et al.,* 1984; Frayha *et al.,* 1989; Malinowski and Hostoffer, 2001). Nasal washings and aspirate in few studies are associated with high rates of viral recovery (HPIV-3) (Wong *et al.,* 1982); these methods are recommended even in young infants for optimal virus isolation. Human Parainfluenza virus type 3 lose infectivity rapidly when

the temperature rises above 4 to 8^0 C. Specimens (swabs or 2 to 4 ml nasal wash or aspirates) should be collected and placed in viral transport medium (2 to 3ml), either veal infusion broth or minimum essential medium supplemented with some protein source (not serum) such as 0.5% bovine serum albumin. The transport medium should contain antibiotics and antifungal agents to decrease contamination and be buffered to yield a PH of 7.5 to 8.0 after addition of the clinical sample (Kelly, 2003). The specimen in transport medium should be kept at 4ºC until tissue culture inoculation. When delay of more than 24hr is anticipated, the specimen should be frozen. Centrifugation at 1,000 revolutions per minute for 5 minutes prior to inoculation is also helpful in removing debris (Wong *et al.,* 1982).

Human serum can also be use for viral isolation or detection when serum samples are required, the first serum sample (acute phase) should be collected as soon into the person's viral illness as possible. The second serum sample (convalescent phase) should be drawn when the peak antibody production occurs (Kelly, 2003).

Viral Detection and Isolation

Human Parainfluenza virus type 3 where best grown in primary monkey kidney (PMK) cells. The most popular cell line for growing HIPV-3 was rhesus MK cells; however, this cells line is not always the most sensitive (Frank *et al*., 1973). Cynomolgus and African green Monkey cells lines are also used. There is an easy-to use secondary cell line (LLC-MK2) which is excellent for continued passage and almost as good as PMK cells for primary isolation. LLC-MK2 cells need trypsin (2-3 Ng/ml) added to the maintenance medium to recover HPIV-3. Trypsin may be helpful even for PMK cell lines. Human

Parainfluenza virus type 3 is detected 10 days or longer after inoculation. PMK cells may start to degenerate between 10 and 14 days, especially if trypsin is in the medium (Kelly, 2003).

Cytophatic effect (CPE) is rarely demonstrated during primary isolation of HPIV-3 in tissue culture, and so this is not a reliable or useful method to detect positive cultures. Hemadsorption (HAD) of guinea pig red blood cells directly onto the tissue culture monolayer is the most common method to detect the presence of HPIV-3. It is detected in only longer than 3 days, of inoculation. (Frank *et al.,* 1973). Human Parainfluenza virus type 3 being the most aggressive is able to destroy more than 50% of tissue culture monolayer almost by 3 days.

Electron Microscopy

Microdrops of Secretions or garglings are placed directly on carbon-coated electron microscopy grids and stained with phosphotungstic acid. Virions typical of the *Paramyxoviridae* (HPIV-3) may be observed. However, this study yields poor sensitivity (Arden *et al*., 2006).

Immunofluorescense

Indirect Immunofluorescence is normally used with antisera against HPIV-3; however, Immunofluorescence finding cannot be used as the sole diagnostic criterion, as it had a poor sensitivity. Human Parainfluenza virus type 3 was detected in only 31% of virus-Culture-Positive throat secretions using immunoflourescense technique (Arden *et al*., 2006).

Detection of Viral Antigens

Direct detection of Human Parainfluenza virus type 3 antigen in specimens can be carried out using enzyme-linked immunoassay (ELISA), radioimmunoassay, fluoroimmunoassay, and Immunofluorescence. Antigens may be detected in exfloated nasophryngeal cells. Synthesized recombinant HPIV-3 nucleocapsid proteins in the yeast *Saccharomyces cerevisiae* are used as sources of antigens for the detection of HPIV-3 (Juozapaitis *et al.,* 2008). The test is rapid and specific but less specific than viral isolation (Jawetz *et al.,* 2007). Shell vial assay is another method for average sensitivity of 84% in testing against standard tissue culture- positive HPIV-3 cases.

Serologic Diagnosis

A 4-fold rise or drop in titer is generally thought to signify acute infection if the testing is performed at the same time on paired acute and convalescent- phase serum samples. Hemagglutination inhibition, neutralization, complement fixation, ELISA, radioimmune assays, and Western blotting are frequently used antibody-based serological tests for diagnosis of HPIV-3 infections (Mariana *et al.,* 2008).

Genomic Detection

Human Parainfluenza virus type 3 RNA can be detected directly with Northern hybridization or a dot blot analysis using virus- specific DNA probes. Polymerase chain reaction (PCR) assay is sensitive and specific in detecting HPIV-3. A multiplex reverse-transcriptase PCR (RT-PCR*)* assay for detecting HPIV-3 has been developed *(*Puppe *et al.,* 2004). Reverse Transcriptase PCR enzyme hybridization assay (RT-PCR-EHA) is

available for detecting HPIV-3. The reported sensitivity was 95-100% and specificity was 97-100% compared to the results of tissue culture. The RT-PCR-EHA is also fast as it yield results in approximately 7 hours (Specter *et al.,* 2001). A multiplex RT-PCR assay kit under dual priming oligonucleotide system (DOP) that is used to detect 12 common viruses causing respiratory tract infections in children has recently become available at Korea (Kate *et al.,* 2004; Yoo *et al.,* 2007; Marshall *et al.,* 2007; Lam *et al.,* 2007).

Other Ivestigations

Other investigations that can help diagnose HPIV-3 infections include:

Complete Blood Count

The complete blood count is usually within the reference range. Lymphocytosis may be present (Arden *et al.,* 2006).

Imaging Studies

In the present time other approaches has been developed for diagnosis of HPIV-3 infection. These include the following:

i) Radiographs of the neck or chest are important if epiglottitis, croup, or pneumonia is a possibility (Pierangeli *et al.,* 2007).

ii) Anteroposterior views of the neck may demonstrate subglottic swelling with narrowing of the air shadow of the trachea (steeple sign) with croup; in patients with epiglotttitis, views may demonstrate enlargement of the epiglottis and ballooning of the lypopharynx (Hamid *et al.,* 2007; Mariano *et al.,* 2008).

Histological Findings

The epithelium of the respiratory tract may show inflammation and necrosis. Subglottic tissues in particular may appear to be involved (Iwata, 2005).

2.8 MORTALITY OF HPIV-3

Mortality associated with HPIV-3 is usual in developed countries, and is seen almost entirely in young infants, the immunocompromised, and the elderly. However, the preschool populations in developing countries have considerable risk of HPIV-3 induced death either due to the primary viral disease or by facilitating secondary bacterial infections in malnourished children (Berman, 1991; Gulen *et al.*, 2007).

2.9 TREATMENT

Treatment of infection cause by HPIV-3 utilizes substances found to have an in vitro antiviral activity against the virus which include neuraminidase inhibitors (example Zanamivir), protein synthesis inhibitors (puromycin) nucleic acid synthesis inhibitors, benzthiazole derivatives, 1,3,4-thiadiozol-2-ylcyanamide, carbocyclic-3-deezaa-denosine (Cc3Ado), ascorbic acid, calcium elonate, and extracts of *Sanicular europaea* leaves (White *et al.*, 1986; Greengard *et al.,* 2000; Murrell *et al.,* 2001; Porotto *et al.,* 2001). Potent inhibitors of HPIV-3 syncytium formation in tissue culture have been found by screening hundreds of small synthetic peptides. These peptides, which are found in a specific region on the F protein, may provide a novel approach to the development of antiviral therapies for HPIV-3 (Lambert *et al.,* 1996; Ghosh and Shae, 1998; Postey *et al.*, *2000*). Ribavirin has both in vitro and in vivo activity against HPIV-3 (Sidwell *et al.*, 1975; Browne, 1981; Stankova *et al.,* 2007). Furthermore, there have been anecdotal reports of decreased HPIV-3 shedding and clinical improvement when infected immunocompromised patients were treated with aerosolized and oral ribavirin (Sparrelid

et al., 1997; Charkrabarti *et al.,* 2000; Chakrabarti *et al.,* 2001; Malinowski and Hostoffer, 2001; Stankova *et al.,* 2007). However a review of Fred Hutchinson cancer centre demonstrated that established HPIV-3 pneumonias responded poorly to aerosolized ribavirin (Nichols *et al.,* 2001).

Corticosteroids

Numerous studies, as summarized in a recent meta-analysis, have demonstrated that oral or systemic steroids are effective at improving symptoms of croup as early as 6 hours after treatment (Somani and Evans, 2001; Donaldson *et al.,* 2003; Segal *et al.,* 2005; Tanaka *et al.,* 2006; Weinberg, 2006).

Antibiotics are used only if bacterial complications (example Otitis, Sinusitis) develop. Corticosteroids are used to treat respiratory symptoms (Cruz, *et al., 1995),* and to help reduce the inflammation and airway edema of croup (James, 2003; Segal *et al.,* 2005; Wang *et al.,* 2007)

Prednisone, prednisolone, and dexamethasone are commonly used glucocoticoids. Dexamethosone, because of its high potency and prolonged intramuscular half-life, is the preferred anti-inflammatory drug for croup (Donaldson *et al*., 2003).

Sympathomimetics

Epinephrine solution causes alpha-adrenergic-receptor mediated vasoconstriction of oedematous tissues, thereby reversing upper airway obstruction. It is highly effective when delivered via nebulizer. Nebulizers are air-or oxygen-powered devices that deliver

medications directly to mucosal surfaces of respiratory tract and smooth muscles (Prendergast *et al.*, 1994).

2.10 PREVENTION

Prevention of HPIV-3 infection involved contact isolation precautions which are necessary to manage nosocomial outbreaks of HPIV-3 infection (Stankova *et al.,* 2007). These include restriction of visitors, isolation of infected patients, and gowning and hand washing by medical personnel (Jawetz *et al., 2007)*.

Both subunit vaccines and live attenuated type 3 virus vaccine are being tested. Field trials of formalin- killed whole HPIV-3 vaccines failed to protect children against natural infection in late 1960_s (Chin *et al*., 1969; Fulginitis *et al.,* 1969). The majority of children develop antibodies to HPIV-3, but the levels were considerably lower than those seen following natural infection (Mao *et al*., 2008). Current approaches to HPIV-3 vaccines include intranasal administration of live attenuated strains, subunit strategy using the HN and F proteins, recombinant bovine/human viruses, and strains engineered using reverse genetics (Craig, 2003; Greer *et al.,* 2007).

Antigenically and genetically stable attenuated strains of HPIV-3 have been developed by cold attenuated (CA). The stability of these CA vaccine strains is enhanced because of multiple markers of attenuation in tissue culture (Belshe and Hisson, 1982; Bially *et al*., 2000). Higher–passage CA HPIV-3 strains demonstrated immmunogenicity and attenuation in younger infants, but symptom still developed. Finally, after further attenuation, a passage of CA HPIV-3 was found that demonstrated good infectivity, immunogenicity, attenuation (no disease), and stability (Belshe *et al*., 1992; Karron *et al.*,

1996; Durbin *et al.*, 1999; Skiadopoulos *et al.*, 2000). In addition, CA HPIV-3 appears to be more immunogenic than BPIV-3 (Schmidt *et al.,* 2000).

Protease activation mutants represent another approach to developing attenuated strains of HPIV-3. Infection with HPIV-3 requires that F protein be cleaved by a trypsin like protease (Craig, 2003; Pierangeli *et al.,* 2007). The envelop glycoproteins (HN and F) of HPIV-3 have been cleaved from whole virus to create subunit vaccines that have demonstrated efficacy in rats, lambs, mice, and hamsters (Ambrose *et al.,* 1991; Ray *et al.,* 1985; Surman *et al.,* 2007). Different routes of immunization have been used to develop systems able to evaluate current like HPIV-3 vaccines in terms of specific mutations and hopefully introduce specific mutations into wild-type strains in the future (Durbin *et al.,* 1997; Hoffman *et al,* 1997). An example of this is the production of a recombinant HPIV-3 that contains the HN and F for HPIV-1 and the specific CA mutation found in the L gene of HPIV-3 (Tao *et al.,* 1999).

CHAPTER THREE

3.0 METHODOLOGY

3.1 **Study Design**

The study was a descriptive cross-sectional study. Children 12 – 60 months of age were studied; they were screened for natural infection using questionnaire.

3.2 Preliminary Investigation

The selected clinics were visited to discuss the feasibility of the study. The consent and cooperation of the management, clinicians (pediatricians) and laboratory staff was obtained. Ethical clearance was also obtained from the Ethical committee of ABUTH, Zaria.

3.3 Study Area

The study area covers Zaria metropolis comprising Zaria and Sabon Gari Local Governments areas located 84km from the Kaduna State capital.

Zaria Local Government has a population of about 369,800 inhabitants (National Population Census, 1991). It is located on longitude and latitude $9^{0.}$ It is an urban set up bounded on the east by Soba Local Government, on the west by Giwa Local Government, on the north by Sabon Gari Local Government, and on the south by Igabi Local Government.

Sabon Gari Local Government is an urban set up with a population of 350,000 inhabitants (population census, 1991). It is along longitude 8^0 and $9^{0,}$ latitude 10^0 and $11^{0.}$ It is bounded by Soba Local Government on the east, Giwa Local Government on the west, Makarfi Local Government on the north, and Zaria Local Government on the south.

3.4 Sample Size

There was no known records of any research on Human *Parainfluenza virus* type 3 infection carried out in this state, as such in the calculation of sample size a prevalence rate of 50% was used to calculate the sample size with the equation below (Lwanga and Lameshow,1991; Sarmukaddam and Garad, 2006)

$$n = \frac{Z^2 pq}{L^2}$$

Where ‘n is the sample size

'Z' is the standard normal distribution at 95% confidence interval =1.96

'p' is the prevalence rate, which is taken as 50% =0.5

'q' is 1-P =1-0.5=0.5

'L' is the allowable error, which is taken as 5% =0.05

Therefore,

$$n = \frac{(1.96)^2 x 0.5 x 0.5}{(0.05)^2}$$

= 384.16

384 blood samples were collected from General out Patient Department ABUTH, Institute of Child Health Banzazzau and Sabon Gari Primary Health Clinic. 77 samples were collected from General Out Patient Department ABUTH, 205 samples were collected from Institute of Child Health Banzazzau Clinic and 102 samples were collected from Sabon Gari Primary Health Clinic. The number of samples collected from each hospital was on the basis of statistical data of patients attendance for each hospital obtained from Medical Record and Statistic Department ABUTH.

3.5 Interview

Information was collected by means of specially designed proforma for this study. The informants were the parent or adult care givers of the children under study. The Age, Sex of the child, occupational and educational level of the parents were obtained and filled into appropriate section of the proforma. History relating to presence of respiratory symptoms, parental smoking as well as sickle cell disease was also obtained and filled into appropriate section of the proforma.

3.6 Sample Collection and Processing

Hospitals were visited after obtaining ethical approval from ABUTH (appendix III). Children 1-5 years of age were all included in the study. The samples were collected only from children whose parent/guardian gave a written informed consent.

A dry sterile plastic syringe (2ml) with 23SWG (standard wire gauges) needle attached to was used for blood sample collection. Blood was collected by applying soft tubing tourniquet to the arm of the patient to enable the veins seen and felt. The punctured site was cleaned using methylated spirit and allowed to air dried. The needle was inserted to the selected straight vein with the bevel of the needle directed upward in the line of the vein. Steadily the plunger of the syringe was withdrawn until 2ml of blood was obtained. The tourniquet was released and the needle was removed from the punctured vein. Pressure was applied to the punctured site to secure homeostasis. The needle was removed from the syringe and the blood was transferred to a clean dried plain specimen bottle and labeled. The used syringes and needles were disposed appropriately.

The blood samples were centrifuged at 1500 revolution per minute for five minutes (1500 rpm/min for 5min) and the sera collected into a clean and dry plain specimen bottles using clean and dry Pasteur pipettes and stored at -20^0c until needed for analysis (Cheesbrough, 2000). All tests were carried out in the Department of Microbiology, Ahmadu Bello University Zaria. Using Enzyme Linked Immunosorbent Assay (HPIV-3 IgG SERION ELISA *classic*, INSTITUT VIRION\SERION, GmbH Germany), samples were assayed for specific IgG antibody against the HPIV-3. Manufacture's instructions were duly followed. The optical density (OD) values were read at 405nm, using Sigma Diagnostic EIA Multi well Reader II.

3.7 PRINCIPLE OF THE ASSAY

The HPIV-3 IgG antibody test kit is on the principle of the enzyme Immunoassay (EIA). HPIV-3 antigen is bound on the surface of the micro titre strips. Diluted patient serum or ready to use standard are pipetted onto the wells of the micro titre plate. Binding between the IgG antibodies of the serum and the immobilized HPIV-3 antigen takes place. After one hour incubation at 37ºC, the plate is rinsed with wash solution, in order to remove unbound material. Then ready to use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes at 37ºC, inducing the development of blue dye in the wells. The color development is terminated by the addition a of stop solution, which changes the color from blue to yellow. The resulting color is measured spectrophotometrically at the wave length of 405nm. The concentration of the IgG is directly proportional to the intensity of the color.

3.8 ASSAY PROCEDURE

The procedures according to manufacturer's instruction were used to carry out the assay. Sufficient amount of microtitre wells for the standards, controls; samples as well as substrate blank were prepared in duplicate. About 10µl each of diluted samples and ready-to-use standards and controls respectively were pipetted into the wells. The first well was left empty as blank, mixed by gentle shaking and incubated for one hour at 37ºC. The wells were emptied after incubation and washing solution was added into the wells. This procedure was repeated three times. The left over of the washing solution was afterwards removed by gentle tapping of the microtitre plate on a clean tissue paper. Approximately 100µl each of ready-to-use conjugate reagent was added into the wells. The substrate blank was left empty. The plate was incubated at 37ºC for 30 minutes, the same procedure for washing was followed, and 100µl each of the ready-to-use substrate was pipetted into the wells. This time the substrate was pipetted into the blank, incubated at 37ºC for 30 minutes. 100µl of stop solution was finally pipetted into the wells to terminate the substrate reaction. The plate was thoroughly mixed and the bottom wiped, the reading of the absorption at 405nm was made using ELISA reader machine (SIGMA DIAGNOSTIC).

3.9 CALCULATION, INTERPRETATION AND QUANTITATIVE ESTIMATION OF HPIV-3 IgG RESULT

The mean values for the measured absorption are calculated after subtraction of substrate blank value. The calculated absorptions for the sera are compared with the value for the cut-off standard. If the value for the sample is higher than the cut-off value, there is a positive result. For a value below the cut-off standard, there is a negative result.

For a quantitative evaluation of the absorptions of the standards and controls are graphically drown against their concentrations. From the resulting reference curve the concentration values for each patient sample were extracted in relation to their absorptions. Quantitative antibody titers < 65 IU/Ml were reported as negative and >65 IU/Ml as positive.

3.10 STATISTICAL ANALYSIS

The Chi-square was used at 95% confidence interval ($P< 0.05$) to test whether there is association between sex, age, respiratory tract symptoms and the presence of HPIV-3 antibodies among the study population. All statistical analysis was performed using (SPSS version 10). The results were expressed as descriptive study regarding the prevalence of IgG antibody to HPIV-3.

CHAPTER FOUR

RESULTS

Five (1.3%) blood samples out of the total 384 samples collected were observed to have lysed. Because of this 379 serum samples were tested for the presence of HPIV-3 antibody.

Of the 379 samples tested for HPIV-3 IgG antibody, 323 were positive giving an overall seroprevalence of 85.2% (table 4.1).

4.1 Distribution of HPIV-3 IgG Antibody by Age

Table 4.1 shows the seroprevalence of HPIV-3 IgG antibody among the studied population with respect to age. Of the total samples tested, 56 (14.8%) were aged 0-12 months, 117 (30.9%) were aged 13-24 months, 78 (20.6%) were aged 25-36 months, 54 (14.2%) were aged 37-48 months and 74 (19.5%) were aged 49-60 months. The seroprevalence was highest (26.1%) in age group 13-24 months and lowest in age group 0-12 months (7.4%). The result shows increased seropositivity with age. $X^2 = 71.69$, $P < 0.05$.

Table 4.1: Distribution of HPIV-3 Antibody by Age

Age (months)	Number of Children Tested	Number of Seropositive	% seropositive Per Each Age Group	% seropositive for Overall Total
0-12	56	28	50.5	7.4
13-24	117	99	84.6	26.1
25-34	78	75	96.2	19.8
35-46	54	50	92.6	13.2
47-60	74	71	95.9	18.7
Total	379	323		85.2

X^2 =71.69, P value =0.000 at 95% CI

4.2 Profile of Human Parainfluenza virus Type 3 Antibody Titers According to Age Group

Table 4.2 indicates that 56 (14.78%) of the children had no detectable HPIV-3 IgG antibodies with a titer less than 65 IU/ml while 67 (17.78%) had a maximum antibodies level of 866-1065IU/ml, a level of 666-865 was found in 26 (6.86%), 466-665 IU/ml level was found in 38 (10.03%), level of 266-465 IU/ml was found in 50 (13.19%) and a relatively low level of HPIV-3 antibodies was found in 142 (37.47%) with a titer of 66-265IU/ml. The protection rate against HPIV-3 seems to be lower in children ages 1 year or less.

Table 4.2: Profiles of HPIV-3 Antibody Titers According to Age.

		Titers (IU/ml)					
Age (months)	No. of subjects	<65	66-265	266-456	466-665	666-865	866-1065
0-12	56	28	20	1	0	1	6
13-24	117	18	45	20	10	6	18
25-36	78	3	28	13	11	5	18
37-48	54	4	21	3	6	7	13
49-60	74	3	28	13	11	7	12
Total	379	56	142	50	38	26	67

Keys: IU = International Unit

<65 IU/ml IgG Antibody = Negative;

≥65 IU/ml IgGAntibody = Positive

4.3 Distribution of HPIV-3 IgG Antibody by Sex

Among the 379 children tested, 190 (50.1%) were males and 189 (49.9%) were females. Seropositivity was observed in 163 (43.0%) males and 160 (42.2%) of the females. This shows that there was slight male predominance over females, which was not statistically significant, (P value= 0.343). X^2 =0.09.

Table 4.3: Distribution of HPIV-3 Antibody by Sex

Sex	Number of children tested	Number seropositive	% seropositive
Male	190	163	43.0
Female	189	160	42.2
Total	379	323	85.2

$X^2 = 0.09$, P value= 0.343

4.4 Distribution of HPIV-3 Antibody by Symptoms of Catarrh/Running Nose

Of the total 379 children tested, 268 (70.7%) had symptoms of catarrh/running nose while 111 (29.3%) had no such symptoms. Seropositivity was observed in 229 (60.4%) of those with catarrh/running nose while 94 (24.8%) of those without these symptoms were seropositive. This is however not statistically significant ($X^2 = 0.097$, P value = 0.434)

Table 4.4: Distribution of HPIV-3 Antibody by Symptoms of Catarrh/Running Nose

Catarrh /R. Nose	No. tested	Seropositive	% seropositivity
Presence	268	229	60.4
Absence	111	94	24.8
Total	379	323	85.2

X^2 =0.036, P value =0.481

4.5 Distribution of HPIV-3 Antibody by Symptom of Cough

Out of 379 children tested 266 (70.2%) were having symptoms of cough while 113 (29.8%) were without symptom of cough. Seropositivity was observed in 219 (57.8%) of those with symptom of cough and 104 (27.4%) of those without symptom of cough. This was found to be statistically significant with a P value =0.009.

Table 4.5: Distribution of HPIV-3 Antibody by Symptom of Cough

Cough	No. tested	Seropositive	% seropositive
Presence	266	219	57.8
Absence	113	104	27.4
Total	379	323	85.2

X^2 =5.931 P value =0.009

4.6 Distribution of HPIV-3 Antibody by Sickle Cell Disease

Table 4.6 shows the seroprevalence of HPIV-3 IgG antibody among sickle cell children tested in the study. Of the total number of 379 children tested, 9 (2.37%) were with sickle cell disease and 370 (97.6%) were those that have no sickle cell disease. Seven (7) of those with sickle cell disease (1.85%) were observed seropositive. Although this was not statistically significant with P value =0.394. X^2 =0.406.

Table 4.6: Distribution of HPIV-3 Antibody by Sickle Cell Disease

Sickle cell	No. tested	No. Seropositive	% Seropositive
Presence	9	7	1.85
Absence	370	316	83.4
Total	379	323	85.2

X^2 =0.406 P value =0.394

4.7 Distribution of HPIV-3 Antibody According to History of Parental Smoking

Of the total 379 children tested in the study 46 (12.1%) were those with history of parental smoking and 333 (87.9%) were those without parental smoking. Seropositively was observed in 45, (11.9%) of these with parental smoking and 278 (73.4%) in those without parental smoking. It was found to be statistically significant with P value = 0.004. X^2 =6.602.

Table 4.7: Distribution of HPIV-3 Antibody According to History of Parental Smoking

Parental smoking	No. tested	Seropositive	% seropositive
Presence	46	45	11.9
Absence	333	278	73.4
Total	379	323	85.2

X^2 =6.602 P value =0.004

CHAPTER FIVE

5.0 DISCUSSION

Human *Parainfluenza virus* type 3 is a medically important respiratory pathogen with no predilection for any race and second only to Respiratory syncytial virus as a major cause of lower respiratory tract illness in infants and young children (Sigh-Nag *et al.*, 1990). They are responsible for 30 - 40% of all acute respiratory tract infections in infants and children worldwide (Santanu *et al.*, 2004). Although re infection in healthy older children and adults are typically less severe, serious lower respiratory tract illness caused by HPIV-3 has been reported among immunocompromised individuals (Legoff *et al*., 2005).

The present study found 323 (85.2%) of the subjects to be seropositive for HPIV-3 IgG antibodies. This is slightly above the finding of Mariana et *al* (2008) who demonstrated 83.76% prevalence in Brazilian children. This is not supprising because the two study were carried out around the same season (rainy season). It may also be attributable to the comparable living standard of the two study areas. It contrasts with the finding of Palern *et al* (2005) who reported (18.6%) prevalence in Cuban children even though the study was conducted around the same season of the year. The possible reason may be the differences in the living condition between Cuba and the present study area. The presence study showed that a significant number of children remain unprotected against HPIV-3 in Zaria.

The study also revealed an increase in seropositivity with age. This finding conforms to those of Laurichesse *et al* (1999), Hohenthal *et al* (2001) and Arden *et al* (2006) who demonstrated that HPIV-3 seropositivity increase with age. This shows that children of

older ages developed higher antibodies that protect them from severe form of subsequent infection with HPIV-3 which could be due to booster effect on the immunity of older children resulting from recurrent infection.

In assessing the sex distribution of HPIV-3 antibodies, seropositivity was observed to be slightly higher in males (43.0%) than in females (42.2%). This also agrees with the finding of Spring (2005) although the result was not statistically significant (P> 0.05). This could be due to the fact that HPIV-3 has no predilection for sex.

In assessing the symptom of catarrh/running nose distribution of HPIV-3 antibodies, seropositivity was observed to be higher (60.4%) in those with the symptoms which agrees with the work of Mariana *et al* (2008) which may due to the present of active infection, although the result was not statistically significant (P > 0.05).

This study also showed that seropositivity is higher in those children with symptom of cough (57.8%) than in those without the symptom (27.4%), which was found to be statistically significant (P< 0.05). This conforms to the work of Subhash and Thomas, (2007) who also showed statistical significant association between IgG seropositivity of HPIV-3 and respiratory tract infection symptom of cough. It could be due to an immune response by the host to clear the airways blockages cause by caused by inflammatory reaction due to the HPIV-3 infection.

It was observed from this study that out of 2.37% of the patients with sickle cell, 1.85% were seropositive subjects but this was not statistically significant (P > 0.05). This is in

contrast with the work of Welliver *et al* (1986) who reported association of HPIV-3 infection and sickle cell disease. However the lack of statistical significance could be due to fact that the number of sickle cell children in the study population is very small.

In this study, it was observed that children whose parents smoke have increased seropositivity of HPIV-3 infection ($P < 0.05$) which conforms with the work of Laura *et al* (2007) who reported environmental smoke or toxin to be one of the predisposing factors to HPIV-3 infection. It may be as a result of the ability of the smoke/toxin to erode the mucosa surface epithelia providing easy assess to the cell.

The profile of HPIV-3 antibody titers indicate that only 67 (17.78%) showed very high titers ranging between 866 – 1065 IU/Ml. It is clear that any detectable HPIV-3 IgG antibody is an indication of immunity. This high IgG level may be due to the booster effect as a result of continuous re-infection with the HPIV-3. Some children with low protective HPIV-3 such as those with titer less than 65 IU/Ml which form (14.78%) of the studied population may stand the risk of severe HPIV-3 infection. Higher number of children from the population with IgG antibody titers between 866 – 1065 IU/Ml was observed in age groups 1 – 2 and 2 – 3 respectively, which suggest previous exposure of the individuals to HPIV-3 infection or acute infection.

CONCLUSION

The present study has revealed the importance of HPIV-3 as an etiologic agent of Respiratory tract infection in Zaria children. The study emphasizes the need to rapidly diagnose viral respiratory infections for clinical management of infected children. It also provide epidemiological data that may be useful in control effort and vaccine trials, mostly in developing countries where less information regarding respiratory virus is available.

RECOMMEMDATION

In view of the high prevalence rate of HPIV-3 Seropositivity, it is recommended that attention should be given to this virus for proper management of patients with respiratory tract infection especially in children with symptoms of cough, within the age of 1-2 years and children who have history of exposure to environmental smoke/toxin from the parents.

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APPENDIX I