AN EPIDEMIOLOGICAL STUDY OF ACUTE RESPIRATORY VIRAL INFECTIONS IN CHILDREN IN IBADAN - NIGERIA

BY

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DEDICATION

DEDICATED

To GOD the Father, the Son and the Holy Spirit
in whom all things are possible.

CERTIFICATION

I certify that this work was carried out by Atanda Olayinka Olabode in the Department of Virology, College of Medicine, University of Ibadan, Ibadan.

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ABSTRACT

A survey for the aetiological agents of acute respiratory infections (ARI) was carried out in Ibadan in order to determine the relative importance of these agents in the epidemiology of acute respiratory diseases.

Three hundred and fifty nasopharyngeal aspirates and two hundred and eighty sera were collected from children suffering from acute respiratory infections at the University College Hospital (U.C.H.) and a community health centre at Idikan, both in Ibadan between 1985 and 1987 for detection of antigens and antibody to six respiratory viruses namely:- Respiratory Syncytial Virus, Influenza A and B, Parainfluenza types 1 and 3 and Adenoviruses.

Antigen detection was carried out by an indirect fluorescent antibody technique. In addition, RSV antigens were detected in specimens by ELISA. Sera were assayed for antibody by complement fixation test.

Out of 350 children examined, 74 (21%) had at least one viral antigen in their nasopharyngeal aspirate. In all, 7% of nasopharyngeal aspirates tested were positive for RSV, 4%, 4%, 3%, 2% and 1% were positive for Influenza A, Parainfluenza 3, Parainfluenza 1, Influenza B and Adenovirus respectively. Of the

350 nasopharyngeal aspirates 230 were examined in ELISA for RSV antigen; 18 (8%) out of these contained RSV antigen. In a comparison of FA and ELISA tests for RSV antigens in 100 nasopharyngeal aspirates, 4% were positive by the two methods.

Of the 280 sera from children tested for complement fixing antibody for the 6 respiratory viruses, 167 (56%) were positive. The prevalence of complement fixing antibody to the individual viral antigens tested were as follows:- The highest prevalence of antibody was to RSV (20%) followed by Adenovirus 16%, Parainfluenza type 1, 4%. Only 1% of sera tested had Influenza A and B complement fixing antibody.

Statistical analysis showed that there was no significant difference in the prevalence of respiratory viral infections among the hospital based and community based patients ($P > 0.05$).

This study showed that viruses very important agents of acute respiratory infections in Ibadan with Respiratory syncytial virus as the most prevalent.

List of Abbreviations

ARD	Acute Respiratory Disease
ARI	Acute Respiratory Infections
C.F.T.	Complement Fixation Test
FUDR	5-Fluoro-2'-deoxyuridine
nM	Nanometer
IUDR	5-Iodo-2'deoxyuridine
Flu A	Influenza A
Flu B	Influenza B
Para 1,2,3	Parainfluenza types 1,2,3
RSV	Respiratory Syncytial Virus
CPE	Cytopathogenic Effect
ELISA	Enzyme Linked Immunosorbent Assay
NA	Neuraminidase
NP	Nucleoprotein
NPA	Nasopharyngeal Aspirate
HN	Haemagglutinin and Neuraminidase
HI	Haemagglutination Inhibition Test
NT	Neutralization Test
FAT	Fluorescent Antibody Technique
FITC	Fluorescein Isothiocyanate

CHAPTER ONE

INTRODUCTION

Acute respiratory infections represent an important cause of morbidity and mortality in developing countries, especially among the malnourished children. Poor sanitation and overcrowding play a role in this mortality. More than 40 million children die of pneumonia annually. This figure represents 30% of the 14.25 million deaths of children under 5 years of age that occur in the developing world each year (Gwatkin, 1980; W.H.O. HLTH. ORG. 1983; Leowsky, 1988). In India, 500,000-750,000 children die of acute respiratory infections each year (Steinhoff and John, 1983).

The aetiological agents of acute viral respiratory infections include Respiratory Syncytial virus (RSV), Influenza A (Flu A), Influenza B (Flu B), Parainfluenza type 1 (Para 1), Parainfluenza type 3 (Para 3) and Adenoviruses (Adeno). Some other viruses, bacteria and fungi are also known to cause respiratory infections (Berman and McIntosh, 1985). Syndromes produced by respiratory pathogens include the common cold, or upper respiratory illness, tonsillitis or pharyngitis, croup, tracheobronchitis, bronchiolitis and pneumonia. Fever, rapid breathing (tachypnoea), nasal flaring, intercostal recession, cough, cyanosis, grunting and failure to feed. The clinical

manifestations of a given acute respiratory infection reflect the extent of respiratory tract involvement and the severity of the infection (Berman and McIntosh 1985).

In Nigeria, there is little information on the aetiological agents of viral respiratory infections. However, David-West and Cooke isolated 13 strains of influenza A virus (A/Nigeria/1/73) during the 1974 influenza epidemics. These strains were shown by haemagglutination inhibition test to be closely related to A/Port Chalmer/1/73 virus. Antibodies to the epidemic strains developed rapidly in the population and 80%-95% of all age groups tested possessed high antibody levels.

In order to provide the much needed information on the epidemiology of acute viral respiratory infections in Nigeria, a survey for the causative agents of acute viral respirotory diseases was carried out in hospitalized patients as well as out-patients attending a community health centre.

AIMS AND OBJECTIVES

(a) To determine the prevalence of antibody to respiratory viruses, namely: Respiratory syncytial virus, influenza A, influenza B, parainfluenza type 1, parainfluenza type 3 and adenoviruses in the Nigerian children.

(b) To elucidate the role of respiratory viruses in te overall picture of respiratory diseases in hospitalized patients as well as those from the community.

(c) To determine the spectrum of clinical signs and symptoms associated with acute respiratory infections in Nigerian children.

(d) To compare results of Enzyme linked Immunosorbent Assay (ELISA) and the Fluorescent antibody technique (FAT) in the detection of respiratory syncytial virus.

CHAPTER TWO

LITERATURE REVIEW

Influenza

This is an acute infectious respiratory disease of man, commonly encountered in epidemic form, caused by one of the influenza viruses. Clinically, there is a sudden onset with fever, pharyngitis, cough, leukopaenia and severe aching. The course is usually self-limiting in 3 to 4 days. Complications by bronchitis and bronchopneumonia are frequent. Epidemics are characterized by rapid dissemination, high morbidity and low mortality; however, there may be pandemics and of such severity as to attain catastrophic proportions (Francis and Magoon, 1965). Three distinct immunological types of influenza virus, A, B and C have been classified according to the plan of Horsfall et al., (1940). Influenza A and B are the most frequently encountered and have been the best studied.

History

From 1510 to 1930, some 30 of the prevalences were clearly considered pandemic and numerous outbreaks of lesser extent were described. For example, between 1800 and 1875, Hirsh (1883) recorded 55 years in which influenza was epidemic in various parts of the world. The outbreak of 1743 was considered a

virulent pandemic, and Jordan (1927) stated that the actual number of deaths occurring in London during the peak of th epidemic was as great in proportion to the population, as it was in the American cities in 1918. It was in this epidemic that the name "Influenza" was derived from the Italian phrase attributing the origins of the disease to "un influenza di freddo". That of 1782 was a great pandemic in Asia and Europe with apparently a high frequency of complications (Mulder and Masurel, 1950). The entire history of influenza was climaxed by the greatest of the pandemics in 1918-1919 which resulted into the death of about 20 million persons. The extensive observation of that episode and efforts to explain it, has since then been the concern of students of respiratory disease and epidemiology. They have been reviewed by Thompson and Thompson (1933, 1934).

Shope, (1931) isolated Swine influenza virus while the first type A virus from humans was isolated by Smith Andrew and Laidlaw (1933). The first type B influenza from human was isolated by Francis (1940). Serological evidence suggested that the pandemics of 1918-1919 were caused by type A viruses (Davenport et al., 1969; Dowdle et al., 1974; Masurel and marine, 1973).

In the temperate zones influenza epidemics of either type occur mainly from late fall until spring but in the tropical

areas the season of prevalence is less well defined: however David West and Cooke (1974) described influenza epidemic during Harmattan and Wet seasons in Nigeria.

The epidemics of influenza A viruses are of veterinary, as well as a public health problem. Type A strains are found in horses, pigs and many species of birds both wild and domestic. Types B and C viruses have been isolated only from humans. Unpredictable antigenic mutability of influenza A and B viruses and the teroretical possibility that type A viruses may emerge in some form, from animal reservoirs have made the laboratory surveillance of these viruses an important component of early warning and defence against epidemic influenza (Doudie et al., 1977).

Clinical

Influenza is transmitted through inhalation of virus containing droplets, expelled from the respiratory tract of symptomatic or asymptomatic individuals. Crowding and environmental conditions during winter or harmattan may enhance transmission. The incubation period varies from 1-4 days but is usually 2 days. Influenza multiplies in the ciliated columnar epithelium of the upper and lower respiratory tract; causing cell

necrosis and sloughing. The greatest period of viral shedding occurs a day before to 3-4 days after the onset of illness. Reduced quantity of virus may be shed for 1 week or more.

Influenza may cause symptomatic infection ranging from a minor respiratory illness to fatal pneumonia. Malaise, fever and a mild sore throat or dry cough are typical signs during the first 2-4 days of illness (David-West and Cooke 1974).

Acute influenza virus pneumonia may give rise to alveolar fluid accumulation, hypoxia and death. Reye's Syndrome characterized by non inflamatory encephalopathy, fatty infiltration of abdominal viscera and elevated transaminase and ammonia concentrations may occur after influenza B infection (Corey et al., 1976) and less commonly after influenza A infection (Ruben and Michael 1975; Morris and Kilberg 1966).

Pathology

The pathology of uncomplicated influenza was described by (Mulder and Hess 1972) as patchy desquamation may occur in the ciliated columnar epithelium of the respiratory tract. In influenza pneumonia, virus is found in the epithelial cells of the distal bronchiolitis and alveoli which may be lined with hyaline membrane; the air spaces are filled with fluid, erythrocytes and leucocytes. Submucosa capillaries and

lymphatico may become distended, with accumulation of interstitial fluid and lymphocytes, (Mulder et al., 1972).

Characteristics

Influenza viruses contain a single-stranded RNA genome, enclosed within a viral modified host. (Pons, 1976; Ritchey et al., 1976). The largest 3 RNA segments contain the genetic code for the polypeptides (P_1, P_2 and P_3), believed to be required for virus specific RNA dependent RNA polymerase activity. The 3 intermediate sized segments of the viral genome contain the code for haemagglutinin, nucleoprotein (NP) and neuraminidase (NA) polypeptides; and the 2 smallest RNA segments contain the code for an internal structural virion protein (M protein) and a nonstructural (NS) popypeptide found only in virus infected cells, (Simmock, 1969; Buckler-White and Murphy (1986). Influenza B probably resemble Influenza A in these general properties. The precise information is lacking about the number of genes in Influenza C viruses or their coding assignments. Inflenza C contains haemagglutination and receptor destroying activities, these do not appear to have chemical specificity for sialic acids containing substrates demonstrated for Influenza A and B viruses (Kendal, 1975).

Morphology

Influenza viruses are pleomorphic, virions are spherical or filamentous with an approximate diameter of 100nm. The greatest degree of pleomorphism and aggregation is usually found with newly isolated viruses grown in eggs. Surface projection of usually 8-10nm long and spaced at 8nm intervals may usually be observed in influenza viruses examined by negative staining technique. Influenza C may, in addition possess a reticular structure on the surface, although this may not be reliably observed on newly isolated virions (Martin et al., 1977). Influenza A and B may be packages as a large coil, about 50nm in diameter and of variable length(Almeida and Waterson, 1970).

Classification

Influenza virus nomenclature is based on antigenic type and epidemiological information, including host, origin, place and year of isolation. For Influenza A viruses, an antigenic description follows the strain designation and indicates the antigenic character of the haemagglutinin and nucleic acid subtypes. For example, prototype strains for four human haemagglutinin subtypes are A/Perto Rico/8/34(H_0N_1), A Fort Monmouth/1/47(H_0N_1), A/Singapore/1/57(H_2N_2) and A/Hong Kong/1/68(H_3N_2). Additional 11 different haemagglutinin subtypes

and 7 different NA subtypes are recognized among type A viruses isolated from horses, pigs and birds. These are designated by host of origin such as A/equine/Prague/1/56(Heq 1 Neq1) an A/tern/South Africa/61(Hav Nav). Certain haemagglutinin and
1 2
Nucleic acid subtypes are shared by strains isolated from different species. Antigenic heterogeneity may be exhibited e.g. A/Hong King/8/68 and A/victoria/3/75 cross react by immunodiffusion and are both considered as H N strains, yet they
3 2
may be readily differentiated by haemagglutination inhibition (HAI) and neuraminidase inhibition tests (NI).

Type of Specific Antigens:

The nucleoprotein and the membrane or matrix protein associated with the inner structure of virion envelopes is also type specific (Pereira, 1969; Schild, 1970).

Strain Specific Antigens

Antigenic variation occurs in the surface antigens of virions. The variation is mediated by various determinants in the haemagglutinin and nucleic acid which are dependent antigens (Smith et al., 1933; Schulman and Kilbourne 1969). The degree of variation in Influenza A is greater than that of Influenza B while antigenic variation in Influenza C viruses has been studied for only few isolates (Pereira, 1969). There are, "antigenic

shift", when there is a sudden change in antigenic composition of influenza viruses and in the case of haemagglutinin, it is usually associated with pandemics such as in 1957 with the Asian H_2 and in 1968, with the Hong Kong (H_3) Viruses. More gradual changes in the antigens within a subgroup are described as "antigenic drift", which may or may not be associated with epidemics. Cross reactivities are recognized by haemagglutination inhibition tests and immunological priming studies (Davenport et al., 1957 and Dowdle et al., 1969).

Host Range

The developing chick embryo is the most widely used animal host for the growth of influenza viruses (Burnet, 1940. Ferrets are susceptible when inoculated intranasally. They produce an illness typical of human disease (Smith et al., 1933). Influenza type C has not been recovered from the lungs but only from the nasal turbins. Mice are susceptible when the source of the virus is mouse lung suspension (Shope, 1954). Influenza C has not been adapted to grow in mouse lungs. Hamsters and guinea pigs are susceptible to experimental infection with adapted strains of Influenza (Shope, 1954).

Influenza A Influenza B or C viruses in nature may have as their normal host avian, equine, swine, or procine species

(Pereira, 1969). Evidence has shown primates, cow and chicken to be susceptible to Influenza A viruses. (Kundin and Easterday 1972; Aymard et al., 1974).

LABORATORY DIAGNOSIS

Direct Examination of Clinical Material

Fluorescent antibody (FA) staining of respiratory epithelial cells first demonstrated by Liu (1956), is the only method of direct examination to gain general acceptance for documentary Influenza infections. The examination of intact cells that are free of mucus and present in sufficient number has shown a high correlation between FA staining and virus isolation (Kerr et al., 1975). Discrete, specific staining is visible in the cytoplasm or the nucleus depending on the stage of viral infection in each cell. The use of rapid methods of Influenza diagnosis for hospitalized patients may allow infected patients to be segregated from high risk patients or new-born nurseries, thus potentially decreasing nosocomial infection (Gardner, et al., 1974).

Virus Isolation

In general, Influenza viruses are reliably isolated in eggs and usually can be isolated in primary monkey kidney cells (PMK).

Swine influenza-like viruses affect man and can also grow in PMK cells (Smith et al., 1933).

Identification Method

All serological methods for identification of influenza viruses are geared towards those based on either reactions of components that are type specific or those based on components that are strain specific (i.e. nucleoproteins or haemagglutinins). Tests available for type components include complement fixation test (CFT), HI test and double immunodiffusion (Fabiyi et al., 1958; Davenport et al., 1969 and Dowdle et al., 1980).

Chemotherapy

Amantadine (1-adamantanamine) can inhibit an early step in the multiplication (uncoating) of some influenza viruses e.g. it provided 50% protection in a clinical trial with (H_2N_2) influenza. Its clinical usefulness is limited, however, because its therapeutic value has been less striking than its prophylactic effect, its effectiveness is restricted to influenza A virus alone. It has neurological toxic effects (particularly in the aged) (Davis et al., 1980).

Ribavirin (1β-D ribofuranosyl-1,2,4 triazole-3-Carboxamide,

virazole) which inhibits synthesis of viral RNA by blocking guanine biosynthesis has been more effective than amantadine in preventing experimental influenza A in cell cultures and animal models. Clinical trials suggest that it may be therapeutically effective (Davis et al., 1980).

Immunity

Blood Stream or Surface Protection

In virus infection, the efficiency of antibody depends largely on whether the virus passes through the blood stream in order to reach its target organ in which case the disease course has a long incubation period e.g. paramyxoviruses. In comparison with another group of virus diseases with a short incubation period such as the influenza viruses, they do not pass through the blood stream, so their target organ is their site of entry to the body, namely the respiratory mucus membranes. In this type of infection, even a high blood level of antibody will be relatively ineffective against these viruses in comparison with its effects on the blood born viruses; (Weir 1977; Davis et al., 1980).

Prevention and Control

Vaccination is the only highly effective measure of control

now available for the prevention of influenza. The short incubation period, the abrupt onset and the high infectiousness of influenza, together with the increased rate and speed of travel make application of isolation and quarantine measures difficult. The delayed introduction of influenza to Australia in 1918-1919 was considered to be an effect of quarantine by Cumpston (1919).

In 1943 a vaccine prepared in classic study with an alternate placebo controls conducted in Army Specialized Training programme units clearly showed that subcutaneous vaccination with concentrated, formaline-inactivated virus A + B from chick allentoic fluid prevented influenza A and influenza B epidemics (Meiklejohn et al., 1952). In 1947, a vaccine of the same composition with that of 1943 was found to be ineffective in an epidemic caused by the newly emergent A prime strain thus the significance of major strain variation in relation to vaccine induced immunity was convincingly demonstrated. The vaccine stimulated good antibody response to its component strains but not to the epidemic strains, while the disease stimulated antibodies to both. The question remained as to how much variation could be practically covered by a vaccine containing a

given strain. In 1949-1950 a PR8 vaccine appeared to have a mild influence, on an A-prime incidence (Meiklejohn et al., 1952). Suggestive effects were also noted with a polyvalent A vaccine containing no A_2 virus in 1957 (Gundelfinger et al., 1958).

The vaccine studies have been the most effective assessment of the immunological significance of serological variations (Francis, 1952). This information has been reviewed extensively by Francis, (1950, 1954). Since that time, studies have continued with varied formulae of monovalent and polyvalent vaccines.

Influenza A (H_1N_1) viruses were isolated in many parts of the world in the 1986-1987 season. (Masurel, et al., 1986). These isolates were all antigenically similar to A/Singapore/6/86. Consequently, it is recommended that vaccines for use in the 1987-1988 season contain an A/Singapore/6/86 like antigen. During the winter of 1985-1986 new antigenic variants of type A(H_1N_1) virus were detected, and although very few type A(H_3N_2) viruses have been isolated in 1986-1987 thus far, several appear similar to variants from 1985-1986. These isolated are very poorly inhibited by ferret serum to the A/Bangkok/1/79 strain (recommended for use in the influenza vaccines from 1980-1981 to 1984-1985), and are inhibited to significantly reduced

titres (compared to the homologous titre) by serum to A/Mississippi/1/85, the type A (H_3N_2) component of vaccine recommended for use in 1986-1987. However, sera to the above isolates react well with the A/Hissisippi/1/85 as with themselves. In view of the continued isolation of virus resembling the A/Stockholm-A/Leningrad variant, it is recommended that influenza vaccines for use in 1987-1988 season should contain a representative of this variant in place of A.Mississippi/1/85 (WHO, 1987).

Influenza B viruses were isolated infrequently during the 1986-1987 season. Strains isolated were antigenically heterogeneous but all were antigenically similar to B/Ann Arbor/1/86. Consequently, it is recommended that vaccines intended for use in the 1987/1988 should contain a B/Ann Abor/1/86-like antigen (WHO, 1987). WHO, (1987) recommended that influenza vaccines for use in the 1987-1988 season should be trivalent and contain the following antigens.

An A Singapore/6/86 (H_1N_1) - like antigen

An A Leningrad/360/86 (H_3N_2) - like antigen

An B/Ann Abor/1/86 - like antigen.

A considerable proportion of the population, with the exception of young children, is likely to have been infected with Influenza A(H3N2), Influenza A(H1N1) and Influenza B viruses in recent years. As a consequence, one dose of inactivated vaccine should be adequately immunogenic for individuals of all ages except young children. The latter, who may not have received influenza vaccine in the past may require two doses of vaccine, with an interval between doses of at least four weeks to ensure a protective antibody response to vaccination (Davis et al., 1980).

Parainfluenza

These are important respiratory tract viral pathogens first isolated from laboratory mouse (Sendai virus, a murine agent). Eventually, the types which infect man were recognized with the application of tissue culture and haemadsorption technique (Beale et al., 1956; Chanock 1956. Chanock et al., 1956; Johnson et al., 1960).

Parainfluenza share many properties with influenza viruses but differ also in many ways. Parainfluenza are larger than influenza viruses. They have a larger nucleoprotein containing double helix 18nm to the 9nm, for the influenza viruses and unlike the influenza viruses Parainfluenza are capable of

haemolysing certain types of erythrocytes (Waterson et al., 1961; 1962).

Parainfluenza viruses have common antigens which are not shared by influenza viruses. However, Mumps and newcastle diseases share antigenic properties with paramyxo viruses including antigenic relationship, (Cook et al., 1956; Dennis and Walker 1963; Vanderreen and Zonderkamp (1965). Alexander (1960) showed that the avian viruses, except for N.D.V., exhibit little if any antigenic relatedness to the human Parainfluenza viruses.

There are four distinct serological types which have been isolated from man. They are called Parainfluenza 1,2,3 and 4.

History

The first recognized influenza virus strain (Sendai or HVJ) was recovered in Japan from mice inoculated with lung tissue obtained at autopsy from infants with pneumonia (Kuroya et al., 1959). Mice in China, and Russia are commonly infected with this agent (Fukumi et al., 1959).

The first human case was isolated from infants with Croup (acute laryngotracheobronchitis) Chanock 1956; Beale et al., 1958). Previously, this syndrome had been called viral croup because of failure to recover pathogenic bacteria from most

patients (Rabe 1948). Parainfluenza viruses have been isolated from pigs (Johnson et al., 1960). Other Parainfluenza viruses have been recovered from other animal species. Simian virus (SV5) caused croup in dogs; Simian virus 41 (SV41) in monkeys and shipping fever virus (SFH) in cows (Hull et al., 1956; Chanock et al., 1961a; Abinanti et al., 1961). Each of the viruses recovered from these species is antigenically related to one of the human parainfluenza-virus types.

Characteristics

Under an electron Microscopy, parainfluenza viruses (1 and 3) are pleomorphic and vary in size from 120nm-800nm (Horne and Waterson, 1960; Waterson et al., 1961; Waterson, (1962). They are therefore larger than influenza viruses which by the same technique measure 00-120nm. The paroinfluenza virion consists of a coiled inner helical component surrounded by an envelope studded with numerous spike-like projection. The inner component is a single stranded helix with a diameter of 15 to 10nm and a hollow core of 5nm (Choppin and Stoeckenius, 1964). These viruses mature by budding from the surface of infected cells (Chanock and McIntosh, 1905). The chemical nature of the inner helix has not been determined by direct analysis; however,

failure of FUDR (5 fluoro-2-deoxyuridine) and IUDR (5 iodo-2-deoxyuridine) to inhibit replication of parainfluenza viruses (types 1,2,3 and 4) suggests that it consists of ribonucleoprotein (Choppin and Stoeckenius, 1964).

Effect of Physical and Chemical Agents

Paraflu viruses are usually unstable at 37°C and above. The rate of inactivation is markedly influenced by the composition of the suspending medium (Kamparian et al., 1963): Serum proteins tend to protect these viruses from heat inactivation. Parainfluenza viruses can be kept for several years at 60°C if 0.5% bovine albumin or 5% chicken serum is incorporated in the suspending medium.

The parainfluenza viruses are rapidly inactivated at pH 3.0 (Kamparian et al., 1963), infectivity is also completely destroyed by exposure to 20 percent ether for 18 hours at 4°C, indicating that lipid is essential for the integrity of the virion (Kamparian et al., 1963).

Epidemiology

Types 1-4 Parainfluenza viruses have a wide geographical distribution. Types 1, 2 and 3 have been identified in most areas, when appropriate tissue culture and haemadsorption

frequently and illness are usually mild and limited to the upper respiratory tract (Canchola et al., 1964).

Parainfluenza infection generally occurs very early in life and approximately 80% are infected by the age of 4 with para 3 (Chanock et al., 1963). Infection with type 1 and 2 generally occur later in life. Majority of children are infected with type 2 virus by 4 years of age and over 75% with type 1 virus by 5 years. Infection with type 3 occurs often in the 1st months of life while infant still possesses maternal antibody (Chanock et al., 1960).

The parainfluenza viruses are particularly troublesome as causes of infection in the hospital. Children who are admitted into the hospital for non respiratory tract illness are often infected during their hospital stay, and this is often associated with the development of serious lower respiratory tract disease (Mufson, et al., 1973).

Host Range

Naturally occurring strains of Parainfluenza viruses grow poorly or not at all in the embryoneted hen's eggs. Thus far, only 3 strains of parainfluenza type 2 have been recovered in eggs (Von Eufer et al., 1963). However, the tissue culture isolates of the 4 types have been adapted to grow in the

technique were applied to the study of childhood respiratory tract disease. (Chanock et al., 1960; Kapikian et al., 1963).

Each of the 4 Parainfluenza virus types can cause acute respiratory tract disease in human. The aetiological relationship is indicated by two observations. First, each of the virus types has been recovered significantly more often from patients with respiratory diseases than from individuals free of such illness (Cancholo et al., 1964; Chanock et al., 1961; 1958; 1963; Parrot et al., 1962; Johnson et al., 1960, Lewis et al., 1961). Second, types 1, 2, 3 and 4 viruses have produced upper respiratory tract infections and illness when administered to adult volunteers (Bloom et al., 1961).

The parainfluenza rank second to respiratory syncytial virus as important causes of lower respiratory tract disease in young children, and they commonly reinfect older children and adults to produce upper respiratory tract disease (Chanock et al., 1969; Glezen et al., 1978). Parainfluenza 1 is the principal cause of croup in children while para 3 is second to RSV in causing pneumonia and bronchiolitis in infants less than 6 months of age. Para 2 resembles para 1 clinically but serious infection is less frequent. Infections with para 4 virus are detected less

amniotic sac of 8-10-day-old eggs (Chanock, 1956; Chanock et al., 1958) Jensen et al., 1962; DeMelo, 1963).

When administered intranasally, parainfluenza viruses infect guinea pigs without producing overt disease (Cook et al., 1956; Johnson et al., 1960). Hamsters are also sensitive to intranasal infection with types 1,2, or 3 virus (Craighead et al., 1960; Cook and Chanock, 1963). Mice are also susceptible. Monkeys serve as natural host for type 2 (Hull et al., 1956).

Antigenic Composition

Specific antigens are present on the envelope of the Parainfluenza viruses. Types 1, 2 and 3 viruses appear to be antigenically homogeneous (Chanock et al., 1958, 1960 Canchola et al., 1964). The viral surface proteins HN and F are glyproteins. In vitro studies suggest that immunity to both the HN and F surface antigens play a role in resistance (Chanock and McIntosh 1985). The viruses appear to be antigenically more stable than Influenza A and B (Chanock et al., 1963; Spurrier et al., 1985). The Parainfluenza share related antigens. Despite antigenic relatedness, these viruses can be differentiated easily by complement fixation test, tissue culture neutralization test using post infection Guinea pig serum and haemadsorption

inhibition test. Homotypic serum titres are higher than heterotypic sera which are usually less than 1:10 (Cook et al., 1963; Spurrier et al., 1965; Van de Veen, 1965).

Diagnosis

Electron microscopy and immunofluorescence, using the nasopharyngeal aspirate have been used extensively in the diagnosis of the Parainfluenza viruses (Doane et al., 1967; Gardner et al., 1971).

Rhesus monkey kidney cell cultures are the preferred system for the recovery of Parainfluenza viruses (Canchola et al., 1964). Human embryonic kidney cell cultures are also suitable for recovery of types 1,2 and 3 viruses, but are considerably less sensitive (Wargocko, unpublished data). All four parainfluenza viruses produce intracytoplasmic, eosinophilic inclusion bodies in monkey kidney cells. These inclusion bodies are particularly striking when infected cultures are fixed in Bouin's solution and stained with haematoxylin and eosin (Johnson et al., 1960; Brandt, 1961).

Cultures infected with types 1-4 parainfluenza viruses are capable of haemadsorbing guinea pig erythrocytes. This property is fundamental to the recognition of naturally occurring strains of types 1,3 and 4 viruses (Chanock et al., 1961). In addition,

haemadsorption is more sensitive than cytopatic effect for detection of type 2 strains (Lewis et al., 1961).

Other test includes serological diagnosis such as CFT, and HI. The serological response of humans to Parainfluenza infection is generally less specific than that exhibited by infected animals such as guinea pigs or hamsters. In part, this appears to be because of exposure to members of Parainfluenza group and with mumps virus. It is unusual to find individuals after the first year of life without antibody to at least one of the Parainfluenza viruses (Parrott et al., 1962). Heterotypic complement fixation and haemadsorbing type antibody responses occur commonly following type 1 infection during childhood (Chanock et al., 1958; 1960).

An enzyme linked immunosorbent assay has been used for detecting Parainfluenza and preliminary results showed that it is more sensitive than the CF. test (Bishai and Galli, 1977).

Consequences of Infections and Effect of Antibody

Type 3 neutralizing antibody provides protection against both infection and illness (Chanock et al., 1961b). In addition, the length of time type 3 virus is recoverable from the pharynx is also inversely related to the level of pre-exposure neutralizing antibody (Chanock et al., 1959).

The risk of febrile disease during primary type 3 infection is 78 percent, and the risk of pneumonitis or bronchitis is 33 percent. Febrile illness occurs during one half of primary type 1 infections (Chanock et al., 1963). However, croup develops during only a small proportion of such infections (Chanock et al., 1963).

Reinfection of adults as well as children with parainfluenza viruses has been recognized on a number of occasions particularly with type 3 virus (Chanock et al., 1961b). Although neutralizing antibody confers some protection against subsequent infection, this protection is not complete (Chanock et al., 1961b). Illness usually occurs less often and is less severe during reinfection than during primary infection.

Prevention and Control

Reducing the attack rate of respiratory diseases is an important social and economical goal. Experimental vaccines have been prepared for type 1 (hominis) virus in eggs and for type 3 (bovis) virus in bovine kidney tissue culture (Jensen et al., 1962). Nevertheless, an effective vaccine evoking an antibody response in the respiratory tract, would be of value for young children, especially in hospitals and institutions (Ginsberg et al., 1980).

Respiratory Syncytial Virus (RSV)

Strains of respiratory syncytial virus (RSV) were first recognized in 1956 when the virus was isolated in Chimpazees with coryzal respiratory illness (Morris, et al., 1956). The natural habitat of the virus was the human respiratory tract, and it was observed that extensive syncytial areas developed in cell cultures infected with this virus. Hence the name 'respiratory syncytial virus (RSV)' was selected instead of "Coryze agent" as it used to be (Chanock et al., 1957).

Morphology

Respiratory syncytial virus ranges from 65nm to 300nm. These estimates were derived from sucrose density gradient centrifugation studies (30-120nm), from electron micrographs of sectioned infected cells (65nm), and from phosphotungstic acid negatively stained virus (120-300nm) (Chanock, et al., 1957; 1962; Waterson, et al., 1962). Like the influenza and the Newcastle disease viruses, they have an inner coiled component surrounded by a spike-armed envelope (Cruickshank, et al., 1964 unpublished data). The inner helical component is approximately the same with the inner helix of the Parainfluenza viruses. The virus has a sucrose density of 1.9-1.20 and in Cesium chloride

1.23 (Chanock et al., 1957; Coates and Chanock, 1964 unpublished). The failure of FUDR and IUDR to inhibit virus replication suggests that it contains ribonucleic acid (Hamparian et al., 1963).

Effect of Physical and Chemical Agents

Respiratory Syncytial virus is very unstable at temperatures of 37°C and above. In a suspending medium containing 5% chicken serum, the half life of viral infectivity is 7.2 hours at 37°C and 2.6 minutes at 56°C (Bennett and Hamre, 1962).

Approximately 90% of infectivity is lost following slow freezing. However, the virus is frozen rapidly and then stored at 70°C. The virus is rapidly inactivated at pH 3.0 (Hamparian et al., 1963). Lipid is essential for the integrity of the virion (Chanock et al., 1957).

Biological Properties

Unlike Parainfluenza virus, RSV has not been grown in embryonated hen's eggs nor has haemagglutination or haemadsorption been demonstrated (Chanock et al., 1957). These distinctions have now grouped RSV into the genus pneumovirus (Doyle et al., 1980).

Virus specific antigens can be visualized in infected cells by immunofluorescence (Kiech et al., 1982). Such antigens are

found only in the cytoplasm and is first detected 10 hours following infection. Fluorescent antibody studies with unfixed infected cells indicate that a large quantity of virus specific antigen is present at the cell membrane (Klach et al., 1962). Prominent intracytoplasmic inclusions are found in infected Hela or Hep-2 cells.

Infection in Animals

A number of animal species can be experimentally infected with RSV - These include chimpanzees, baboons, monkeys, ferrets, mink, chinchillas, guinea pigs, hamsters and mice. Only the chimpanzees developed clinical symptoms. The disease in chimpanzees occurs without fever and is limited to the upper respiratory tract (Morris et al., 1958; Coats and Chanock 1962). RSV can be recovered from nasal turbinates of a symptomatically infected ferrets (Coates and Chanock 1962). In addition, multinucleated cells containing eosinophilic cytoplasmic inclusion bodies form in the respiratory epithelium of infected ferrets (Coates et al., 1962).

Pathology and Pathogenesis

The most prominent changes in the fatal illnesses were extensive necrotizing or interstitial pneumonitis emphysema and

necrosis of the tracheobronchial epithelium (Beem et al., 1960; Holzel et al., 1963; Parrott, 1964).

The pathology of nonfatal infections include involvement and inflamation of the mucus membranes of the nose and throat. Paranasal and eustachian tube obstruction may occur (Holzel et al., 1963). In young infants, there is tendency for necrotizing bronchiolitis and pneumonitis. Infants who exhibit many allergic manifestations are more likely to develop bronchiolitis than infants free of such symptoms (Freeman and Todd, 1962). Autopsy examination of infants dead of RS virus infection has shown in addition, cytoplasm inclusion bodies and by immunofluorescence, virus has been detected from the lungs (Chanock et al., 1976; Davis et al; 1980).

LABORATORY DIAGNOSIS

Diagnosis of RSV infection can be made by virus isolation and/or demonstration of a rise in antibody titre during convalescence. Virus is present in the nasal and the pharyngeal secretions of infected individuals and can be isolated with greatest efficiency when specimens are inoculated directly into cell cultures without freezing (Beem et al., 1960).

Immunofluoreacence technique for rapid diagnosis has been developed for the respiratory syncytial virus. Specific staining as visible in the cytoplasm of the cell (Lauer, 1982; Minnich and Ray 1980; McIntosh, et al., 1982) described an ELISA for detection of respiratory syncytial virus infection in clinical samples.

Serological diagnosis ordinarily is most reliable and, easiest, using C.F (the most convenient and economical) or neutralization titrations.

Because RSV is highly liable, isolation is most efficient when nasopharyngeal aspirate are inoculated directly from the patient into culture of human continuous cell lines (Hep-2, Hela or KB). Characteristic giant cells develop within 2-14 days.

Infectivity of RSV is completely destroyed during storage at -20 °C for only several days. Viral suspensions can be preserved by addition of proteins (5%-10% normal serum or albumin), freezing rapidly and maintaining at -70 °C (Davis, et al., 1980).

Epidemiology

RSV is a major cause of respiratory disease in children. Infections have a worldwide distribution. The virus spreads rapidly through the susceptibles in a community so that epidemics

are, sharply circumscribed and relatively brief. The outbreaks are commonly found in children and infants between late fall and early spring in Britain. Indeed, RSV is the only recognized virus that preferentially produces severe disease and has its maximum impact during the first six months of life. Although infants have a poor antibody response to infection, about one-third of infants in the United States develop antibodies in the first year of life and 95% by 5 years of age (Parrott, et al., 1974, Davis et al., 1980).

Prevention and Control

The need to control Respiratory Syncytial Virus as a causative agent of acute respiratory infection in young children is desirable but the experience with the alum precipitated formalin-inactivated vaccine has been discouraging (Davis et al., 1980). However, it is clear that inactivated whole virus vaccines containing viruses with lipid envelopes must be used with caution.

ADENOVIRUS

HISTORY

The first isolations of Adenovirus was reported by Rowe et al., (1953) from fragments of human adenoids grown in tissue culture. After a prolonged incubation, cytopathic change was noticed in the epithelial-like cells of some of the cultures, and passage revealed the presence of a virus. Almost simultaneously, Hilleman and Werner (1954) isolated by tissue culture technique, a cytopathogenic agent from army recruits sick with an influenza-like or grippe-like illness. An increase in neutralizing and complement fixing antibodies to the virus was shown to occur in the patients. This disease had been described by several investigators as catarrhal fever, febrile catarrh and acute respiratory disease of recruits (ARD).

The adenovirus group is composed of at least 45 immunologically distinct types of viruses that are related by a major cross reacting antigens.

Characteristics

The adenoviruses are non-enveloped viruses and are 70-90nm in diameter. They have molecular weight of 170-175 x 10^6 and buoyant density of 1.33 - 1.35gm/cm^3 in cesium chloride. The capsid proteins are arranged in an icosahedron having 20

triangular faces and 12 verticles (Ginsberg et al., 1966). Each virion has 240 hexons and 12 pentons. The hexons are diepereed on the triangular faces and edges and the 12 penton are located in the verticles of the icosahedron. Each penton conelets of a base and a fiber which is rod-like outward projection with a terminal knob (Levine et al., 1967).

The virion contains a single molecule of double stranded DNA in a linear form. The molecular weight and the Guanine + Cytosine base composition of the genome for the different immunotypes ranges from 20-30 x 10^6 and 40-61% respectively (Green, 1962).

Both human and nonhuman adenoviruses exhibit a single type of morphology, a similar chemical composition, replicate in the cell nucleus and have a tendency towards species specificity. They produce unique and characteristic cytopathic effects (CPE) (Ginsberg, 1962).

The Adenoviruses do not haemadsorp erythrocytes. they do not replicate in chicken eggs and with the exception of avian adenoviruses, all possess at least 1 family reactive antigenic determinant (Philipson et al., 1974).

The family Adenoviridae include 2 genera Mastoiadenovirus and Aviadenovirus based on the absence of any immunological cross

reactive antigens in mammalian and avian adenoviruses (Norrby, 1976).

Antigenic Composition

33 antigenic groups have been known to affect man: 2 other candidates, types 34 and 35 and many other naturally occurring intermediate strains which possess hexons of 1 prototype virus and fiber antigens related to another type have been recognized in human infections: however no classification status has been assigned to these strains (Crawford et al., 1960; Hatch et al., 1965; Wigand, 1971). Types 1,3,4,21,14,7 are known to cause acute respiratory diseases (Vanderveen et al., 1957; Davis et al., 1980.

Pathogenicity in Animals

Most human adenoviruses do not induce acute clinical infections in common laboratory animals. Intravenous inoculation of adult mice with adenovirus type 5 results in death of animal within 3-4 days (Postlethwaite, 1979). Hamsters, when inoculated subcutaneously showed fatal infections in 4-10 days. Respiratory tract inoculations of types 1,2,5 and 6 in piglets (Blacklow et al., 1962) and type 4 in dogs (CarMichael et al., 1961) produce inapparent infections.

LABORATORY DIAGNOSIS

Electron microscopy:-2 types of electron microscopial methods for the identification of adenovirus in clinical specimens have been described (Edwards et al., 1975; Hammar et al., 1967 and Valtera et al., 1975). In one procedure, fixed and stained tissue sections were examined for arrays of matured virions in the cell nucleus and in the other method, concentrated throat washing specimens incubated with immune serum were observed for aggregates of virus particles. This method is not used routinely. The other method is immunofluorescence microscopy. this technique has been of immense use in the rapid diagnosis of infections caused by adenoviruses. (Sarkkinen et al., 1981).

Virus isolation

Human embryonic kidney (HEK) cells provide optimal isolation of adenoviruses. They contain Adeno-associated viruses which can interfere with the replication of adenoviruses (Hoggan, 1972). But they are not readily available in most laboratories. The hep-2 Hela and KB are highly sensitive WI-38 is commonly employed to isolate adenoviruses. Adenoviruses cause CPE of grape-like type in tissue culture (Ginsberg, 1962).

Serological tests for identification of Adenovirus include the complement fixation test, haemagglutination inhibition test and neutralization test (Bell et al., 1960).

Other tests procedures have been used in the diagnosis of adenoviruses. These include colorimetric neutralization test (Johnson et al., 1957). Single radial diffusion, was described by Gradien et al., (1975). Adenovirus antigens can be detected by Fluorescent antibody technique in the nuclei and cytoplasm of infected cells (Hayashi, 1977).

Epidemiology

Man provides the only known reservoir for strains of adenoviruses that infect humans. Person-to-person spread in respiratory and ocular secretions is the most common mode of viral transmission. Despite the large number and the worldwide distribution of adenoviruses their clinical importance is largely restricted to epidemics of acute respiratory diseases. Infections are observed throughout the year but most commonly in late fall and winter. Type 7, 4 and 3 are the viruses most frequently responsible for epidemics of acute respiratory infections. Types 11, 14 and 21 have been increasingly implicated in epidemics. Type 4 adenovirus commonly cause ARD in military recruits.

A relatively high proportion of adults have antibodies to one or more types of adenoviruses indicating previous exposures. Epidemiological studies indicate that adenoviruses annually cause at most (4-5)% of viral respiratory illness in civilians Davis et al., 1980.

Prevention and Control

Isolation of sick persons has little or no effect on the spread of adenoviruses since many healthy carriers exist. Different types of vaccines have been used in adults particularly in the army recruits. In closed populations, such as chronic disease hospitals or home for orphans, a vaccine containing types 1-7 may be useful for infants and young children (Davis et al., 1980). A suitable vaccine without the putative oncogenic danger (Types 3 and 7 are oncogenic for animals) may be provided by using the purified hexon and fiber capsid proteins which are present in abundance as soluble antigens in infected cells and can stimulate the production of neutralizing antibodies (Davis et al., 1980). The studies of Huebner et al., (1955) Bell et al., (1958); Hilleman et al., 1958 and Wilson et al., (1960) have demonstrated that a satisfactory vaccine can be prepared containing 2 or 3 types of adenovirus types (3,4,7). The vaccines are antigenic and 1 dose apparently gives almost maximum antibody response.

CHAPTER THREE

MATERIALS AND METHODS

Areas of Study

Ibadan

Ibadan, the capital of Oyo State of Nigeria has a population of more than 2 million people. It is situated at an altitude of about 250 metres above sea level. The geographical location is about 3° 52' East and 7° 22' North of the equator. The annual rainfall and humidity are high. The vegetation is lowland Rainforest (Fagbami et al., 1972; Mabogunje, 1974; Udo, 1974). The wet months of the year are between April and September.

Nasopharyngeal Aspirate

Nasopharyngeal secretions were collected from children suffering from acute respiratory infections and attending the University College Hospital (U.C.H.) and a rural health centre clinic of Idikan by aspiration through a plastic tube into a mucus collector containing the tronoport medium. The tube was passed along the base of one nostril into the nasopharynx. The distance from the nostril to the nasopharynx is half the distance from the nostril to the base of the ear. The mucus was thus collected and the process repeated for the second nostril. The mucus collector contained the antibiotics (Penicillin and

Streptomycin) as transport medium. The negative pressure of the suction pump did not exceed 1.5kg/cm^2. Serum samples were also obtained from patients for serology.

Blood was collected by venepuncture from the antecubital fossa of patients into sterile vacutainer tube and allowed to clot at room temperature. The samples were kept at 4°C overnight and centrifuged at 1,500 revolutions per minute (r.p.m.) for 10 minutes. Sera were separated and stored frozen until tested.

IMMUNOFLUORESCENT ANTIBODY (IFA) STUDIES

Materials

Antisera

The antisera used were previously raised against RSV, Influenza, A and B, Parainfluenza types 1,3 and adenovirus and were commercial products of Wellcome Biotechnology Company, England. The RSV, Influenza A and Parainfluenza 3 antisera were raised in bovines while Influenza B, Parainfluenza 1 and adenovirus antisera were raised in chicken.

EVANS BLUE

A 1:30 dilution of the stock of 1:1000 was made to obtain 1:30,000 needed for counterstaining.

Glycerine buffer

8 parts of pure glycerol was mixed with 1 part PBS pH. 7.2.

Phosphate Buffered Saline pH 7.2

Sodium chloride	-	8.00g
Potassium chloride	-	0.20g
Potassium dihydrogen phosphate	-	0.20g
Disodium hydrogen phosphate (anhydrous)	-	1.15g
Double distilled water	-	1000cm^3

Diluent for Antisera

Sterile distilled water.

Transport medium

Transport medium was made of hanks balance salt solution containing 1% Albumin, Penicillin, and Streptomycin.

Antigens

Preparation of Smears for Antigen Detection by F.A.T

Nasopharyngeal aspirates collected from children at the U.C.H. and Idikan Health Centre were centrifuged at 1,500 revolutions per minute for 10 minutes as mentioned above. The pellets contained both cells and mucus. This was resuspended in 2ml of PBS and broken up by pipetting with a Pasteur pipette until a smooth suspension was obtained. The process was repeated twice at 1,500 rpm for 10 minutes each and the supernatant fluid was carefully removed and discarded. If the final cell

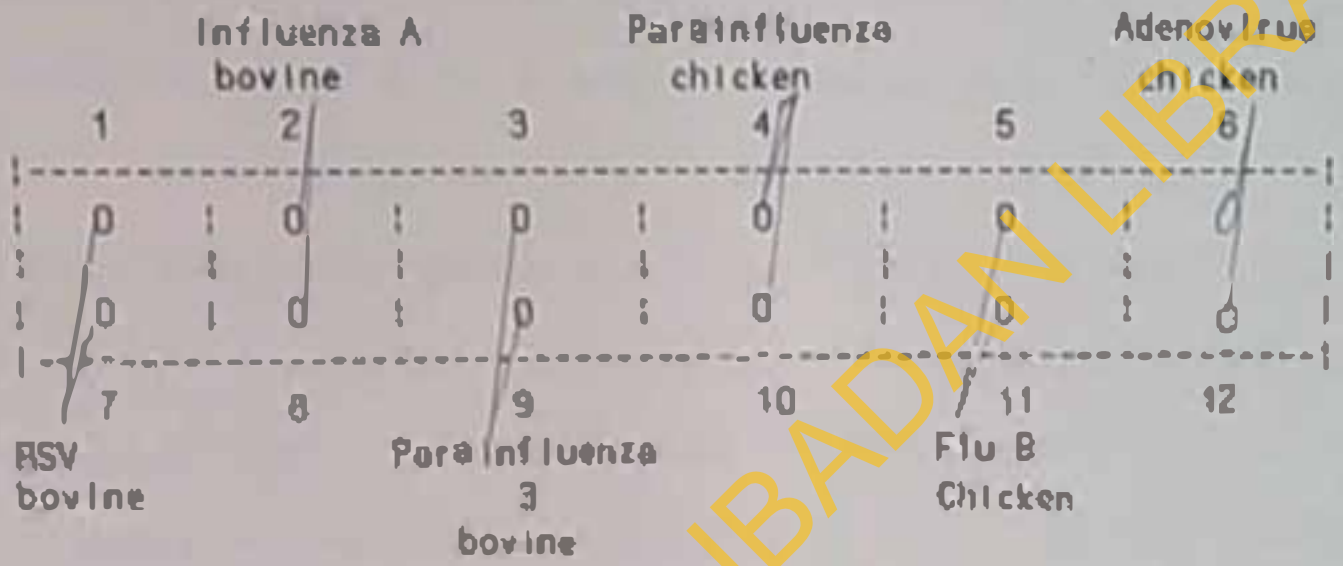
suspension still contained much mucus the process of washing was repeated until a moderately opaque but not sticky suspension was obtained. The cell pellet was used for smear preparation.

Teflon-templated microscope slides (Erie Scientific) with twelve "spots" per slide were cleaned with alcohol. Using microtitre droppers, 20ul of the suspended cells was placed on each spot. The drops were made as flat as possible to avoid aggregation of cells in the centre. The cells were allowed to air dry on the slides and fixed in acetone for 10 minutes. Slides were then stored in the Revco (-70 C) until used.

Indirect Immunofluorescent Antibody Test (IIFA)

The IIFA test was carried out using the method previously described by Orstavik, et al., (1984). On each spot was placed 20ul of appropriate 1:10 dilution of unlabelled viral antiserum as shown in Fig. 1. The slides were placed in a moist chamber and incubated at 37 C for 30 minutes.

FIGURE 1 SHOWING THE LAYOUT SLIDE



Slides were given three washings of 10 minutes each in PBS and air dried. FITC antiglobulin of same species as the unlabelled antiserum (bovine or chicken) was added to the appropriate wells. Slides were placed in the moist chamber and incubated at 37°C for 30 minutes and washed three times in PBS for 10 minutes each thereafter. Counterstaining of smears in Evans Blue (1:30,000 was carried out for 5 minutes after which they were mounted in glycerine buffer. The preparation was then examined with a Leitz Fluorescent Microscope equipped with a transmitted light fluorescence illuminator. A 200 Watt Ultra high pressure mercury lamp was used as the light source. A 3mm BG3 filter served as the exciting filter in addition to a K490 suppression filter. 12.5 oculars and 54X oil immersion objectives were used.

ELISA For Detection of RSV Antigen in Nasopharyngeal Aspirates

Materials Used in ELISA Test

(1) RSV antibody tubes

RSV HRP - Conjugated Monoclonal Antibody

Sample diluent

Positive control Antigen

Negative control Antigen

Substrate buffer

Chromogen

Assay treatment buffer

High quality deionized water

1 Normal Sulphuric acid

Spectrophometer

These are supplied in the ELISA kit ready for use.

Preparation of reagents for use

1N H_2SO_4

Concentrated Sulphuric Acid	14.0mls
Distilled Water	486.0mls

Working Colour Treatment

Substrate buffer	1cc
Chromogen	1 drop

Principle of ELISA TEST

Viral antigens present in the specimen bind to a polyclonal anti RSV- antibody coated on the plastic tube. When added to the tube, a pool of soluble peroxidase conjugated antibodies specific for the RSV nucleocapsid antigen binds to the antigen captured on the tube wall. Following a washing step to remove unbound sample and conjugate, a perodiase substrate added to the tube generates a blue colour if virus is present in the specimen. Visual or spectrophotometric evaluation of the specimen determines the presence or absence of RSV. Observation of blue colour or an absorbance value equal to or greater than the cut-off value indicates the presence of RSV antigen in the patients specimen. The absence of a blue colour or an absorbance value less than or equal to the lower cut off value indicates the absence of detectable RSV antigen in the patients specimen.

Test Procedure

One antibody tube each was labelled for the negative control, the positive control, and patients specimen. A drop of assay treatment buffer was also added to each tube. One hundred ul HRO-conjugated monoclonal antibody was added per tube. Three hundred ul of sample diluent was added to the negative control tube and the same quantity of positive control antigen was added

to the positive control tube. Three hundred ul of each patients specimen was placed in the appropriately labelled tube. The tubes were mixed gently and incubated at room temperature for 60 minutes.

Samples were carefully aspirated from the tubes and each tube was washed 6 times with a high quality distilled water. After the final wash, excess moisture was removed from the tubes by inverting the tubes and blotting the rim on paper towels.

0.5 ul of working colour reagent (substrate) was added to each tube. The tubes were mixed gently and incubated at room temperature for 15 minutes. The tubes were read visually. Observation of blue colour in the positive control tube and any 'test', sample indicated a positive result while negative results remained colourless.

Complement Fixation Test

PREPARATION OF MATERIALS USED IN COMPLEMENT FIXATION TEST

Sheep Red Blood Cells

Sheep Red blood cells were used as indicator of complement fixation. Sheep was bled from the jugular vein and blood was collected in an anticoagulant (Alsever's solution). Blood cells were washed twice in physiological saline and twice in veronal

buffer (VB). Cells were suspended as 4% in VB and kept at 4°C until used. The composition of the solutions are as follows:

(a) Alsever's solution

Dextrose	50.5g
Sodium Chloride NaCl	4.2g
Citric Acid	0.55g
Sodium Citrate	8gm
Distilled water	1000cm^3

The solution was sterilized in the autoclave for 10 minutes at 10lbs pressure.

(b) Veronal buffer

Tablets - One tablet was dissolved in 100mls of warm distilled water.

Formular for Oxoid (1969) CF diluent Tablet

Barbitone	0.575g
Sodium chloride	8.50g
Magnesium chloride	0.160g
Calcium chloride	0.020g
Barbitone soluble	0.185g

All dissolved in 1 litre of distilled water to give a pH of 7.2.

Haemolysin

As anti-sheep red blood cell prepared in rabbit was used to sensitive red blood cells. It was titrated and used at 4-6 mean hoemolytic doses (MHD). The dilution of haemolysin was carried out as shown below.

In the titration of hoemolysin one volume of each dilution of haemolysin was added to one volume of 4% sheep red blood cells.

Haemolysin Titration

Serial fold dilutions of hoemolysin are made as follows: Add 1 volume neat serum plus 9 volumes of Diluent to get 1:10 dilution.

1:10 1 volume of neat hoemolysin + 91 vols of VB 1:10

1:50 1 volume of 1:10 + 4 volumes of Veronal buffer 1:50

1:100 1 volume of 1:10 + 9 volumes of VB 1:100

1:100 1:200 1 vol. of 1:100 + 1 vol of VB 1:200

1:400 1 vol. of 1:100 + 3 vol of VB 1:400

1:800 1 vol. of 1:100 + 7 vol of VB 1:000

1:1000 2 vol of 100 + 18 vol of VB 1:1000

1:1500 2 vols of 1:1000 + 1 vol of VB 1:1500

1:1000 1:2000 1 vol of 1:1000 + 1 vol of VB 1:2000

1:2500 2 vol of 1:1000 + 3 vol of VB 1:2500

1:3000 1 vol of 1:1000 + 2 vol of VB 1:3000

1:4000 1 vol of 1:1000 + 3 vol of VB 1:4000

1:5000 1 vol of 1:1000 + 4 vol of VB 1:5000

and incubated in water bath at 37°C for 15 minutes. the dilution of 1:10 to 1:800 were removed and allowed to stand at room temperature and observed for agglutination.

Agglutination should not occur in 6 MHD of haemolysin and preferably should not exceed 1:100 dilution of haemolysin. To the dilution of 1:1000 to 1:5000 of haemolysin was added 1 volume of 1:10 dilution of complement. Incubation was carried out at 37°C for 15 minutes. The highest dilution showing complete haemolysis was taken as 1MHD.

Complement

Fresh or reconstituted lyophilized guinea pig serum was the source of complement. The complement titration was carried out as follows.

Complement was diluted 1:30 and 8 master tubes set up as shown below.

PROCEDURE FOR CARRYING OUT THE MASTER DILUTION OF COMPLEMENT

Reagents	Tubes							
	1	2	3	4	5	6	7	8
Complement 1:30	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Veronal buffer	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2

PROCEDURE FOR THE FINAL TITRATION OF COMPLEMENT

Reagents	Tubes							
	1	2	3	4	5	6	7	8
C' Complement (Master Dilution)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Veronal buffer	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sensitized Cells	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Final vol of 1/30 Complement in tube	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08

The tubes were incubated in water bath at 37°C and the results of the titration was read after 30 minutes. The tube showing complete haemolysis was taken as the dilution containing 1 unit of complement. The formula used for calculating the dilution required to give two units of complement is shown below:

Reciprocal of Original Complement Dilution

Twice the amount of complement in the tube with x 0.1 complete haemolysis.

e.g. If complete haemolysis was in tube 5, the complement dilution needed was $\frac{30 \times 0.1}{0.05 \times 2} = 1:30$

Complement Fixation Test

A modification of the microtitre technique of Sever, (1962) and that of Yale Arbovirus research Unit (YARU) was used. Commercially prepared antigens were used in the test. The sera were diluted 1:4 with Veronal buffer diluent, inactivated at 56°C for 30 minutes and further diluted in increasing double fold dilution. The antigens were also used in 2 fold dilutions and a checkerboard type of CF was performed using known positive controls sera packed along with the antigens by the manufacturers. The sera were dispensed into appropriate wells using a micro dropper which delivered 0.025ml per drop. Appropriately diluted antigens were dispensed one drop

each into appropriate wells. One drop of complement containing 2 units was added to each well, according to the protocol of the test. Serum control, was set up for the test, so also were the antigen, and diluent controls. A back titration of the complement was made by placing 2 drops of complement from the original master tube to a drop of diluent in each well.

The plates were incubated at 4°C overnight. the next morning, they were placed a 37°C for few minutes before adding one drop of sensitized sheep red blood cells per well. The plates were incubated at 37°C for 30 minutes and shaken at intervals of 10 minutes. The plates were finally placed at 4°C to allow cells to settle before reading. A reading of 4 (No haemolysis) or 3(25% haemolysis) was taken as positive. Reading of 2(50% haemolysis) or less were negative.

CHAPTER FOUR

RESULTS

Survey For Antibody To Respiratory Viruses

Out of the two hundred and eighty patients tested for complement fixing antibody, 104 (37%) were community based and 176(63%) were hospitalised at the University College Hospital both in Ibadan. One hundred and fifty seven (56%) were positive for complement fixing antibody to one or more viruses. The highest prevalence of antibody was to RSV (28%). The prevalence antibody to other respiratory viruses were as follows: Adenovirus (16%), Influenza A (1%), Influenza B (1%), Parainfluenza type 1, (4%) and Parainfluenza type 3, (5%) (Table 2).

Survey for Respiratory Viral Antigens in Nasopharyngeal Aspirate

A total of three hundred and fifty nasopharyngeal aspirates obtained from patients from Idiken community health centre and patients with acute respiratorydisease requiring hospitalisation at U.C.H were examined for presence of antigen to Respiratory syncytial virus, Influenza A and B, Parainfluenza types 1 and 3 and Adenoviruses by fluorescent antibody technique.

Respiratory viral antigens were found in the nasopharyngeal aspirates of 74 out of the 350 patients (21%) tested. A

TABLE 1

Specimens tested for Respiratory Viral Antigens and Antibody Using Nasopharyngeal Aspirates and Sera of Patients Suffering from Acute Respiratory Infections

Specimen Type	Tests	No	Year of Collection	Source
Nasopharyngeal Aspirate	Fluorescent Antibody Technique	350	1985/1987	U.C.H. and Idikan
	ELISA	230	1985/1987	U.C.H. and Idikan
Sera	C.F. Test	280	1985/1986	U.C.H. and Idikan Ibadan

TABLE 7

Analysis of Results of CF Test Performed on SIRA of children suffering from Acute Viral Respiratory Infections

Age Distribution in Months	No of Sera Tested	RSV	No. (%) ART CFT POSITIVES					
			Flu A	Flu B	Para 1	Para 3	Adeno	Total
1 - 6	70	23 (32)	1 (1)	2 (3)	5 (7)	7 (10)	17 (24)	55 (79)
7 - 12	57	11 (19)	-	-	1 (2)	1 (2)	8 (14)	21 (37)
13 - 18	21	3 (14)	-	-	-	-	2 (10)	5 (24)
19 - 24	26	11 (42)	-	1 (4)	-	1 (4)	7 (27)	20 (77)
25 - 30	21	8 (38)	-	-	1 (19)	2 (10)	3 (14)	17 (3()
31 - 36	22	8 (36)	-	-	1 (5)	-	1 (5)	10 (45)
37 - 42	21	4 (19)	-	-	-	1 (5)	3 (14)	8 (39)
43 - 48	22	5 (23)	1 (5)	-	1 (5)	1 (5)	1 (5)	9 (41)
49 - 54	9	3 (33)	-	-	-	1 (11)	1 (11)	5 (56)
55 - 60	5	- (0)	-	-	-	-	2 (40)	2 (40)
61 and above	8	1 (17)	2 (33)	-	-	-	1 (17)	5 (83)
Total	280	77 (28)	4 (1)	3 (1)	12 (4)	14 (5)	46 (16)	157 (56)

Overall Prevalence 56%

RSV = 28%
Adeno = 16%
Para 3 = 5%
Flu A = 1%
Para 1 = 4%
Flu B = 1%

breakdown of the FA positive patients shows that RSV infection is the most prevalent respiratory viral infection in Ibadan (7%); followed by Parainfluenza type 3 (4%); and Influenza A, (4%).e The percentage of positives to other viruses are as follows: Parainfluenza type 1 (3%), Influenza B (2%) and Adenovirus (1%) (Table 3).

In the community, 26 out of 150 (17%) were positive while 48 out of 200 (24%) were positive in the hospitalised patients. In all, 74 out of 350 (21%) were positive for viral antigens.

Statistical analysis showed that there was no significant difference in the prevalence of respiratory viral infection among the hospital based cased and community based patients ($P > 0.05$). Eleven of the children had multiple infections. Nine, were hospital patients and 2 were from the community (Table 4a). Among the hospital patients, 8 were infected by 2 viruses and one by 3 viruses (Table 4b). The two community patients were infected by 2 viruses (Table 4b).

Parainfluenza type 3 was found to be the virus most frequently associated with multiple infections. Seven of the eleven patients were infected with Parainfluenza type 3 associated with Parainfluenza type 1, Flu B, Flu A or RSV.

TABLE 3

Results of FA Test for Detection of Viral Antigens and the Prevalence of the Causative Agents of ARI in Nasopharyngeal aspirates of Nigerian children suffering from Acute Respiratory Diseases

ANTIGENS	COMMUNITY PATIENTS		HOSPITAL PATIENTS		TOTAL	
	Tested	No (%) Positive	Tested	No (%) Positive	Total Tested	Total (%) Positive
RSV	150	7 (5)	200	18 (9)	350	25 (7)
Flu A	150	6 (4)	200	8 (4)	350	14 (4)
Flu B	150	1 (1)	200	9 (5)	350	8 (2)
Para 1	150	4 (3)	200	6 (3)	350	10 (3)
Para 3	150	6 (4)	200	9 (5)	350	15 (4)
Adeno	150	2 (1)	200	2 (1)	350	4 (1)
Total	150	26 (17)	200	48 (24)	350	74 (21)

Acute Respiratory Infections (ARI).

TABLE 4a

Results of FA Showing Multiple Infection in Children Suffering from Acute Respiratory Infections in Ibadan

Patient's Side No.	RSV	Flu A	Flu B	Para 1	Para 3	Adeno.	Age	Sex	Location
/84	+	-	-	+	+	-	17	M	Hospital
/ 1	-	+	-	-	+	-	7	F	Hospital
/ 5	-	-	-	+	+	-	3	F	Community
/48	-	-	+	-	+	-	2	M	Hospital
/127	+	-	-	+	-	-	24M	M	Hospital
/216	-	+	-	+	-	-	24M	M	Hospital
/229	+	-	-	+	-	-	3	M	Hospital
/53	-	-	+	-	+	-	8	F	Hospital
/224	+	-	-	-	+	-	-	M	Community
/293	+	-	-	-	+	-	9	F	Hospital
/267	+	-	-	-	-	-	8	F	Hospital
Total 11	6	3	2	5	7	0		6M	Hospital 9
								5F	Community 2

TABLE 4b

Results of FA showing Multiple Infections in Children suffering from Acute Respiratory Infections in Ibadan

	No. of samples	RSV	Flu A	Flu B	Para 1	Para 3	No. (%) with 2 viruses	No (%) with 3 viruses
Hospital	9	5	3	2	4	5	8(12)	1(0.3)
Community	2	1	-	-	1	2	2(0.5)	0
Total	11	6	3	2	5	7	10(3)	1(0.3)

Figure 2 shows the monthly variation of respiratory viral antigens in the nasopharyngeal aspirate of Nigerian children. The infection rose gradually and peaked in December. There was a fluctuation between January and March and it rose to a high level again in April. It then dropped gradually during the months of May and June.

Table 5 shows the age distribution of children whose nasopharyngeal aspirates were tested for viral antigens. The percentage of nasopharyngeal aspirate positive for respiratory viral antigens are as follows:- 1-6 months (29%); 25-30 months (30%), 37-42 months (24%). Lower prevalence of respiratory viral antigens was found in children aged 31-36 months (10%), 13-18 months (9%) and 19-24 months (0%).

RSV infections were seen most commonly in children aged 1-6 months and those aged 43-48 months. Percentage of positive nasopharyngeal aspirate in these age groups were 11% and 10% respectively. The highest prevalence of infections to the other viral antigens were found in the following age groups:- Influenza A 37-42 months; Influenza B 31-36 months; Parainfluenza 1, 31-36 months; Parainfluenza 3, 25-30 months; and adenovirus 61 months and above.

MONTHLY VARIATION OF RESPIRATORY VIRAL ANTIGENS IN NASOPHARYNGEAL ASPIRATES OF PATIENTS

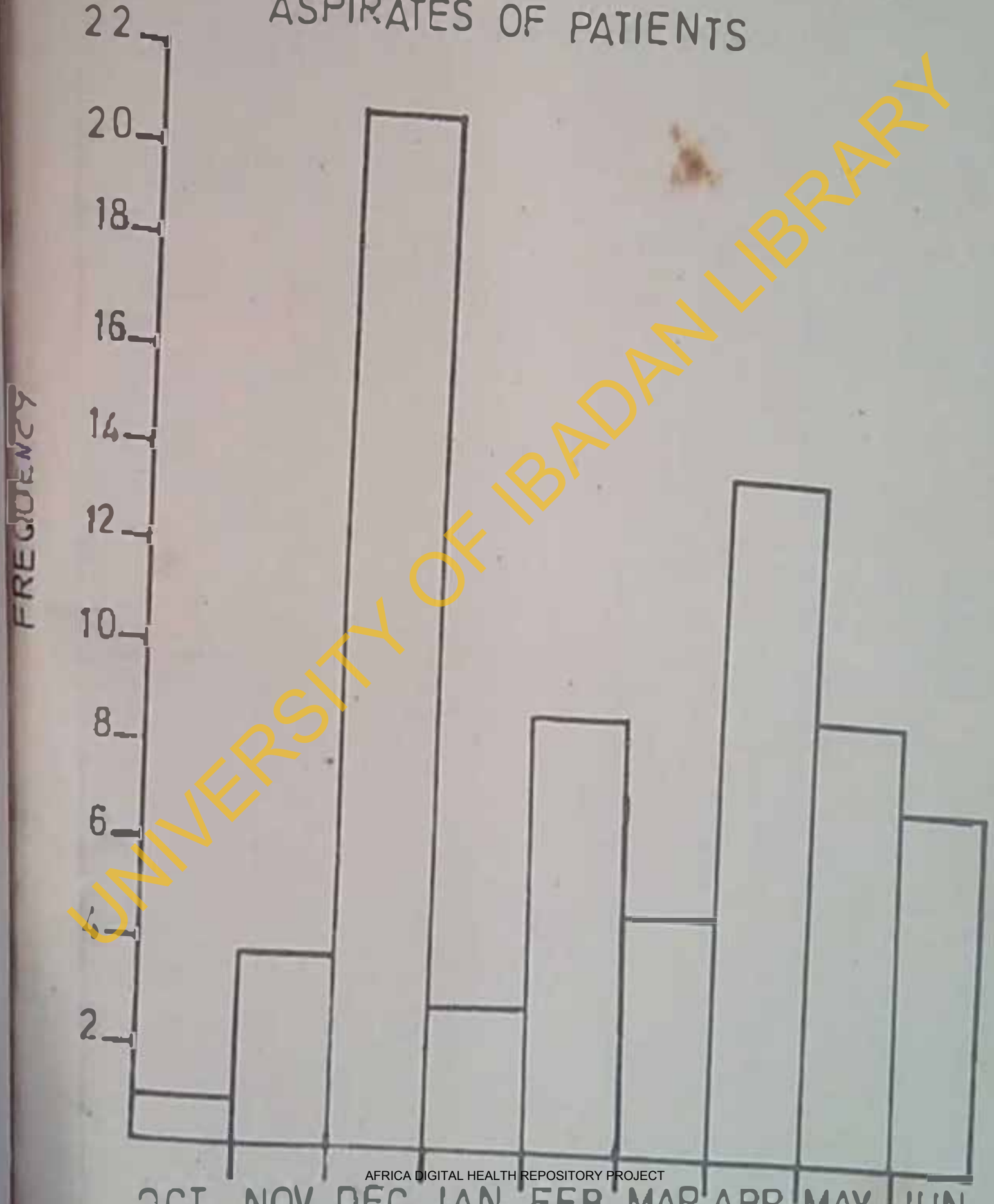


TABLE 5

RESULTS OF FA TEST FOR DETECTION OF VIRAL ANTIGENS AGE DISTRIBUTION AND % POSITIVITY

Age Distribution in Months	No tested	RSV	Flu A	Flu B	Para 1	Para 3	Adeno.	Total
1 - 6	79	9(11.0)	4(5)	1(1.0)	4(5)	5(6.0)	0(0)	23(29
7 - 12	61	2(3.0)	2(3.0)	0	6	4(7.0)	0(0)	8(13)
13 - 18	33	1(3)	0	1(3)	0	1(3)	0	3(9)
19 - 24	39	2(5.0)	2(5.0)	0	1(3.0)	1(3.0)	0	6(15)
25 - 30	23	2(0.0)	1(4.0)	0	1(4.0)	2(8.0)	1(4.0)	7(30)
31 - 36	30	0	0	1(3.0)	2(7.0)	0	0	3(10)
37 - 42	17	1(5.0)	2(12.0)	0	0	1(6.0)	0	4(24)
43 - 48	29	3(10.0)	2(7.0)	0	0	0	1(3.0)	6(21)
49 - 54	13	1(0.0)	0	0	0	0	0	1(8)
55 - 60	5	0	0	0	0	0	0	0(0)
61 and above	10	1 (10)	0	0	0	0	1 (10)	2(20)
Age Unknown	11	3	1	3	2	1	1	11(0)
Total	350	25	14	6	10	15	4	74(21)

TABLE 5

RESULTS OF FA TEST FOR DETECTION OF VIRAL ANTIGENS AGE DISTRIBUTION AND % POSITIVITY

Age Distribution in Months	No Tested	NSV	Flu A	Flu B	Para 1	Para 3	Adeno.	Total
1 - 6	79	9(11.0)	4(5)	1(1.0)	4(5)	5(6.0)	0(0)	23(29)
7 - 12	61	2(3.0)	2(3.0)	0	0	4(7.0)	0(0)	8(13)
13 - 18	33	1(3)	0	1(3)	0	1(3)	0	3(9)
19 - 24	39	2(5.0)	2(5.0)	0	1(3.0)	1(3.0)	0	6(15)
25 - 30	23	2(8.0)	1(4.0)	0	1(4.0)	2(8.0)	1(4.0)	7(30)
31 - 36	30	0	0	1(3.0)	2(7.0)	0	0	3(10)
37 - 42	17	1(5.0)	2(12.0)	0	0	1(6.0)	0	4(24)
43 - 48	29	3(10.0)	2(7.0)	0	0	0	1(3.0)	6(21)
49 - 54	13	1(8.0)	0	0	0	0	0	1(8)
55 - 60	5	0	0	0	0	0	0	0(0)
61 and above	10	1 (10)	0	0	0	0	1 (10)	2(20)
Age Unknown	11	3	1	3	2	1	1	11(0)
Total	350	25	14	6	10	15	4	74(21)

ELISA results showed that 18 (6%) of the patients were positive for respiratory syncytial virus antigen. However, in a comparative study carried out to determine the sensitivity of ELISA technique and FA test for the detection of respiratory syncytial viral antigens, only 4 were positive by the two test systems (Table 8).

TABLE 6

COMPARISON OF RESULTS OF ELISA AND FAT FOR THE DETECTION OF RESPIRATORY SYNCYTIAL VIRAL ANTIGENS IN NASOPHARYNGEAL ASPIRATES

SYSTEM USED	FA +	FA -	TOTAL
ELISA +ve	4	8	12
ELISA -ve	12	76	88
TOTAL	16	84	100

Clinical Signs and Symptoms in Patients Infected with Respiratory Viruses

The clinical signs and symptoms at presentation varied with individual patient. However, infection with all the viruses were characterized by fever, cough and runny nose. Only those with Influenza A and occasional combination with Respiratory Syncytial Virus were tachypoeic. Infections with RSV, Influenza A, Adenovirus and those with multiple infections also had chest retraction wheezing, bronchial breathing, refusal to feed and increased crepitations.

CHAPTER FIVE

DISCUSSION

The results of survey for C.F. antibody showed that several respiratory virus infections occur in the children population of Ibadan Nigeria. RSV appeared to be responsible for most infections as revealed by the results of the present serological survey. The highest prevalence of C.F. antibody was demonstrated to RSV.

In a similar work carried out by Ogunbi (1970) complement fixing antibody to RSV (11.3%) adenovirus (5.6%) and parainfluenza (5.6%) was found in children suffering from bronchiolitis in Lagos. In Ibadan, David-West and Cooke (1974) found influenza A antibodies in 60-95% of patients following an epidemic of acute respiratory infections using the haemagglutination inhibition test (HI).

Although the prevalence of complement fixing antibody to RSV and Adenovirus reported in this study is high when compared to previous findings by Ogunbi (1970) in Lagos, the prevalence of parainfluenza antibody in both studies are similar. A survey for respiratory viruses in nasopharyngeal aspirate of children presenting with acute respiratory infection in U.C.H. and Idikan community revealed that RSV, Parainfluenza types 1 and 3, Influenza A, Influenza B and Adenoviruses were present in these patients. This is in agreement with the results of the serological survey for antibody to these viruses which showed that antibody to these viral agents was prevalent in the children population (see Table 2).

The age distribution of respiratory viral infections in children with ARI is shown in (table 5). The highest prevalence of respiratory infections was in patients of 1-6 months of age. The percentage of positive to individual respiratory virus antigens at this age was RSV (11%) Parainfluenza type 3 (6%) Influenza A (5%) Parainfluenza type 1 (5%) Influenza B (1%).

Although RSV infections frequently occurred in young children aged 1-6 months, infections by other respiratory viruses were more frequently seen in older age groups. The highest prevalence of infections to Para 3, Para 1, Influenza A and B were found in children aged 25-42 months and adenovirus in those aged over 61 months.

These results are similar to the findings of Chanock et al., (1963); Holzel et al., (1963); Parrott et al., (1974) who showed that most children were infected with respiratory viruses early in life. In the comparison of ELISA and FA technique for the detection of RSV, 4 out of 100 samples tested were positive in the ELISA and Fluorescent antibody technique. There were some non agreement results as shown in Table 8). This is consistent with the work of Gradien et al., (1985) who showed that when ELISA and FA techniques are used for the detection of Respiratory Syncytial Virus in the same specimens, the results obtained have always been comparable. However, McIntosh et al., (1982) and Gradien et al., (1985) identified the possible cause of non agreement results as overdilution of specimens during collection,

transportation and processing. Hendry et al., (1986) advocated the use of whole nasal secretion because washed cells gave lower positive results.

In the present study however, the causes of non agreement results of FA and ELISA may be due to problems of handling, storage and washing of cells.

CHAPTER SIX

CONCLUSION

A survey for complement fixing antibody to 6 respiratory viruses was carried out in Ibadan. Results showed that antibody to these viruses was present in sera of children tested suggesting that all these viruses are circulating in Ibadan and causing human infections. Highest prevalence of antibody was found to Respiratory Syncytial Virus. It is probable that RSV may be the commonest virus causing respiratory disease in Ibadan children.

Tests for viral antigens in nasopharyngeal aspirates of children with acute respiratory infection confirmed the serological data. All 6 viral antigens were found in the nasopharyngeal aspirates of children with acute respiratory diseases. The most commonly identified antigen by Fluorescent antibody technique is RSV. This emphasizes the important role of this virus in the aetiology of Acute respiratory infections in Nigeria.

A higher prevalence of infection was found in children with severe respiratory disease requiring hospitalization than in outpatients attending community health clinics. Symptoms commonly seen in children suffering from acute respiratory

diseases in Nigeria include fever, cough running nose, refusal to feed, wheezing, grunting tachypnea and crepitations. Some children may vomit. The body temperature may become as high as 40°C.

The present study revealed that viruses are very important agents of acute respiratory infections in Nigeria. It is advocated that laboratory diagnosis of Respiratory syncytial virus, Influenza A and B, Parainfluenza types 1 and 3 and adenovirus be included in routine investigations in cases of Acute respiratory diseases.

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