Viral and Chlamydial Etiology of Acute Respiratory Infection in Infants in Addis Ababa

A thesis presented

to the School of Graduate studies

Addis Ababa University

In Partial Fulfillment of the

Requirements for the Degree of Masters

of Science in Zoology

by

DESALEGN ENARO

June , 1993

ADDIS ABABA UNIVERSITY

School of Graduate Studies

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by

DESALEGN ENARO

Faculty of Science

Approved by Board of Examiners:

External Examiner	
Internal Examiner	
Advisor	
Chairman	

A C K N O W L E D G E M E N T S

My deepest gratitude to Dr. Beyene Petros , my advisor, Department of Biology, Addis Ababa University for attaching me to carry out this study . His concern in follow ups of the study and reviewing of the final report .

My thanks to Dr. Lulu Muhe , my advisor , Department of Pediatric and Child Health, Addis Ababa University for the co-ordination of the administrative affairs at the Ethio-Swedish Children's Hospital and WHO, Geneva and for giving access to computer use. His resourcefulness made the study much easier . To all pediatricians who participated in clinical diagnosis and sample collection .

My thanks to Progressor Monica Grandien and Miss Anna Lena Hammarin of the National Bacteriological Laboratory (NBL) , Department of Virology of Stockholm , WHO Collaborating Centers for Rapid Viral detection , for their technical advice ,kindly supplying of kits and reagents , control slides for quality control and for the collaboration of NBL as an external reference laboratory which received stained and /or unstained duplicates of study slides and for providing the training in the immnofluorescent antibody technique .

My indeptedness to Dr. Sandy Gove , the World Health Organization (WHO) ARI Control Program Co-ordinator for kits and reagents , laboratory equipment and assignment of external specialist as a technical advisor . For arranging for financial assistance WHO .

To the School of Graduate Studies, for partial financial assistance for locally purchased laboratory supplies and transportation expenses.

Due thanks to National Research Institute of Health (NRIH) administration for facilitating my work in various ways. I also thank Ato Nigussie Gezahegn and the NRIH Staff of the Immuno-Heamatology Department for their encouragement and technical support.

My deepest gratitude to W/o Almaz Ayano, my wife for her steadfast encouragement throughout the study period. To my sister - in - law, Alemtshay Ayano who made the initial computer typing of the manuscript.

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ABSTRACT

A case - control study was carried out to establish the etiologic agents causing acute respiratory infections in infants in Addis Ababa using the immunofluorescence technique. Special attention was given to viruses and *Chlamydia trachomatis*. The influence of the level of education, passive smoking, breast feeding, and meteorological factors in association with viral infection in acute respiratory infections was investigated using the questionnaire data.

Nasopharyngeal aspirates of 225 cases and 178 control infants were studied for the presence of respiratory syncytial virus, parainfluenza virus, influenza virus types A and B, adenovirus and *Chlamydia trachomatis* between January, 1992 and December, 1992. The overall viral and chlamydial detection rate was 48.9%. The total viral detection rate was 32.9%, out of which respiratory syncytial virus accounted for 31.1%. *Chlamydia trachomatis* was detected in 16%. In control infants 2.8% of viral detection was observed. Five control infants had detectable viral antigens but no control infants had detectable *Chlamydia trachomatis* antigens. Infants having acute respiratory infection showed significantly higher rates of viral detection than healthy control infants (Chi-Square Yates corrected = 52.64, $p<0.001$). Viral detection demonstrated higher rates than that of *Chlamydia trachomatis* (Chi-Square with Yates corrected = 54, $p<0.001$). Mixed infections of virus and *Chlamydia trachomatis* was evident in 6.2%.

The rate of viral detection showed an inverse relationship with increasing age.

Those infants aged up to 2 months demonstrated higher frequencies of viral detection compared to the 3-12 months of age . Slightly higher frequencies of viral detection were observed in females than in males, and males were more affected than females by chlamydial infections . In acute upper respiratory infections a similar frequency of viral detection rates were observed in comparison with that of the pneumonia cases .

Respiratory syncytial virus was the most prevalent etiologic agent detected , and *Chlamydia trachomatis* was the second most important etiologic agent .

The socio-economic factors thought to contribute to viral infections have not been established ; however , meteorological factors tended to be associated with the prevalence of viral infections .

This is an original study that establishes the importance of viral and chlamydial etiology of acute respiratory infections in infants in Ethiopia .

1. INTRODUCTION

Acute respiratory infection (ARI) is an infection of the respiratory tract and the alveoli of the lungs caused by invasion of viruses, bacteria, fungi and parasites (Berman, 1983). According to World Health Organization (1991) ARI is clinically categorized as acute upper respiratory infections (AURI) and acute lower respiratory infections (ALRI). AURI is an acute infection involving the nose, throat and middle ear while ALRI consists of an acute infection involving the epiglottis, larynx, trachea, bronchi, bronchioles or the lung. Important clinical syndromes of AURI are the common cold, pharyngitis which is an inflammatory infection of the pharynx and otitis media. On the other hand important clinical syndromes of ALRI are epiglottitis, bronchitis, bronchiolitis and pneumonia. Epiglottitis is an acute infection of the epiglottis and the adjacent cells. Acute bronchitis is an inflammatory infection of the bronchi while bronchiloitis is an acute infection of the bronchioles which causes swelling and narrowing leading to the development of pneumonia. Pneumonia refers to the inflammatory infection of the lung and it has differing degrees of severity (WHO, 1991).

In infants and young children throughout the world, ARI is one of the three major diseases (diarrhoea, ARI and protein calorie deficiency) which results in infant and young child morbidity (Savage, 1987, Pio *et al.*, 1986 and Shann *et al.*, 1986). It is estimated that of children under the age of five years, 15 million perish due to these diseases (WHO, 1983 and Editorial, 1985). Gwatkin (1980) indicated that ARI alone accounts for the death of about 5 million infants and young children all over the world each year while Leowski (1986) and Woodhead and his colleagues (1987) reported that it is an

important cause of morbidity and mortality in infants. Mounla (1987) and Forgie *et al* (1991) reported that in developing countries, ARI is more severe and mortality rates are reported to be approximately 10 to 50 times greater than that of the developed countries. This is because of host and environmental factors which are associated with the socio-economic conditions (Denny and Lodda , 1986 and Sunakorn and colleagues , 1990).

Beslshe *et al* (1983) and Dym *et al* (1986) reported that there are well documented studies on the etiologic agents of ARI in infants in the developed countries. However, WHO (1980) and Ong *et al* (1982) reported that in developing countries, because of inadequate laboratory facilities, the etiologies of ARI remain unreported. Consequently, in 1976 a global control programme of ARI was launched by the WHO with the objective of decreasing ARI morbidity and mortality in developing countries (WHO, 1977).

In Ethiopia, ARI in infants and young children is reported to be one of the causes of frequent visits to health care units (Taffesse , 1973). Anonymous (1989) hospital annual Report 1988-1989 showed that ARI was a major cause of infant deaths. A study by Desta and co-workers (1991) conducted in Butajira , in rural Ethiopia , on mortality pattern in the under fives also indicated that ARI is a major probable cause of infant and young child death. However , so far the etiologic agents of ARI have not been established, especially with respect to viruses and *Chlamydia trachomatis.*

Diverse etiologic agents of ARI have been described by several workers; some of the agents that causing severe ARI but occurring only occasionally are *Mycoplasma pneumoniae* (Maletzky *et al* .,1971) , *Pneumocystis carinii* (Hughes ,1991) and *Ureaplasma* spp. (Stango *et al.*, 1987) . In addition ,*Trichomonas vaginalis* , *Ascaris lumbricoides* ,*Actinomyces* spp. and *Nocardia* spp. are also reported as rare causes of ARI in young children (McLearen and his colleagues , 1983 and Medoff and Kabayashi, 1981). Major bacterial agents such as *Streptococcus pneumoniae* and *Hemophilus influenzae* type b (Hib) are also important etiologic agents of ARI (Eskola *et al* . , 1990 and Madore *et al* . , 1990) . Additionally, *Staphylococcus aureus* is also an important bacterial agent of ARI (Esperson and Gabrielson , 1981). The bacterial agents that are less frequently associated with ARI are *Staphylococcus epidermidis* , beta-hemolytic Streptococcus , alpha-hemolytic Streptococcus , *Streptococcus pyogenes* , *Streptococcus pneumoniae* , *Klebsiella pneumoniae* , the coliforms and many of the gram negative bacilli (Falkon , 1980 and Yuccum , 1989) .

Hall (1988) and McConnochie (1990) reported that important viral agents causing ARI in infants are respiratory syncytial virus (RSV) , parainfluenza viruses (PIV) , influenza virus types A and B (Flu - A and B) and adenoviruses. Less frequently reported agents causing ARI in infants are enteroviruses , cytomegaloviruses and rhinoviruses . In addition , several researchers have reported that *Chlamydia trachomatis* , a bacterial agent which is related to viruses , is an important cause of ARI in infants (Stango , 1987 , Nunez *et al.*, 1988 and Graham and Blanco , 1990) .

A. Major Viruses Causing ARI

Influenza viruses belong to the genus *Orthomyxovirus* of the *Orthomyxoviridae* family and were given a variety of names when they are originally isolated in 1933 (WHO, 1980). The nomenclature of influenza viruses is dynamic (Matthew 1979 and Matthew, 1982). According to the Committee on Infectious Diseases (1989) the three immunologic types of influenza viruses which to date have been described are type A , B and C .

According to Kandal (1985) there are a total of 12 different hemagglutinin (HA) subtypes and 9 different subtypes of neuraminidase (NA) described among type A isolates . However , the Committee on Infectious Diseases (1989) reported that three distinct HA (H1 , H2 and H3) and two NA (N1 & N2) subtypes have been recognized as causing human infection . Major changes in H1 to H2 subtypes bring about antigenic shift while minor changes in N1 and N2 cause antigenic drift . Kandal (1985) described the peculiar ability of influenza viruses to display major antigenic changes . Consequently , these viruses cause pandemic diseases in non-immune populations throughout the world .

Hirsch (1883) as cited in Knight (1973) pointed out that historical records established epidemic influenza virus type A as early as 1173 B.C. Since then , a continuous resurgence of Influenza A epidemic occurring at irregular intervals of 1-3 years has been observed . On the other hand, Influenza B epidemic occurs at an interval

of 3-7 years and C causes few epidemics.

Parainfluenza virus was first isolated in 1953 and thereafter, was soon associated with ARI in children (Clark and Fineberg, 1957, cited in McIntosh and Clark, 1985).

The genus *parainfluenza virus* (PIV) belongs to the family *paramyxoviridae* (Melnik, 1985). It displays antigenically distinct types of PIV-1, PIV-2, PIV-3 and PIV-4 of which PIV-4 has been characterized into subtypes of 4A and 4B (Canchola *et al*, 1964). PIVs are antigenically stable and virions display hemagglutinin and neuraminidase activity. PIV-1 causes severe epidemic while those due to PIV-2 are less severe and less frequent. PIV-3 causes infections of longer duration. (McIntosh and Clark and 1985 and Waner, 1991).

Respiratory syncytial virus (RSV) belongs to the genus *Pneumovirus* of the *Paramyxoviridae* (Brown, 1989). It is an RNA virus with a helical symmetry. It shares properties in common with paramyxoviruses and orthomyxoviruses, but, show a number of differences in the size and diameter of the helix. Cells infected by RSV contain dense cytoplasmic inclusion bodies which contrast sharply with the large, more loosely arranged nucleocapsid which form the inclusions of paramyxoviruses. Neuraminidase and hemagglutinin properties to date have not been recognized in RSV (Melnick, 1991). According to McCnnochie and others (1990) RSV subtype A accounts for more severe morbidity and higher mortality rates in infant pneumonia.

Morris *et al.* (1956) cited in Knight (1973) first isolated RSV from a chimpanzee which was suffering from a cold. The agent isolated was named chimpanzee coryza Agent (CCA). A year later Chanock and his collaborators (1957), identified RSV in secretions of a child suffering from lower respiratory tract infections. After repeated demonstration of CCA from children with ARI, it was renamed respiratory syncytial virus.

The genus *Adenovirus* belongs to the family *Adenoviridae* (Fenner, 1976) and was initially isolated from adenoid tissue which had been surgically excised from asymptomatic children (Rowe *et al.*, 1953 cited in Cooney, 1985). Hambraeus and Wadell (1991) described adenoviruses as a diversified genus. The genome is a double stranded DNA molecule which is resistant to ether, sensitive to heat and has stable to pH (Cooney, 1985).

According to Marchant (1989) over 45 serotypes of adenoviruses have been described, of which serotypes 1, 2, 3, 5 and 7 are most frequently detected clinical samples.

Viruses require special culture systems for their nutritional and laboratory growth requirements. This is because they only multiply within living cells. There is evidence as to the pathogenic process occurring after implantation of viruses. In PIV infections, local multiplication of viruses, veraemia(dissemination of viruses from the primary site of infections to other structures) and reversible desquamation are reported. In RSV

infections , proliferation and necrosis of the bronchiole , blockage of airways from damaged epithelium and increased mucus secretions are reported (Price 1990). The narrowing of the lumen, hyperinfiltration of lung epithelium and complete blockage of airways in infants subsequently leads to inflated lung volume and resistance to expiration (Wohl *et al.*, 1969). These processes may lead to secondary infection by bacterial agents , of which *Staphylococcus aureus* is a primary causative agent (Urquhart and Gibson , 1970).

Sources of viral agents include infected respiratory or oral secretions which come into direct or indirect contact with the respiratory tract of a susceptible person . Transmission may take place horizontally from person to person by kissing , coming in contact with contaminated items such as hands , clothings , eating utensils etc . It may also be transmitted by direct proximity to droplets expelled with coughing , sneezing or any forceful exhalation which contaminates the environment with virus contaminated aerosols (droplet nuclei) which are suspended in the air (Donohue *et al.*, 1955). Subsequently , to cause an infection the concentration of virus in the aerosol , its persistence of viability , its infective dose and the susceptibility of the host cells are the major preconditions (Marchant , 1989 and Melnick, 1985).

Adenoviruses are also reported to be transmitted via the fecal-respiratory tract route or birth canal-respiratory tract route (Knight , 1973). Bear and colleagues (1971) reported there were indications that cross infections of human epidemic Influenza A also occurred in pigs , horses as well as wild and domestic birds .

Standard techniques of viral isolation in cell culture and its procedure are described by Jennings and Grant (1967) and Hughes *et al* (1988). In addition, there are several methods presently being used for viral detection, different generations of enzyme linked immunosorbent assay (ELISA) (Obert and Beyer and 1988 and Perceivalle *et al.*, 1989) and Western blot (Jankowski *et al.*, 1990) have been recently introduced.

A number of virological investigations of nasopharyngeal aspirate (NPA) have detected various types of respiratory viruses in infants diagnosed with ARI. In developed countries, such as West Virginia, Belshe *et al* (1983), Yugoslavia, Jelic and Jelic (1990) and Austria, Kellner *et al* (1990) smears using immunofluorescence (IF) staining of NPA samples from infants having ARI have showed the etiologic importance of viral agents. In contrast to these studies, there are limited virological investigations carried out in developing countries (Hazlett *et al.*, 1988).

Viral pneumonia is well reported in developed countries: from Colombia, Central America, Nunez *et al.* (1988), from Europe, Kellner *et al* (1988) and Ostraivik *et al* (1984), from America, Abzu *et al* (1990) reported that viral infections concurrent with other pathogens are immediately acquired from the community after the first week of life. Hall (1984) and Cherian *et al* (1990) and Jelic and Jelic (1990) also reported the importance of bronchiolitis caused by viral agents.

Paisley (1984), Stango (1987) and Brasfiel (1987) and their co-investigators indicated the importance of viral pneumonia in infants in developing countries.

The control of viral ARI appears to be more complex than with many other infectious diseases. Improved socio-economic standards of living, alleviating overcrowding, reducing household smoking and general indoor air pollution,as well as increasing the availability of health facilities may reduce the occurrence of ARI caused by viral agents (WHO, 1991). In addition, the introduction of vaccines is strongly recommended as a mechanism which could serve to combat factors contributing to increased infant morbidity and mortality rates.

B. *Chlamydia trachomatis*

Chlamydiae are classified in the order *Chlamydiales* of a single family *Chlamydiaceae* and a genus *Chlamydia* (Edwards and Ewing, 1976 cited in Barnes, 1989). The three species which have been characterized so far are *Chlamydia trachomatis*, *Chlamydia psittaci* and Taiwanian acute respiratory (TWAR) agent (Grayston *et al.*, 1986) which has been recently renamed as *Chlamydia pneumoniae* (Campbell, *et al.*, 1987). Strains of *Chlamydia trachomatis* exhibit very close relatedness at DNA level. *Chlamydia psittaci* and *Chlamydia pneumoniae* seem to be genetically diverse and less related to *Chlamydia trachomatis*. *Chlamydiae* are related to most of the gram negative bacteria, suggesting that they were related to eubacteria in origin (Moulder, 1966).

According to Barnes (1989), *Chlamydiae* are obligate intracellular parasites which depend on the host cell machinery for their energy requirements. They do not multiply in cell free cultures but primarily propagate within membrane bound inclusions in the cytoplasm of the parasitized cell. Schachter (1991) described that *Chlamydiae* differ from the viruses by possessing both RNA and DNA while cell walls are quite smilar in structure to those of gram negative bacteria. They are susceptible to many broad spectrum antibiotics, possess a number of enzymes, and have a restricted metabolic capacity.

Campbell *et al.* (1987) described that the elementary body of (EB) of Chlamydia have an outer membrane similar to that of many Gram negative bacteria. They also showed that the major outer membrane protein (MOMP) makes up most of the dry weight of the outer membrane. Stephens *et al*. (1982) determined that the MOMP is a membrane protein antigen which has genus- specific, species specific sub-species-specific and type- specific reactive antigens with the monoclonal antibodies (Mabs).

Nurminen and co-investigators (1985) and Brade (1987) reported that the outer membrane is also composed of a lipopolysaccharide (LP) antigen which is structurally similar to that of the LP of *Salmonella minnesota* strains. Dhir *et al*. (1971) described that the extractable chlamydial LP is the antigen detected in genus- specific tests for *Chlamydiae*.

According to Rice (1969), cited by Barnes (1989), *Chlamydia trachomatis* has a very limited host range of only human beings and mice. However, *Chlamydia*

psittaci is reported to have a wide host range of different vertebrates of veterinary importance , such as birds , sheep , cattle and others. The limited host specificity as well as morphologic and in vitro virulence characteristics distinguish *Chlamydia trachomatis* from other species of the genus . It has a characteristic elementary body (EB) . It is vacuolar , generally single , refractile in bright field microscopy and has a granular appearance on Giemsa staining due to a deposition of glycogen which makes its detection possible using iodine staining .

Grayston (1975) and Wang *et al*. (1985) serologically classified *Chlamydia trachomatis* strains into 3 serotypes of *Lymphogranuloma venerum* (LGV) , and 12 serotype of trachoma. Syva (1987) also described 15 human serotypes of oculo-genital (A, B, Ba, C, D, E, F, G, H, I, J, K) and *Lymphogranuloma-venerum* (LGV) serotype of L1,L2, and L3 on the basis of the antigenicity of MOMP. According to Dement'eva *et al*, (1990), these oculo-genital serotypes of B to K strains are the ones which are potentially transmitted during the process of birth from the genital tract of infected mothers to their neonates resulting in pneumonia .

Identification of a number of distinguishable serotypes has been facilitated with the use of monoclonal antibodies and resulted in a more detailed knowledge of the antigenic similarities within *Chlamydia trachomatis* strains (Wang *et al.* ,1985) .

According to Schachter (1978) , Todd *et al* (1985) and Schachter (1984) the replicative cycle of *Chlamydiae* begins with the ingestion of a spore like non-vegetative

elementary body (EB). The EB attaches itself to the surface of susceptible host cell receptors and stays within the parasitized cell. It enters the host cell in a phagocytotic vesicle derived from the host cell surface membrane where it reorganizes itself into dividing, metabolically active, initial forms of reticulate body (RB). Within the membrane bound vacuole, RB synthesizes new materials and divides by binary fission. It ceases dividing in 18-24 hours. The overall replicative cycle requires approximately 48-72 hours. Hundreds of daughter organisms in various stages of EBs and intermediate forms are the features of its multiplication. During this process all the stages remain within the expanding vesicles. By the time the stages mature to infectious EBs, the vesicle either ruptures or is lysed resulting in new infective EBs being released.

Chlamydia trachomatis grows in a special culture of cells such as monolayer cell culture of certain cells like buffalo green monkey kidney cells were developed for research and diagnostic activities. The isolation of *Chlamydia trachomatis* in embryonated chicken's egg culture, was developed and used for detection in the late 1950s (Jones *et al.*, 1959 and Gordon *et al.*, 1969). Standard procedure presently utilized for chlamydial culture techniques is described in CDC (1985) and it remains as one of the methods of choice due to its sensitive in identifying of the organism.

Beem and Saxon (1977) and Alexander and Harrison (1983) found that nasopharyngeal aspirates (NPA) are recommended clinical samples for the detection of the etiologic agents of *Chlamydia trachomatis* in infant pneumonia.

Infections caused by *Chlamydia trachomatis* are currently recognized to cause one of the most prevalent and insidious of the sexually transmitted diseases (STDs) (Schiefer and Krauss, 1990). It is reported that the infection is approximately 2.5 times more prevalent than the gonococcal infections, and yet, most infections remain asymptomatic (Thompson and Washington 1983 cited in CDC, 1985). Its infections affect more women than men and result in mucopurulent cervicitis (MPC), pelvic inflammatory diseases (PID) and non-gonococcal urethritis (NGU). The organism has been isolated in 30 to 50% of the women who experience cervico-vaginal discharges (Brunham *et al.*, 1984). Mothers who developed cervical chlamydial infections potentially transmit the infectious agent in utero or during delivery to their neonate as passage occurs through infected cervix (Schiefer and Krauss, 1990). The acquisition of the infectious agent reportedly occurs in 50% of infants vaginally delivered from infected mothers (Alexander, 1983 and Schiefer and Krauss, 1990). However, Dement'eva *et al* (1990) reported only approximately 20.8% of the infants had the potential to develop chlamydial pneumonia during the first 28 days of their post-natal period.

According to Graham and Blanco (1990), in the United States, each year 155,000 infants are exposed to *Chlamydia trachomatis* infection during pregnancy and delivery, of which more than 100,000 infants are at risk of infection. Of the total 30,000 reportedly will develop pneumonia. Roblin *et al* (1989), Obert and Beyer (1988) and Numazaki *et al* (1989) pointed out that *Chlamydia trachomatis* as an etiologic agent of neonatal pneumonia presented an increasing health problem.

Meguro *et al* (1988) also reported super-infection by *Chlamydia trachomatis* of viral pneumonia in RSV infections. Chandwani *et al* (1983) reported that RSV infections in Human Immunodeficiency Virus (HIV) infected infants developed pneumonia with a mortality rate of 20%.

C. Immunofluorescence Technique And Its Application In Viralt And Chlamydial Etiology Of ARI

The rapid method of immunofluorescent antibody technique (IFAT) was first pioneered for the detection of RSV in 1972. Subsequently it has been utilized for virological investigations in infants and young children having ARI (Ostravik *et al.*, 1980 and 1984). Adequately collected NPA, followed by processing and fixing cells on slides, in acetone for viral detection or in methanol for chlamydial detection, makes the antigens and cell components permeable to reacting monoclonal antibodies.

Immunofluorescence antibody technique is performed using either a direct or an indirect method (Pozzeto *et al.*, 1988 and Roorda *et al.*, 1990). The direct method is originally devised by Coons, *et al*. 1942 cited in Gardner *et al* (1980). In this method the staining of smears makes use of fluorescein isothiocyanate (FITC) conjugate labelled monoclonal antibodies (Mabs). The antibodies are allowed to bind with the antigen in the sample, are incubated at 37 o C in a humidified chamber. The smears are then washed three times to remove any unreacted conjugated Mabs.

The indirect method was first introduced by Waller and Coons in (1954) cited in Gardner *et al* (1980). Here the staining involves smears using unlabelled Mabs

which are allowed to bind with the antigens in the sample. Again, incubation occurs at 37 o C in a humidified chamber. After washing the smears three times a second Mabs, labelled with FITC conjugate is added and allowed to fix to the bound antibody. The bound antibody acts as an antigen, which reacts specifically with the gamma globulin fraction of the Mab and forms a complex which gives a brighter fluorescence (Gardner *et al.*, 1980).

According to WHO (1981) this antigen-antibody reaction allows the antibody molecules to chemically react and bind without destroying its immunologic specificity. When conjugated antibody is added to the antigen-antibody complex molecule, it is deposited onto the antigenic sites (epitopes).

The specific viral antigen-antibody complexes can be observed under a fluorescent microscope. Cytoplasmic fluorescence with cytopathic effect (CPE) of inclusion-like bodies is observed in RSV and PIVs (Perceivalle *et al.*, 1989). In influenza, viral fluorescence can either be nuclear or cytoplasmic while in adenoviruses only nuclear fluorescence is observed. These characteristics, of diagnostic value, are due to individual patterns of multiplication and assemblage inside the cytoplasm and/or nucleus of the infected cells (Gardner *et al.*, 1980).

World Health Organization (1981) recommends that the choice of utilization of either direct or indirect method be based on the number of antigens expected in the sample. If few antigens are being assayed, the direct method is recommended, whereas, when several antigens are to be detected, the indirect method is preferred.

On the other hand, according to Pozzetto *et al* (1988), the choice is a matter of personal preference. However, according to Rompalo *et al* (1987) and Paisley *et al* (1986), the direct method is a recommended method of choice for the detection of *Chlamydia trachomatis* in NPA samples. Over 90% of the viral agents causing severe ARI (WHO, 1981) and up to 92% or more of *Chlamydia trachomatis* causing infant pneumonia (Barnes, 1989) are detectable with the IFAT.

D. Purpose Of Invetigation

This study attempted to establish the importance of viral and chlamydial etiology of ARI in infants. The study is part of a Multicenter Collaborative WHO study on CLINICAL AND ETIOLOGICAL AGENTS OF PNEUMONIA, SEPSIS AND MENINGITIS IN YOUNG INFANTS. It is based at the Ethio-Swedish Children's Hospital (ESCH) and facilitated by the Department of Pediatrics and Child Health, Addis Ababa University (AAU). The results of the study are to be used by the WHO to develop ARI control strategies for developing countries. It is also to provide information to national health planners to design control strategies in Ethiopia.

Specific objectives of the study are:

. to detect the frequency of respiratory syncytial virus, parainfluenza virus, influenza virurs types A and B and adenoviruses using the indirect immunofluorescence method in NPA smears of ARI cases and healthy control infants;

. to detect the infection rate of *Chlamydia trachomatis* using the direct immunofluorescene method in NPA smears of the cases and controls;

. to define age and sex distribution of the etiologic agents detected in relation to the clinical diagnosis ;

. to assess the seasonal distribution of the detected viral agents during the study period and assess the association of viral infections with the meteorological factors ;

. utilize a questionnaire for the assessment of preliminary socio-economic factors as potential risk factors associated with viral ARI .

II. MATERIALS AND METHODS

A . Study Area And Population Characteristics

Addis Ababa, with an estimated population of 2 million, is the capital city of Ethiopia . The city has a subtropical climate and the months of heavy rains extend from June to September or October. ARI is commonly occurs during these rainy months .

Most of the study population were from a low to middle level of socio-economic standard of living . Most of the families lived in a crowded situation several people shared a bed and/or lived in the same room . The characteristics of the population on size of family, address , no. of rooms shares , education of mothers, age of the infant, breast feeding of the infant and age of the mother are shown in Annex 7 .

Two hundred twenty five cases (118 males , 107 females) and 178 healthy control infants (male 89 ,female 89) up to the age of one year visited the ESCH, Addis Ababa , for medical care between January 1992 and December 1992 were enrolled for the study . Infants up to the age of 12 months , having typical signs and symptoms of ARI (cough , difficulty in breathing , fever , poor feeding , running nose, etc.) who had not been treated with antibiotics within the preceding 5 days were enrolled as cases of ARI Those of the same age group and residence areas , who visited the ESCH for non-ARI medical care were selected as matched controls . A nurse stationed at the pediatric out-patient department (OPD) screened the study population enrolled in the study. A questionnaire (Annex-8) inquiring on preliminary socio-economic data was filled in . The clinical diagnosis was established by the participating pediatricians .

B. Collection And Processing Of Nasopharyngeal Aspirates

Sterile disposable plastic extractors attached to a feeding tube FG 8 (Medico Plastic , West Germany) was used to collect the sample . A single NPA was aspirated in sequence from each nasopharynx by a hand operated vacuum pump (Nalgene , West Germany) . NPA samples were collected following procedures outlined in Annex- 3 according to the procedures described by Gardsner *et al* (1980) . The aspirates were washed into the tubes using Phosphate Buffered Saline (PBS) of a chemical compostion given in Annex - 2 at a pH of 7.6 and stored at 2-8 o C . Samples were processed following standard procedures (Annex 4) as described by Gardner *et al* (1980) . The smears of the re-suspended pellet cells were made in duplicate on two-eight well slides (WHO, Geneva) for viral detection. Duplicate slides on one-well Erie Scientific slides (Syva , MicroTrak) were made for detection of *Chlamydia trachomatis.* The slides for viral detection were fixed in acetone at + 4 o C for 10 minutes while the other slides for chlamydial detection were fixed in absolute methanol for 5 minutes at room temperature . Slides were stored at - 40 o C in batches of 5 slides each in a box provided by the WHO until IF stained .

C. Viral Assay

The monoclonal antibodies to RSV , PIV , Flu-A , Flu-B and adenoviruses (CDC, Atlanta) were obtained from WHO, Geneva . They were shipped in dry ice and stored at - 20 o C until assayed . The fluorescein isothiocyanate (FITC) conjugate (Dakopatts, Denmark) was also obtained from WHO, Geneva. Additional Mabs were

kindly supplied by the National Bacteriological Laboratory (NBL), in Stockholm, shipped in dry ice and kept at 2-8 o C . In addition , the control slides for each virus were also obtained from NBL and stored at - 40 o C until assayed .

The Mabs to RSV , PIV, Flu-A, Flu-B and adenoviruses were strong stock reagents 1:1 dilution. Working dilutions of Mabs were made with PBS at pH 7.6 . Serial Doubling dilutions of the Mabs were made for monoclonal antiboies to RSV, Flu-A, Flu-B and adenoviruses , starting at 1:50 and proceeding up to 1:1600. For the PIV pool , serial doubling dilutions were made starting at 1:25 , progressing up to 1: 800 dilutions. The conjugate stock reagent 1:1 dilution was diluted starting at 1:5 progressing up to 1:160 . The control slides from NBL were stained following procedures described in Gardner et al (1980)for IIF with the respective dilutions . The optimal dilution of 1:50, 1:100 and 1:20 for PIV , other viral Mabs and conjugate respectively , yielded the best characteristic intense green fluorescence (Annex 1) . The same working dilution is used in viral assays by NBL, Stockholm , Sweden .

D . *Chlamydia trachomatis* Assay

The FITC conjugated Mabs for *Chlamydia trachomatis* Direct Specimens Test were reconstituted using a 2 ml reconstituting fluid . The fixed smears were stained following the DIF staining procedures by Syva (1987) (Annex-6) .

E . Immunofluorescence Staining Technique

Monoclonal antibodies to RSV , PIV, Flu-A, Flu-B and adenoviruses, the conjugate, batch control slides and fixed slides of patient samples were thawed to room temperature. The smears were IIF stained in the manner described by Grandiner *et al* (1980) in (Annex-5) . The slides were examined under 40 X objective of a fluourescence microscope (Will Leitz, West Germany,) under incident UV blue light . Those smears with a characteristic fluorescence of one or more cells were recorded as (1+) , (2+) , (3+) . Representative stained slides with their results and unstained duplicate slides were sent to NBL for double confirmation .

The conjugated Mab for *Chlamydia trachomatis* was reconstituted and the fixed and control slides were brought to room temperature . Following Direct Specimen by Test, syva (1987) the fixed slides were stained (Annex - 6) .

F. Statistical Methods

Laboratory results and other related data were coded into a manageable form and entered into an EPI - INFO Version - 5 microcomputer statistical analysis programme (Dean *et al* ., 1990) based at the Department of Pediatrics and Child Health , Addis Ababa University . Frequencies, Chi-Squares and P- values were subsequently calculated.

III. RESULTS

A total of 74 (32.9%) infants had detectable viral antigens in their samples. Additionally, a total of 36(16%) NPA samples were positive for *Chlamydia trachomatis* antigens. The overall detection rate was 110(48.9%) for viral and *Chlamydia trachomatis* agents. Respiratory syncytial virus found in 70(31.1%) infants was the most frequently detected viral agent. Parainfluenza virus detected in 2 infants (0.9%) and influenza virus type A also detected in 2 infants (0.9%) were the least detected viral agents.

Influenza virus type B and adenovirus were not detected. Of the 178 apparently healthy control infants investigated, only RSV was detected in the NPA smears of 5 infants (2.8%). A Significantly large proportion of the case study infants demonstrated detectable viral antigens compared with the control infants (Chi-Squares Yates corrected = 52.62, $P<0.001$). A Significant difference was observed among study cases in the viral detection rate compared with that of *Chlamydia trachomatis* (Chi-Squares Yates corrected =6.92, $P<0.001$) (Table 1).

Table 1 .Viruses and *Chlamydia trachomatis* and their frequency by IF detection in NPA of 225 cases and 178 control infants .

	C A S E S	CONTROLS
Viruses/ *Chlamydia trachomatis*	frequency of detection +ve (%)	frequency of detection +ve(%)
A. type of virus (IIF)		
Respiratory syncytial virus	70 (31.1)	5(2.8)
Influenza virus type A	2 (0.9)	ND
Influenza virus type B	ND	ND
Parainfluenza virus	2 (0.9)	ND
Adenovirus	ND	ND
Total	74 (32.9)	5(2.8)
B. *Chlamydia trachomatis* (DIF)	36 (16.0)	ND
Total	110(48.9)	5 (2.8)

Key: IF-immunofluorescence, NPA-nasopharyngeal aspirate IIF-indirect immunofluorescence technique, DIF - direct immunofluorescence technique, ( ) parenthesis - percent , ND - not detected

Table 2 shows mixed infections of RSV and *Chlamydia trachomatis* where 14(6.2%) were observed among the 225 cases and not among the controls .

Table 2 . Frequency of mixed infections of viruses and *Chlamydia trachomatis* in 225 cases and 178 controls .

	cases	controls
Etiologic agent	+ve no(%)	+ve no(%)
Respiratory syncytial virus/ *C.trachomatis*	14(6.2)	ND
Influenza virus type A/ *C. trachomatis*	ND	ND
Influenza virus type B/ *C. trachomatis*	ND	ND
Parainfluenza virus / *C. trachomatis*	ND	ND
Adenovirus /*C. trchomatis*	ND	ND
Total mixed infections	14(6.2)	ND

Key: *C. trachomatis* - *Chlamydia trachomatis*, ( ) parenthesis- percent, ND - not detected

Table 3 shows 18.2% viral detection occurred in infants of up to 2 months of age , 6.2% from 3 to 5 months, 5.3% were ages 6-8 months and 3.1% ages 9-12 months . Fifty- seven percent of RSV infections detected were in infants up to 2 months of age indicating a significant difference when comparing the detection rate in infants aged 3 - 12 months ($P<0.05$) . Similarly , *Chlamydia trachomatis* was detected with a significant frequency in infants of up to 2 months of age compared to those aged 3 to

12 months 12.4% and 3.5% respectively (P<0.05). The highest proportion observed in the age group up to 2 months was 18.2% and 12.4%, respectively, for virus-positive specimens and *Chlamydia trachomatis*.

Table 3. Age distribution of positive cases and control infants for the etiologic agents detected in 225 cases and 178 controls.

	cases					controls
	age					
Type of virus	0-2(mo)	3-5 (mo)	6-8(mo)	9-12 (mo)	all ages	all ages
	no (%)	no (%)	no(%)	no(%)	no(%)	no(%)
syncytial Respiratory virus	40(17.8)	14(6.2)	12(5.3)	4(1.8)	70(31.1)	5(2.8)
Influenza virus type A	1 (0.4)	ND	ND	1(0.4)	2(0.9)	ND
Influenza virus type B	ND	ND	ND	ND	ND	ND
Parainfluenza	ND	ND	ND	2(0.9)	2(0.9)	ND
Adenovirus	ND	ND	ND	ND	ND	ND
Total	41(18.2)	14(6.2)	12(5.3)	7(3.1)	74(32.7)	5(2.8)
C.trachomatis	28(12.4)	3(1.3)	1(0.4)	4(1.8)	36(16)	ND
Grand total	69(30.7)	17(7.5)	13(5.7)	11(4.9)	110(48.9)	5(2.8)

Key: Mo - month, *C.trachomatis* - *Chlamydia trachomatis*, ND - not detected

Mixed infections of RSV and *Chlamydia trachomatis* encountered by age groups are shown in Table 4. Six percent of *Chlamydia trachomatis* infected babies showed mixed infection with RSV. In comparison with the rest of the age groups, a significant frequency of RSV and *Chlamydia trachomatis* mixed infections (Chi-Squares Yates Corrected = 8.15, $P<0.01$) in the age group of up to 2 months of age was observed.

Table 4. The distribution of respiratory syncytial virus and *Chlamydia trachomatis* detected in 36 *Chlamydia trachomatis* positive cases by age group.

	R S V and *C. trachomatis*
AGE GROUP (mo)	+ve no(%)
0 to 2	10(27.8)
3 to 5	3(8.3)
6 to 8	1(2.8)
to 12	ND
Total	14(6.2)

Key : RSV- respiratory syncytial virus, *C.trachomatis* - *Chlamydia trachomatis.*
ND - not detected

Table 5 shows the over all viral detection rate in males was 14.7% and in females 18.2% indicating that there is a slightly higher rate of viral infection in females. Conversely, the detection rate for *Chlamydia trachomatis* was 10.7% for males and 5. 3% for females, indicating

a higher rate of infection in males than in females. In both sexes RSV was the most prevalent etiologic agent. *Chlamydia trachomatis* was not detected in any control infant in both sexes whereas, RSV was detected in 5 infants comprised of both sexes from 178 controls.

Table 5. Sex distribution of 225 cases and 178 control infants in whom the etiologic agents were detected

Etiologic agent	cases		controls	
	sex			
	M	F	M	F
	+ve no(%)	+ve no(%)	+ve no(%)	+ve no(%)
Respiratory syncytial virus	31(13.8)	39(17.3)	2(1.1)	3(1.6)
Parainfluenza	1(0.4)	1(0.4)	ND	ND
Influenza virus type A	1(0.4)	1(0.4)	ND	ND
Influenza virus type B	ND	ND	ND	ND
Adenovirus	ND	ND	ND	ND
total	33(14.7)	41(18.2)	2(1.1)	3(1.6)
C.trachomatis	24(10.7)	12(5.3)	ND	ND
TOTAL	57(25.3)	53(23.6)	2(1.1)	3(1.6)

Key: M- male ; f- female , *C.trachomatis* - *Chlamydia trachomatis*, ND - not detected

The association of each clinical diagnosis with each etiologic agent is shown in Table 6 and can be seen that RSV is the most prevalent agent . Similar RSV detection rate of 16% and 14 .2% was observed in AURI and pneumonia respectively.

Table 6 . Association of clinically diagnosed cases with each etiologic agent as detected in 225 cases

Clinical Diagnosis	Type of viruses					
	RSV	PIV	Flu-A	Flu-B	Adeno	*C.trachomatis*
	+ve no(%)	+ve no(%)	+ve no(%)	+ve no(%)	+ve no(%)	+ve (%)
AURI	36(16)	2(0.9)	1(0.4)	ND	ND	17(7.6)
Pneumonia	32(14.2)	ND	1(0.4)	ND	ND	18(8)
Others	2(0.9)	ND	ND	ND	ND	1(0.4)
Total	70(31.1)	2(0.9)	2(0.9)	ND	ND	36(16)

Key: RSV - respiratory syncytial virus, PIV - parainfluenza virus, Flu- A - influenza virus type A, Flu- B- influenza virus type B , Adeno - adenovirus, *C.trachomatis* - *Chlamydia trachomatis* AURI - acute upper respiratory infection, others - sepsis / menin ND - not detected

Of the 225 cases studied AURI accounted for 59.1% of the cases making it the most common clinical syndrome . Of these, 17.3% demonstrated detectable viral antigens . Pneumonia accounted for 35.1% of the cases which made it the second important clinical syndrome . Of the pneumonia cases , 14.7% had detectable viral antigens .

Similar viral detection rates were observed in AURI and in pneumonia. However, there was a significant difference between clinical diagnosis and IF detection for viruses (Chi-Squares Yates corrected = 21.7, $p<0.001$). The diagnosis of AURI and pneumonia caused by *Chlamydia trachomatis* accounted for 7.6% and 8.0% of cases, respectively; showing a similar rate of detection (Table 7).

Table 7. Association of clinical diagnosis with *the* etiologic agents detected in 225 cases

Clinical diagnosis	clinical cases no(%)	virus positive cases no (%)
A. Virus		
AURI	133(59.1)	39(17.3)
Pneumonia	79(35.1)	33(14.7)
Others	13(5.8)	2(0.9)
Total	151(67.1)	74(32.9)
B. Chlamydia trachomatis		
AURI	133(59.1)	17(7.6)
Pneumonia	79(35.1)	18(8.0)
others	13(5.8)	1(0.4)
Total	189(84)	36(16)

Others - Sepsis ,Meningitis, AURI - acute upper respiratory infection, C. trachomatis - *Chlamydia trachomatis.*

Table 8 indicates RSV was detected in 68(31%) of the 216 patients whose residence was in Addis Ababa while 2(22.2 %) of the 9 cases in whom RSV was detected resided outside of the City . Detection of RSV from those residing outside of Addis Ababa compared favorably with detection rate from patients living in the City .

Table 8 . Respiratory syncytial virus infection in relation to residence in 225 cases

		RSV +ve	*C.trachomatis*
Residence	no(%)	no (%)	no (%)
Addis Ababa	216(96)	68(31.4)	35 (16.2)
Outside Addis Ababa	9(4)	2(22.2)	1(11.1)

Key: RSV - respiratory syncytial virus , *C.trachomatis* - *Chlamydia trachomatis*

Table 9 demonstrates the assessed relationship of socio-economic factors to RSV infection. Among mothers with higher level of education, i. e. secondary/ highschool / college, 16.8% of their infants were RSV positive while among those with lower level of education, i.e. illiterate/ elementary education , 14.2% of their infants were RSV positive . This indicates that there is not significant association between viral infections in infant to mothers level of education.

Among infants exposed to passive smoking , 8.9% were RSV - positive; whereas of infants from non -smoking households, 22.2,% were RSV- positive with a higher rate of detection of RSV in non- smoking families. Therefore, the negative effect of passive smoking to RSV infection has not been validated in this study.

Among 193 breast fed infants ,30.5% were RSV positive and of the 32 non-breast fed infants, 34% were RSV -positive; hence, slightly higher RSV infection was observed in non- breast fed infants . Therefore, an indication of the beneficial impact of breast feeding on RSV infection has been traced in this study.

Table 9. Respiratory syncytial virus detected in 225 cases and the relationship of some risk factors

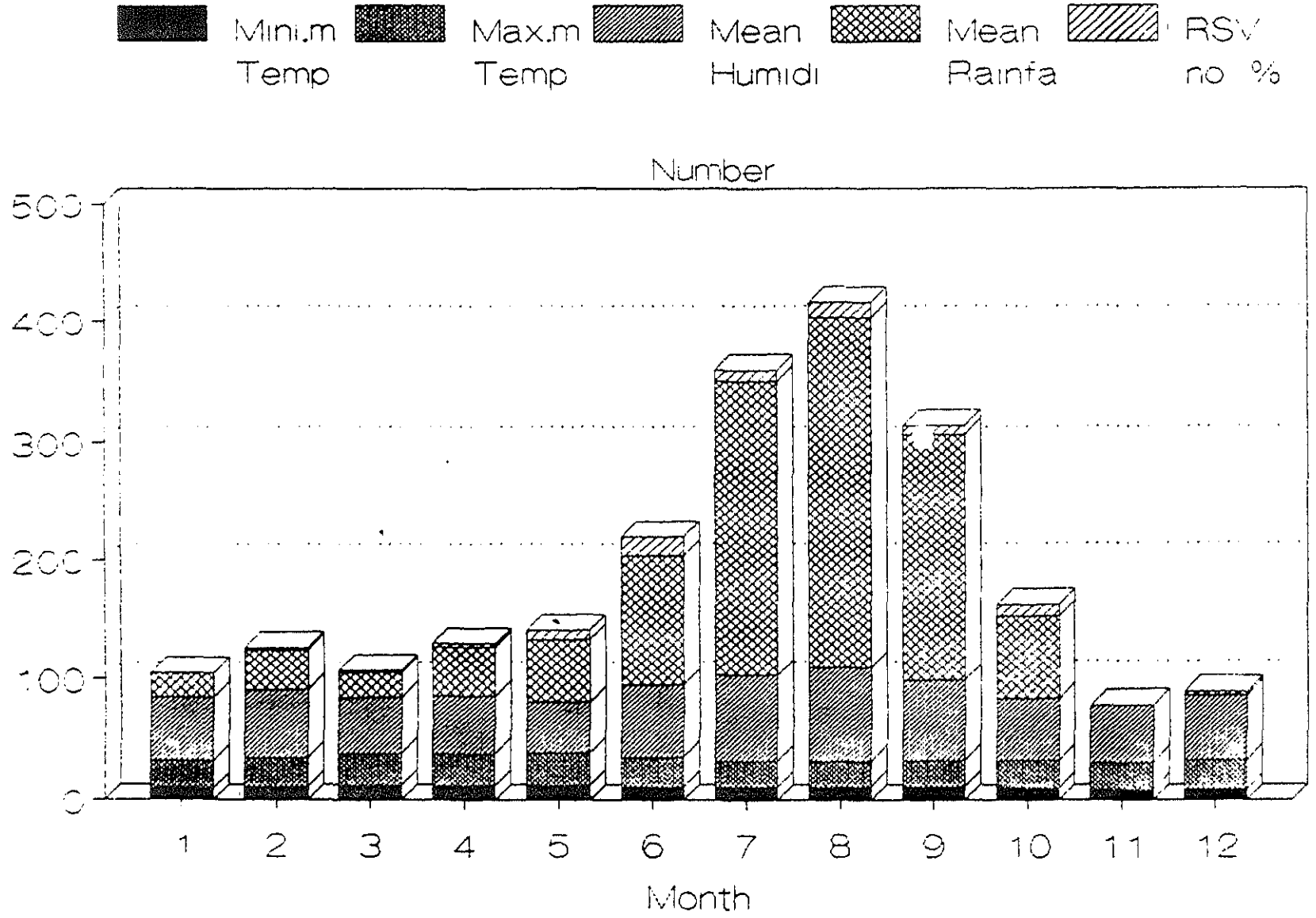
Exposure	RS +ve no(%)
A. Mother' s Education:	
Higher level	38(16.8)
Lower level	32(14.2)
B. passive smoking :	
smoking	20(8.9)
non- smoking	50(22.2)
C. Breast feeding	
breast fed	59(30.6)
non- breast fed	11(34.0)

Key: High level - secondary & High school , college graduates , Low - illiterate & elementary , EDUM - education of mother ,

Figure 1 shows the seasonal distribution of RSV in relation to meteorological factors during the twelve months of the study period. Monthly maximum mean temperatures and minimum mean temperatures were similar throughout the year. Higher relative humidity (RH) and higher amount of rainfall were recorded during June to September months. RSV peak was observed in June with a detection rate of 22.9% out of the total RSV detection. In addition, high detection rate of RSV was also observed around the peak. The peak was observed within a period of marked increase in the amount of rainfall. Beyond June even a much higher amount of rainfall recorded, the peak did not change. RSV was detected throughout the year except in November, December and January when low amount of rainfall was recorded.

FIG 1. Seasonal distribution of RSV positive 70 cases in relation to meteorological factors

Fig.1 Seasonal Distribution of RSV
Jan. 1992 - June 1992



Meteorologoca; data amd RSV detectopm fpr Fig. 1.

Month	Mini.mean temperature	Maxi. mean temperature	mean relative humidity	mean rainfall	RSV +ve no(%)
1	10.0	22.4	52	20.2	0(0.0)
2	11.1	23.3	56	33.3	2(2.9)
3	12.4	25.7	46	20.2	2(2.9)
4	12.4	25.6	47	41.0	3 (4.3)
5	12.7	25.5	42	52.0	8(11.4)
6	11.3	23.7	59	109.1	16(22.9)
7	11.0	20.7	70	248.5	9(12.9)
8	11.8	19.7	77	294.7	13(18.6)
9	10.9	21.0	65	209.4	7 (10.0)
10	10.1	21.7	50	69.7	10(14.3)
11	8.8	21.3	46	0.0	0(0.0)
12	9.3	22.8	52	2.9	0(0.0)

Key: 1- January, 2- February , 3- March, 4- April , 5 - May , 6 - June, 7 - July, 8 - August, 9 - September, 10 - October, 11 - November , 12 - December, Maxi. Temp. - Maximum temperature , Mini. Temp. - minimum temperature, Mean values of temperature in degree o C, rainfall in mm & relative humidity in % .

N.B :- Daily Mean Temperature = $\frac{\text{Max. Temp. +Min. Temp.}}{2}$

Source - National Meteorological Service Agency , Addis Ababa (1992)

IV. DISCUSSION

This study is the first in Ethiopia to use rapid viral detection methods in order to demonstrate the etiology of viruses and *Chlamydia trachomatis* causing ARI in infants. The 32.9% viral detection rate achieved in this study is higher than the 26% detection rate obtained by Kellner *et al* (1990) from Austria, a developed country with temperate climate, and 20% rate by Sutmoller *et al* (1983) from Rio de Janeiro, a developing country, with a tropical climate.

Higher viral detection rates, 71% were reported from India, a developing country in South East Asia, by Cherian *et al* (1990), 54% by Hazlett *et al* (1988) from Kenya, a developing country in East Africa, and 42.4% from Europe by Kellner *et al* (1988). However, this study is in agreement with the 33% detection obtained by Nunez *et al* (1988) from Colombia, Central America which is a developed country.

The 31.1% frequency of RSV obtained in this study was higher than the 20.8% frequency of detection from Nairobi by Wafula *et al* (1985) and the 12.7% from Beijing by Zi-jing *et al* (1986). However, Forgie *et al* (1991) from The Gambia reported 37.5% which is similar to the findings in this study. In contrast, studies by Carlsen *et al* (1983) from Norway, Abzu *et al* (1990) from Colorado, North America, both from developed countries, showed higher frequencies of detection, 58% and 55% respectively.

The fact that RSV is a leading viral etiologic agent of ARI in infants is well documented by the results obtained in a number of previous studies (Gelezen *et al.*, 1973, Henderson *et al.*, 1979 and Ostravik, 1980).

Thus, the frequency of RSV compared with other viral agents detected in this study demonstrates that it is the most important viral agent causing ARI in infancy in Addis Ababa.

The 0.9% detection rate of parainfluenza virus in this study is lower than the 7.5 % detection rate from Colorado, a temperate climate, by Abzu *et al* (1990), a 7 % rate from Rio de Janeiro, by Sutmoller *et al* (1983). The low frequency of detection of PIV in this study is possibly due to its infections being observed more frequently in children greater than 12 months of age (Monto, 1973, cited in Kellner *et al.*, 1988) thereby detection reasonably being limited by the age restrictions established for this study.

In addition, the 0.9% frequency of influenza virus type A in this study is also lower than the 9% frequency result obtained from Latin America by Sutmoller *et al* (1983). However, our result is similar to the 1.1% from China by Zi-jing *et al* (1986) and the 1.1% from East Africa by Wafula (1985).

Influenza virus type B has not been detected in this study. This is comparable to the results obtained from Europe by Kellner *et al* (1988) in infants having ARI. The low rate of detection of influenza virus type A or not detecting influenza virus type B may be explained by RSV interference phenomena by which RSV is the dominant agent, other viral

agents were reported to be suppressed (Glezen , 1973). Or may be because of the possible absence of influenza virus type B infections in the general population during our study period as the prevalence is increased every 3 to 7 years (Knight, 1977).

The detection rate of adenovirus in this study contrasts with the reported frequency of 47% and 12.2% depicted by Sutmoller *et al* (1983) in Rio de Janeiro and Zi-jing *et al* (1983) in China, respectively. The basis for its apparent absence is unclear. Whether there are yearly variations in the prevalence of adenoviruses or whether these viruses play a less important role in ARI in young infants in has not been confirmed and requires further detailed investigation.

On the other hand, adenoviruses were reported to be of low frequency by other studies from developing countries (Forgie *et al.*, 1991 and Ong *et al.*, 1977).

The descending order of frequency of detection, RSV-parainfluenza/ influenza virus pattern, in our study was consistent with the results from Kenya and Uganda (Hazlett *et al.*,1988 and Sobslavisky *et al.*, 1977) respectively. But our results differ from those reported from Kenya which showed adenovirus - influenzavirus - RSV pattern in a decreasing order of importance (Sutmoller *et al.*, 1983). Another order of importance of RSV-adenovirus-parainflunzavirus was also reported in China and in North America (Zi-jing *et al.*, 1986 and Abzu *et al.*, 1990) respectively indicating a different pattern of decreasing order importance .

The 16% frequency of detection of *Chlamydia trachomatis* in this study is lower than the 37 % frequency by Datta *et al* (1988) and the 28% by Hammerschlag *et al* (1990) . Nonetheless , lower frequency of 3.3% is reported from Colombia (Nunez *et al* . , 1988) . However , our finding was favorably comparable with the 14% frequency reported by Forgie *et al* (1991) .

The etiologic importance of *Chlamydia trachomatis* is in infant ARI is in accord with other study results by several workers (Broadbent and O'Leary , 1988 , Numazaki *et al* . , 1989 and Roblin *et al* . , 1989) .

In this study an 18.2% viral detection rate was presented in the ages up to 2 months of age, whereas the frequency of viral detection in ages of 3-12 months accounted for 14.6%. On the other hand , RSV alone contributed to a frequency of 17.8% in ages up to 2 months and 13.3% in ages 3-12 months.. This demonstrates that majority of RSV infections in ARI is age dependent and also shows an inverse relationship as age increases which is in agreement with a study by Roorda *et al* (1990) from Russia. RSV is the most frequently detected viral agent within the age group up to 2 months is also shown by others elsewhere in the world (Glezen and Denny, 1973 and Jelic and Jelic, 1990). The inverse relationship of virus detection rate to age increments may be explained by the possible presence of locally neutralizing antibodies develop as age increases (McIntosh , 1987). This hypothesis is supported by a study report by WHO (1980) that intranasal viral inoculation to volunteer adults indicated the cessation of shedding of viral antigens with the development of detectable locally neutralizing antibodies . Another possible explanation is that as age increases there might be a shift to other respiratory pathogens (Kellner *et al.*, 1988).

Similarly the 12.4 % frequency of detection of *Chlamydia trachomatis* in the age group of up to 2 months when compared with the 3.6% frequency observed in the groups 3-12 months, significantly higher rate of detection ($P< 0.05$) was shown in the age groups up to 2 months. Hence , the etiologic role of *Chlamydia trachomatis* indicated in this study during early infancy is in accord with results from other parts of the world (Harrison *et al.*, 1978, Beem *et al.*, 1977 and Alexander and Harrison , 1983).

The 33 and 41 ratio of viral detection in male and female infants respectively , indicates that females were slightly more affected than males. In contrast to our findings Carlsen and Ostravik from Oslo, (1980) reported that males were more affected than females. However, Forgie *et al* (1991) from The Gambia reported male to female viral detection ratio of 15:17 respectively and RSV male to female ratio of 9: 12 respectively , in which males were less affected than females, was in agreement with our study. Frequency of chlamydial detection ratio in males and females , 10.7% and 5.3% respectively, demonstrates its increased prevalence of infection in males . Our result indicating that males were more affected than females by chlamydial infection is comparable with that of Datta *et al* (1988) from Kenya. This may be explained by the tendency of males to be more susceptible to an infection in a given population because of genetic factors.

The 16% and 14% detection rate of RSV in AURI and pneumonia respectively, reveals that RSV was the most common viral agent with a similar frequency of detection in both clinical syndromes . Zi-jing *et al* (1986) from China reported a similar detection of 11.5% RSV in AURI and Forgie *et al* (1991) from The Gambia reported a higher detection rate of 37.5% RSV infection in pneumonia.

In this study, out of the 59.1% of AURI cases clinically diagnosed, a frequency of 17.3% were due to viruses. The frequency of detection in AURI shown here is in conformity with the 17% frequency result from Uganda by Sobslavisky *et al* (1977). However, Ong *et al* (1982) and Wafula *et al* (1985) showed higher frequencies of detection of 23.7% and 25% respectively.

Out of the 35.5 % pneumonia cases clinically diagnosed in this study, 14.7% were caused by viruses in this study. This frequency is lower than the 28.2% from Kuala Lumpur by Ong *et al* (1982) and the 30.6% by Wafula *et al* (1985) from Kenya. However, our results are comparable with the 17% frequency by Ong *et al* (1982). The low rate of pneumonia diagnose may be due to mis - diagnosis of. clinical case .

The 8.0% of *Chlamydia trachomatis* detection in pneumonia, in this study are lower than the 14% frequency in pneumonia cases from The Gambia obtained by Forgie *et al* (1991), whereas, in contrast, Nunez *et al* (1988) reported even lower frequency of 3.33 %. Several investigators demonstrated the importance of infant pneumonia caused by *Chlamydia trachomatis* within the first few months of the neonatal period (Dement' eva *et al*., 1990, Schiefer and Krauss, 1990 and Limudomprorn *et al*, 1990).

Ninety - six percent of the study cases resided in Addis Ababa. From these residents 31.4% were RSV positive and 16.2% were *Chlamydia trachomatis* positive. Whereas, from the 4% of residents outside of Addis Ababa, 22% were RSV positive and 11.1% were *Chlamydia trachomatis* positive. Almost all of the cases were from Addis Ababa and

very few infants visited the Pediatric Hospital on referal basis outside of the city. Hence, to depicte an actual prevalence of viruses and *Chlamydia trachomatis* in patients from the City and outside the City , a well planned study is needed . This report simply indicates the proportional detection rate of the etiologic agents. The comparison of this result with any previous reports has not been possible as this is an original study in the country.

The relationship of RSV infection among infant of mothers with higher levels of education versus lower levels showed a proportion of 16.8% and 14.2% , respectively . These results in this study may indicate that education as a socio-economic factory has not shown influence on RSV infection , however, the information obtained may not be sufficient to arrive at a reasonable conclusion at this time. The present result might have been biased because of artificial classification of lumping of "secondary, highschool and college " completed mothers as one category versus " illiterate and elementary " as another category . Also some of the mothers were not giving reliable information regarding their level of education. As the result, there is a need for obtaining additional information. On the other hand, Kellner *et al* (1988) from Viena , Austria, reported that the impact of mothers education cannot be validated in their study.

Among households smoking and not smocking the 8.9% and 22.2% frequency of RSV detection respectively shows a higher frequency of RSV in non-smoking families . This result do not in support of the premise that infants from households where

smoking occurs at a higher risk of viral infection. However, the damaging and sensitizing effect of tobacco smoke on the respiratory structure of man is an established fact (Target *et al.*, 1983 cited in Kellner *et al.*, 1988). Also, Selwyn (1990) reported the view that the prevalence of ARI was higher among children in households having one or more smokers. In addition, majority of infants infected by viruses were observed in age group up to 2 months. This indicates that the time of exposure to passive smoking might have been not enough to show its impact. It is reported that Kellner *et al* (1988) also the impact of passive smoking has not been confirmed in their study.

In an assessment of the influence of breast feeding, the frequency of detection of RSV in ARI, observed in breast fed infants and those not breast fed, (30.6% and 34% respectively), reveals a slightly higher rate of RSV infection in the infants who had not been breast fed; indicating that no beneficial effect of breast feeding has been shown. The finding in this study is similar to the result by Eriksson *et al* (1983) and Hall *et al* (1984) in that a clear beneficial effect of breast feeding in association with RSV infection could not be established.

The objective of this preliminary evaluation of these socio- economic factors was to assess their potential impact on RSV infections. Further investigations on additional factors such as low birth weight, over crowding, indoor air pollution, malnutrition and other factors may be required to arrive at a reasonable conclusion.

The meteorological data establishes an evidence supporting the fact that cold winters favor RSV outbreaks in temperate climates; and, similarly cooler temperatures during higher rainfall months also favor RSV infections in tropical climates. Likewise, in this study,

the high rainfall with cold temperature and relatively higher relative humidity resulted in RSV peak occurrence. A marked increase in the amount of rainfall preceded by relatively drier and probably dusty months resulted in RSV peak season.

This study compares well with increased RSV detection during the months of April to July with high rainfall in East Africa, Kenya (Wafula *et al.*, 1985) and in Central America, Colombia (Nunez *et al.*, 1988). However, several other workers demonstrated that RSV peak seasons in temperate climates were during the cold autumn-winter months or during winter and early spring seasons (Abzu *et al.*, 1990, Jelic and Jelic, 1990 and Kellner *et al.*, 1990). On the other hand, Sobslavisky (1977) reported no seasonal variation was observed in Uganda, East Africa.

V. SUMMARY AND RECOMMENDATIONS

In this study , a literature review was undertaken to determine the importance of viral and chlamydial etiologies of ARI in both developed and developing countries. In addition, literature was also reviewed on laboratory methods of choice for the detection of viruses and *chlamydia trachomatis* .

The study results were compared with results of studies undertaken in developed countries with temperate climate and developing countries with tropical climates. Viral detection from countries with temperates climate indicated lower or similar results to those obtained in the present study. This demonstrates that viral infections are important also in tropical or subtropical climates with low level of socio-economic standard of living. The comparative result from other developing countries showed higher rates of viral detections.

The RSV rate of detection was higher from developed countries while the reported detection rate from developing countries with tropical climates was lower. This demonstrates that RSV infections are more important in temperate climates, although RSV infections also continues to be a significant health factor in infants for countries with tropical climates.

Results of detection rate for *Chlamydia trachomatis* from developed countries indicate a lower prevalence of infection whereas higher results were obtained from developing countries .

The fact that viral and chlamydial infections are predominant in ARI in very young infants of up to 2 months of age is demonstrated by the results of studies from both developed and developing countries .

In the present study frequencies of detection of these etiologic agents from NPA samples showed RSV to be the most important viral agent in mixed infections, age, sex distribution and associated clinical diagnosis. On the other hand, *Chlamydia trachomatis* was found to be the second most important etiologic agent, while the rest of the viral agents were found to be present in very small numbers.

This study also attempted to demonstrate the impact of socio-economic factors, such as education, passive smoking and breast feeding on ARI. The impact of these socio-economic factors on ARI could not be established and this finding is comparable to results obtained in other studies.

Conversely, the impact of meteorological factors in acquiring RSV infections was evaluated and a definite association was observed between increased quantities of rainfall and high relative humidity to the frequency of detection of RSV agents. This indicates that months simulating winter seasons in tropical areas favor RSV infections.

RSV and *Chlamydia trachomatis* were shown to be important etiologic agents in infants with ARI, whereas, in healthy control infants, these etiologic agents were not important.

This study confirms that the immunofluorescence antibody technique (IFAT) which was employed is an important diagnostic tool which should be introduced for rapid viral and chlamydial detection, for research and for diagnostic activities.

Based on the finding of the present study, the following recommendations may be made :

1. Further Investigations should be carried out in Ethiopia to determine the prevalence of viral and chlamydial etiologic agents of ARI in infants. Other causativeagents of ARI such as rhinoviruses , *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Ureaplasma* spp. *Pneumocysstis carinii* and bacteria , also be investigated .
2. Introduce IFAT for viruses and chlamydial agents causing ARI at national level for research purposes .
3. Viral culture and ELISA be initiated for comparative studies with IFAT both for research and diagnostic activities at national level.
4. Surveillance may be carried out on the epidemiology of *Chlamydia trachomatis* in pregnant women and assess risk of cross- infection in their infants .
5. Since viruses causing ARI are by and large horizontally transmitted health education programmes should also incorporate viral control strategies .
6. Vaccines be introduced for major ARI viruses where possible incorporating with the existing immunization programmes .
7. An ARI control programme should be established at the national level. .

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VII . ANNEXES

ANNEX - 1 . Assay Determination Of Monoclonal Antibodies And Conjugates On Tissue Culture Control Slides And Study Ssample Slides.

	control slides						sample slides					
	Dilutions of Monoclonal antibodies with PBS at pH 7.6											
	1:50	1:100	1:200	1:400	1:800	1:1600	1:50	1:100	1:200	1:400	1:800	1:1600
RSV	X	+++	+	+/-	-	-	X	+++	+/	-	-	-
Flu-A	X	+++	+	+/-	-	-	X	+++	+/-	-	-	-
Flu-B	X	+++	+	+/-	-	-	X	+++	+/-	-	-	-
Adeno	X	+++	+	+/-	-	-	X	+++	+/-	-	-	-
	1:25	1:50	1:100	1:200	1:400	1:800	1:25	1:50	1:100	1:200	1:400	1:800
PIV	X	+++	+	+/-	-	-	X	+++	+/-	-	-	-
	1:5	1:10	1:20	1:40	1:80	1:160	1:5	1:10	1:20	1:40	1:80	1:160
FITC	X	+++	+	+/-	-	-	X	+++	+	+/-	-	

Key: RSV - respiratory syncytial virus, Flu - A - influenza virus type A, Flu- B - influenza virus type B, Adeno- adenoviruses, PIV - parainfluenza virus, FITC - fluorescein isothiocyanate conjugate , X - fluorescence too strong to read , (+)- weak fluorescence, (+/-) very weak fluorescence, (_) no fluorescence, (+++) - best fluorescence

ANNEX - 2 Preparation Of Phosphate Buffered Saline (PBS), pH 7.6

NaCl	8.5 00 g
Na_2HPO_4	1.280 g
$NaH_2PO_4.2H_2O$	0.156 g

Dissolve in deionized, double - distilled water and bring the volume up to 1 litre with deionized, double - distilled water. **Adjust pH to 7.6 with 0.1N H_2SO_4**

Stored at 4 o C.

Nacl - dried at 160 o C ,

Na_2HPO_4 - dried at 110 o C to 130 o C ,

$NaH_2PO_4.2H_2O$ before use.

ANNEX-3. Collection Of Nasopharyngeal Aspirates

1. The infant is made to sit on the mother's or nurse's arm and the head is immobilized .

2. A sterile polythene or rubber nasogastric feeding tube (size FG 8) with a blunt end and two wide openings near the tip is connected at the other end to a sterile mucus trap and a suction pump giving a negative pressure of not less than 100 mmHg (1 to 2 kg per cm square) .

3. The tube is finger pinched and gently inserted down one nostril until the tip is judged to be at the nasopharynx (approximately half the distance from the nostril to the base of the ear). With this the pump is hand compressed and released.

4. The tube is worked up and down , rotated and finally , withdrawn .

5. The same procedure is repeated through the other nostril until a desired amount of mucus is extracted .

Source : Gardner , P . S . , McQuillin , J and Grandien , M . 1980 . pp. 92-96 .

ANNEX - 4. Processing Of Nasopharyngeal Aspirates

1. Collect the specimen into 4 to 5 ml of phosphate buffered saline (PBS) pH 7.6 in a conical centrifuge tube .
2. Using a Pasteur pipette the mucus and aggregates is sucked up and down or gently shaken by hand about 10 times to break it up .
3. The collected material is centrifuged at about 350g (1500-2000 rev/min .) for 10 minutes .
4. The supernatant is removed using a Pasteur pipette . The pellet cells with mucus is resuspended in 1-2ml PBS and broken up by pipetting gently with a Pasteur pipette until a smooth suspension is obtained . Another 4ml PBS is added gradually while the pipetting continues . Any remaining thick lumps are removed by Pasteur pipette .
5. The cell deposit is resuspended in a few drops of fresh PBS added so that the final cell suspension is opaque , but not "sticky" .
6. Microscope slides are numbered and placed on the table . Drops of concentrated cell suspension about 4 uL is evenly spread on a 2 - eight well slides each and a 2-one well slides each . Out of the duplicates of slides one is stained and the other is kept at - 70 o C .
7. The smears are air dried and fixed in an anhydrous acetone containing 15 g/L of pure Sodium Sulfate for 10 minutes for viral detection and for 5 minutes for chlamydial detection and stored in a plastic box each containing 5 slides provided by the WHO.

Source : Gardner , P . S . , McQuillin and Grandien , M . 1980 . pp. 92-96 .

ANNEX-5. Staining Technique Of Viral Detection For The Indirect Immunofluorescence Method

1. The fixed slide is placed across two parallel bridges of sticks in a humidified chamber of a plastic box or petri dish. Water soaked cotton wool or soft paper or filter paper is kept in the chamber to prevent evaporation of the reagents during incubation.
2. 15-30 uL of unlabeled virus specific monoclonal antibody is applied to the smears and a platinum loop is used to spread the Mabs over the smear.
3. The chamber is closed and the slides are incubated at 37 o C for 30 minutes to allow the virus antigen and the monoclonal antibody to react.
4. The Mab is rinsed off the slides and the slides are washed three times for 10 minutes each in three changes of PBS to remove all the traces of unbound Mabs to the homologous antigens.
5. The slides are drained and dried in air.
6. They are placed in a humidifying chamber and 15-30uL of FITC conjugated anti-mouse Mab is added over each smear (viral Mab bound to homologous virus antigen in the specimen acts as an antigen to the conjugate) . The chamber is closed and the slides are incubated at 37 o C for 30 minutes to allow the antigen-antibody reaction to take place.
7. The conjugate is rinsed off the slides in three changes of PBS each for 10 minutes to remove all the unreacted conjugate leaving behind the virus antigen - antibody complex.

8. The slides are immersed in Evan's Blue 1:30,000 to counterstain for 5 minutes and rinsed in distilled water for 10 seconds. The slides are drained off and air dried. A mounting medium is added to the center of the smear and a cover slip is added carefully removing all the trapped air bubbles.

9. The slides are observed under a fluorescent microscope for the characteristic intense green fluorescence using incident UV blue light, and a 40x objective. The results are recorded as +1or +2 or +3.

Source : Gardner, P. S., McQuillin, J and Grandien, M. 1980. pp 60.

ANNEX-6. Staining Technique Of The Direct Immunofluorescence Method For Chlamydial Detection

1. 30 uL of conjugated Mab is added to each control slide and patient smears making sure the entire area of the well is covered.

2. The slides are incubated for 15 minutes at room temperature in a well-humidified chamber.

3. The excess conjugated Mabs are aspirated from the slides.

4. The slides are rinsed by gently agitating them in deionized or distilled water for 10 seconds. Excess water is gently shaken off and the remaining moisture is drained off from the edges of each slide with a blotting paper, then were allowed to air dry.

5. A drop of mounting fluid is added to the center of each slide well. A cover slip is placed on top of the drop and all air bubbles are removed. The slide is examined with a 40 x oil objective of the immunofluorescent microscope. If characteristic green fluoresceing EBs resembling a starry sky is observed, the result is recorded as a positive test.

ANNEX 7. Characteristics Of The 403 Study Population.

Sum total of size of the family	2463
Mean size of the family	6.11
Address from Addis Ababa	393
Outside of Addis Ababa	10
Household shared only one to two room/family	244
Mean number of rooms/household	2.51
Education of mothers - illiterate	87
- elementary	104
- secondary/high school	178
- College/University	34
Age of the infant (up to 2 months)	173
3 to 6 months	130
7 to 12 months	100
No. of infant breast fed	348
Age of mothers - Youngest (16 years old)	2
- Oldest (48 years old)	1
- Mean age	27.15

ANNEX-8 . Questionnaire On Socio-economic Information

. Age of the infant _____ (weeks) .
2. Residence within Addis Ababa _____ (Kf , Kb) ; _____ outside Addis Ababa .
3. Duration of illness when sample was collected _____ days .
4. Has the infant got antibiotics for this illness within the preceding 5 days _____ (yes , no) .
5. Diagnosis of the illness _____ . (AURI , Pneumonia , others)
6. The infant vaccinated for the age _____(yes , no) .
7. Nutritional status of the infant _____ (low birth weight , undernourished , normal , well nourished etc .) .
8. Is the infant breast fed ? _____ (yes ,no) .
9. Has the infant shared lodgings with anyone having ARI patient in the household ?
10. Number of children under 10 years of age in the household _____ .
11. Age of the mother _____ years .
12. Age of the father _____ years .
13. Marital status of the mother _____ (unmarried , married) .
14. History of Sexually transmitted diseases of mother during Pregnancy or delivery of the infant . (yes , no) .
15. If yes , has the mother been treated for it ? _____(yes , no) .
16. Education of the mother , level of grade completed (elementary , secondary , high school , college) .
17. Occupation of the mother _____.
18. Occupation of the father _____ .
19. Size of the family _____.
. Number of rooms shared by the household _____.
. Other member or members of the family having ARI (cough , fever , difficulty in breathing , running nose etc .) _____ (yes , no) .
Any member of the household smoking (yes , no) .
Cooking facilities of the household (electric , gas , biomass , other) _____ .

ce : Slightly modified after Kellner *et al* . 1988 .