SERO-EP'IDEMOLOGY OF RUBELLA VIRUS
IN IMO STATE OF NIGERA

BY

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THESIS APPROVAL

This thesis by Obioha, Udobuike, Ezeronye meets the regulations governing the degree, Master of Science of Ahmadu Bello University and is approved for its contribution to Scientific knowledge and literary presentation.

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THE PREACHER

"And further, by these, my son,
be admonished: of making many
books there is no end; and much
study is a weariness of the flesh.

Fear God, and keep his commandment:
for this is the whole duty of man.

For God shall bring every work into
judgment, with every secret thing,
whether it be good, or whether it
be evil."

—Solomon—
Eccl 12:12-14
The Holy Bible
(King James Version)

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A B S T R A C T

Nine hundred and thirty one serum samples collected at random from healthy subjects in the four health zones in Imo State were analysed for presence of Rubella haemagglutination-inhibition (HAI) antibodies using the Haemagglutination-inhibition technique. Rubella HAI antibodies were found present in 625 serum samples which gave a prevalence rate of 67%. No difference in percentage seropositive HAI titre was found between the urban and rural population. Immunity to the virus however varied with socioeconomic groupings in Imo State. The distribution of Rubella virus antibodies was also found to be influenced by geographical location. Age distribution pattern of rubella HAI antibodies showed that individuals of age-group 10-14 years and 15-19 years who were born during the civil war or who were minors at this period had a high rubella HAI antibody titre.

Primary isolation studies for Rubella virus was conducted with 120 samples of throat swabs collected from sick children at the Out-patient ward, Pediatric Clinic, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Most of the children had evidence of a mild rash and a few others had posterior cervical lymphoadenopathy typical of Rubella virus infection. On inoculation of these throat swab samples into Vero cell confluent monolayers, cytopathic effect (CPE) was observed in 40% of the 120 inoculated cell cultures after 4 days of incubation. Pronounced degeneration leading to the destruction of the cell sheet was observed after 14 days of inoculation. A standard rubella human antiserum neutralized the observed CPE and an average titre of $10^{5.2}$ $TCID_{50}$/ml was obtained on titration of these isolates. These findings support the actual presence of rubella virus infection in the Nigerian population.

INTRODUCTION

Rubella virus infection (German measles) is a mild & self-limiting infection associated with childhood which produces a generalized exanthema after an incubation period of about two to three weeks (Heggie and Robinsons, 1969). The erythematous maculopapular exanthema following this incubation period may last for three to four days in a majority of the infected persons hence the disease is sometimes called the 'three-day Measles.' Accompanying this exanthema is the swelling of the neck glands and lymphocytic changes in the blood cells (Alford et al., 1975). Protective antibodies which can be demonstrated by a variety of serological techniques have been found to exist in approximately eight-five percent of the world population up to the age of twenty-one (World Health Organization, Collaborative Study Rawls.et al; 1967)

The recognition of rubella as a serious clinical entity results from its severe damaging effect on the developing fetus when infection occurs in utero within the first trimester of pregnancy. This discovery was made by the Australian Ophthalmologist, Gregg [illegible] (1941) who observed an unusual high number of neonates with congenital cataracts in mothers contacting rubella in [illegible] early pregnancy. Viral persistence of infection with continuous shedding of the virus may also occur many months after birth thus damage can be done not only at the period of organogenesis but also after birth. Several organ

systems may be simultaneously affected and multiple syndromes collectively classified as 'Congenital Rubella Syndrome' (C.R.C.) is frequently the result. Lundstrom (1962) reported that the rate at which these embryopathies manifest differ according to the period of gestation at which contact is made with the virus. A decreasing trend is shown from around 33% for infections accurring during the first month of pregnancy to around 4% for infections during the fourth month. The congenital anomalies associated with congenital rubella include cardiac malformations, posterior cervical auricular or occipital lymphadenopathy, Unilateral or bilateral defects of the eye, fetal underdevelopment, idiopathic thrombocytopenic purpura, hepatosplenomegaly, otopathies, hepatitis, stillbirth, cerebral damage causing a breakdown in pyschomotor functions, mental retardation and severe behavioural disorders. These conditions however, may not be typical of rubella and may occur singly or in various combinations, confirmation can only be achieved by virological and serological diagnosis.

The rubella virus disease is poorly diagnosed by clinicians due to its mild constitutional symptoms and in Africa the dark skin make it more difficult to recognize (Andre, 1978). The need for rubella control became necessary after a major rubella epidemic in the United States of America (U.S.A.) in 1964 led to congenital rubella anomalies in an estimated 20,000 infants.

Therapeutic abortion was administered on a large number of women at risk. The World Health Organization in 1967 therefore started a collaborative study on the sero-epidemiology of rubella in various parts of the world. The objective of these surveys was to determine immunity patterns of peoples of different age-group in different areas of the world to rubella virus (Rawls et al., 1967).

Efforts to develop a rubella virus vaccine was intensified after the successful isolation of the virus in cell culture in 1962 (Parkman et al., 1962; Weller and Neva 1962). In 1969 the first rubella virus vaccine was licensed in the United States of America thus ushering in hope for the successful control of this devastating human disease (Parkman et al., 1966).

The growing awareness of postnatal rubella and the congenital rubella syndrome in the human population call for a greater understanding of the epidemiology of these diseases. Rubella epidemiology in Africa is however still in its infant stage hence Bada and Odelola (1978) reported the general lack of clinical recognition and awareness of this disease in Nigeria. In a Symposium held in Cairo Egypt in 1979 on the 'New Developments in Vaccines' Andre (1979) called for a continued epidemiological surveillance of the rubella virus infection in Africa. This work is therefore designed as an additional contribution to the various epidemiological surveys so far conducted in Africa and it is aimed at determining

the distribution pattern of rubella immunity in Imo State of Nigeria. In addition isolation studies were performed in cell cultures.

LITERATURE REVIEW

BIOLOGICAL CHARACTERISTICS OF RUBELLA VIRUS

Rubella virus is the only type species of the genus Rubivirus belonging to the family Togaviridae. Other genera of the family include; Alphavirus, formally called arbovirus A, Flavivirus formally known as arbovirus B, and Pestivirus. All these show common characteristics of small size, spherical shape, presence of an envelope and similar nucleic acid content. This classification was established in the third report of the International Committee on Taxonomy of Viruses (Matthew, 1979).

Holmes and associates in 1969 first recognized rubella as a non-arthropod-borne virus showing morphological and morphogenetic resemblance to arboviruses though serologically unrelated. The viral particles are roughly spherical and have an average diameter of 58±7nm after purification in sucrose density gradient. The diameter of the viral icosahedral core averages 33±1nm (Vaheri and Hovi, 1972) with 11nm ringlike subunits covering their surface. A viral envelope bearing 5 to 6nm long spikes which is easily denatured by heat at 60°C but remain stable at -70°C for up to one year, is also recognized. Rubella virions are sensitive to lipid solvents such as ethylene, chloroform, and bile salts and are inactivated at pH3. The viral genome is a single stranded ribonucleic acid (RNA) with a sedimentation coefficient of 40s in sucrose gradient

(Hovi and Vaheri, 1970). The molecular weight of the RNA based on this sedimentation coefficient is $3x10^6$. The structural protein contains eight species of viral specific proteins grouped into three main areas on the bases of their electrophoretic characteristics on 5% acrylamide gels (Liebhaber and Gross, 1972) Heterogenicity was observed by Liebhaber and Gross (1972) within each region and region II and III proteins contain fucose hence they are called glycoproteins. The viral core contains nucleoprotein which is released on disruption of the virion with neutral detergents. The rubella virion also incorporates a lipid and phospholipid component accounting for 0.25mg weight per milligram of its protein (Bardeletti et al, 1975).

Rubella virus can be cultivated in a battery of animal cell systems though techniques for indicating the presence of the virus in these tissue culture systems vary. The virus replicates in the cytoplasm of the host cell and matures by budding in the cytoplasmic membrane. It has an ability to interfere with the replication of several other viruses, for example; ECHO-virus type 11; Coxsackie virus, type A9, Sindbis virus and New Castle Disease virus. (Parkman et al., 1962; McCarthy et al., 1963; Milfune and Matsue, 1975; Kleinman and Carver, 1977). This interference is indicated by the absence of cytopathologic effect otherwise present on infection of the particular host

systems in which these virus are propagated. Milfune and Matsuo, (1975) however reported the inability of the attenuated vaccine virus strain RA27/3 and a temperature sensitive mutant of rubella to interfere with the New Castle Disease Viruse (NDV)

The virus contains hemagglutinin which is able to agglutinate many avain erythrocytes (Liebhaber, 1970). Complement-fixing antigens have also been found in supernatants obtained from rubella infected cells. There is no antigenic relationship between rubella and any known virus in the family togaviridae (Mettler et al., 1968). The purified virus have been reported by Bardelletti and Gautheron (1976) to have a weak Calcium (Ca^{2+}) independent neuraminidase activity.

CLINICAL MANIFESTATION

The clinical manifestation of rubella virus can be grouped into two based on the nature of contact with the virus. Postnatal rubella contacted naturally or through vaccination and perinatal or congenital rubella contacted in utero by a fetus within the first twenty weeks of pregnancy are the two types of clinical manifestations.

Postnatal rubella infection in most cases are subclinical and hardly noticed due to its sublime constitutional symptoms. The most predominant symptoms are adenopathy lasting for several weeks and rash,

splenomagaly may also occur. Green and associates in 1965 investigated the clinical response of subjects experimentally infected with rubella by injecting intramuscularly or by spraying in the nasopharynx serum from patients ill with rubella or prolonged contact with such patients. The finding show an absence of fever and the appearance of enlarged Lymph nodes about eight days after inoculation. The maculopapular exanthem which follow the period of incubation was found to vary in duration between one to four days. The rashes progress from the face to the hand the whole body and to the legs. No serious complications was associated with this acquired rubella infection. Recent studies however implicate rubella in a number of complications detected in patients with postnatal rubella infection. The most common of these complications is arthritis and arthralgia which tends to involve the fingers, wrists, knees and occur either as the rash is appearing or soon afterwards (Vergani et al., 1980; Smith et al., 1981). Other complications include haemorrhagia which occurs in one out of 3,000 cases of rubella and occurs more often in children. It may occur as a secondary complication to thrombocytopenia and vascular damage, this was suggested to be immunologically mediated (Bayer et al., 1965). Thrombocytopenia may last from weeks to months and may cause serious problems if bleeding occurs into vital areas such as the brain, kidney or the eye (Heggie and Robinson, 1969). Another very rare complication of

postnatal rubella is rubella encephalitis which may occur in one out of 5,000 cases. Bernard and Co-workers (1982) recently reported complications of encephalitis in a sixteen year old boy serologically diagnosed for rubella. The virus has been recovered from the lymph nodes, leukocytes, throat swabs, rectal swabs, serum, urine, conjuctival sac, synovial fluid and lungs of patients with natural rubella acquired after birth (Heggie and Robinson, 1969). Complications resulting from postnatal rubella infection sometimes progressively disappear undetected by the patient due to its mild constitutional symptoms.

The embryopathies associated with intrauterine rubella infection gives it a very serious clinical picture. The risk of fetal damage is very high for primary maternal infections during the first twenty weeks of gestation (Alford et al., 1975). Cradock-Watson and Co-investigators in 1980 however reported that the fetus can be infected at any time during the second and third trimester of pregnancy but the risk of fetal damage varies at different stages. A one to one ratio is shown for inapparent to apparent rubella infection in adult women therefore serological investigation is needed to confirm a case of rubella for women with a history of rubella or occurrence of rash. There is possible reinfection in a small proportion of women with natural immunity (Partridge et al., 1981) and in a much larger

number of those immunized by vaccination (Landes et al., 1980).

A serious problem associated with congenital rubella in neonates is the inability to recognize the disease during the early months of life due to a lack of symptoms. However certain symptoms in rubella infected neonates have a reasonable degree of diagnostic specificity. These include cardiac anomalies, glaucoma, pigmented retina, bone lesions, cataract, immunologic defects other than Immunoglobulin A (IgA) deficiency (ALford et al., 1975). Circulating immune complexes (ClC) have been implicated in the lesions observed in late-onset rubella syndrome and susceptibility to infection was suggested to be related to a disequilibrium between the T and B Lymphocytes (Tardieu et al., 1980). Examining an abnormal male infant born to a twenty-two year old mother, Hoekelman and Anderson (1982) implicated rubella for such diagnosed abnomalies as prematurity, microcephaly, pneumonia, thrombocytopenia, hyperbilirubinemia, hepatosplenomagaly and patent ductus arteriosus.

PATHOGENESIS

Aerosol transmission through the nasal route to the mucosa of the upper respiratory tract is the natural portal of entry of rubella into the susceptible host (Heggie and Robinson, 1969). The virus implants into the respiratory mucosa and multiplies in the respiratory epithelium. It then spreads through the cervical lymph nodes to the blood stream. The presence of the virus in the blood stream causes viremia. Following this viremia the virus is spread through the vascular system to the nasophargeal region from where it is further spread by droplets into the environment. In the absence of a nearby host the cycle of infection is terminated since rubella is inactivated by heat and lack of moisture and does not persist in the environment (Heggie and Robinson, 1969). Heggie (1971) recovered rubella virus in the erythematous maculopapular skin lesions within 24 hours after the appearance of rash in some rubella patients. The role of the virus in the production of the rash was however uncertain though Heggie (1971) proposed that the rash may be a result of an inflammatory reaction in the skin due to viral replication or an antigen-antibody complex produced in the infected tissues. The erythematous rash lasting for three to four days is experienced by the postnatal rubella patient.

Although rubella virus has been implicated as the causal agent of many congenital anomalies in the fetus the pathogenesis of the disorders remained unresolved until viral recovery were made from a number of newborn infants suggesting persistent infection of the fetal tissues. Following prolonged viremia in maternal postnatal rubella patient within the first 20 weeks of gestation, the fetus becomes infected via the transplacental route. The actual mode of infection is unclear, two possibilities however are, the passage of the virus through the placenta into the blood stream of the fetus or the infection of the placenta before the infection of the fetus. The infection of the placenta before subsequent infection of the fetus is however highly favoured (Plotkin, 1975). The constant recovery of the virus from placentas of rubella infected fetus, voluntarily aborted by surgery, rather than from the aborted fetus itself supports the idea that the virus is transmitted by the infection of the placenta before fetal infection (Plotkin et al., 1965; Rawls et al., 1968; Thompson and Tobin, 1970). Another possible mode of infection of the fetus is the ascending route since the virus has also been isolated from the female genital tract (V.heri et al., 1972).

Once the placenta becomes infected the chorionic villi becomes inflammed and this is followed by granulomatous changes. Infection of the fetus by rubella

establishes a non-Lytic chronic pathological change in the developing fetal tissues. Plotkin and fellow workers (1965) first demonstrated this *in vitro* using established cell lines of human embryo fibroblast. Viral persistence was observed in these cultures for weeks and reduced cell numbers was explained as a consequence of mitotic arrest in these human cells. Interference phenomena was also observed in the human cells infected with rubella. This resistance to superinfection observed was proved to be independent of interferon (Rawls and Melnick, 1966). Carrier cultures were derived from congenitally infected infants and shown to exhibit reduced cell division and also resistant to cure with antibody (Rawls and Melnick, 1966). Naeye and Blanc (1965) reported a subnormal size of intrauterine rubella infected newborns. The basis of growth retardation in these infants was proposed to be due to a subnormal number of cells in many of the fetal organs (Naeye and Blanc, 1965). Viral persistence and proliferation in the fetal tissues which consequently inhibits cell multiplication was implicated for the growth disturbance and subsequent postrubella syndrome experienced many months and even years after birth. Necrosis and inflammatory reactions were reported to be rare. This rarity of inflammatory reactions or necrosis in the fetal tissues observed by Naeye and Blanc (1965) was however in contrast with the work of Tondury and Smith (1966) who proposed direct cell damage and

necrosis with inflammatory or fibrotic response in the fetal tissues. Plotkin and associates (1965) however questioned the possibility of rubella having a direct cytopathologic effect *in vivo* within the fetal tissues but not *in vitro* in the human embryo cell cultures. Plotkin (1975) further attributed fetal damage by rubella as a resultant effect of vascular system infection and consequent damage to endothelial cells leading to thrombosis of small blood vessels and necrosis of the surrounding tissues. Rubella virus also has the ability to kill some cells especially those of the lens of the eye which result in the frequent congenital cataracts.

Another possible mechanism of growth inhibition and fetal damage by rubella is the transduction of mitotic materials (Monif et al., 1965). Specific chromosome anomalies such as Mongolism in infants with postrubella syndrome have also been reported (Lundstrom, 1962). Profound breakage of human chromosomes by rubella was demonstrated *in vitro* in human cell cultures (Plotkin et al., 1965). The above findings are however not compelling enough to implicate chromosomal anomalies as a cause of rubella malformations (Plotkin, 1975).

Congenital rubella does not only elicit the fetal immune response but may also interfere with the normal development of the immune system with the resultant

development of a permanent immunodeficiency syndrome (Stiehm, 1975). Excess of IgM termed dysgammaglobulinemia type I is the most common immunodeficiency associated with congenital rubella (Schimke et al, 1969; Cradock-Watson et al., 1980). Fuccillo and Coworkers (1974) have also demonstrated a general absence of cell-mediated immunity to rubella syndrome. Loss of rubella antibody with unreactivity to rubella vaccine suggestive of specific immunologic tolerance have also been noticed in children with immunodefeciency syndrome resulting from congenital rubella (Steihm, 1975). Lymphocytes of normal individuals show a decreased phytohemagglutinin response when infected in vitro (Olson et al., 1968), whereas lymphocytes from infants with congenital rubella syndrome often carry virus for long periods after birth (Jack and Grutzner, 1969; McMurray and Rey, 1981). There is almost a complete absence of response of lymphocytes to phytohemagglutinin stimulation in the presence of typical delayed hypersensitivity skin reaction in children with congenital rubella syndrome (Montgomery et al., 1967). Other workers have however achieved this lymphocyte stimulation with phytohemagglutinin (White et al.,1968; Marshal et al., 1970). Infants with postrubella syndrome show decreased humoral antibody response to antigens such as diphtheria toxoid, tetanus toxoid, blood group antigens and type 1 and 3 poliovirus during

The period of viral excretion but not after (Plotkin, 1975). Tardieu and Co-investigators (1980 and Wolinsky and associate (1982) implicated circulating Immune complexes as responsible for the lesions associated with late-onset rubella syndrome. Tardieu and coworkers (1980) further proposed that susceptibility to infection can be related to a peripheral T and B Lymphocyte disequilibrium.

Immunodeficiency syndrome due to dysgammaglobulinemia type I was explained by Stiehm (1975) as due to viral interference at the critical time when clonal differentiation from IgM Bcells to IgG and IgA Bcells is occurring. In explanation of these rubella immunodeficiences, Plotkin (1975) postulated that the B Lymphocytes which differentiate into the antibody forming cells are only partly damaged in infants with the rubella syndrome. He also suggested that the thymus lymphocytes (T-cells) are prevented from going into mitosis when infected by rubella virus and therefore have reduced ability to destroy infected cell clones. Damage to lymphocytes was explained by Plotkin (1975) as being responsible for the defective pytohemagglutinin response and the slow conversion from IgM to IgG antibody. Lastly antibody to rubella secreted by uninfected B Lymphocytes is stimulated in utero without the development of tolerance. When uninfected clones of T-cells become available they attach to and destroy infected cells, releasing virus

that is then neutralized by the secretions of B lymphocytes. The interferon response remain intact in congenital rubella infants hence viral persistence cannot be explained as a breakdown of the interferon defence mechanism. No explanation could however be given for the absence of cell-mediated immunity to rubella in these infants with congenital rubella syndrome who no longer shed the virus (Plotkin, 1975).

IMMUNITY

The fetus with a functioning cellular immune system is protected by its ability to limit intrauterine viral infections during the gestation period. An example of this is seen in some congenital rubella infants who at birth no longer excrete the virus presumably due to an intact operative cellular immune system (Stiehm, 1975). The passive maternal antibodies which are also constantly transmitted through the placenta to the fetus during late gestation further help to prevent intrauterine infection and damage to the fetus. Mothers who have a high antibody titre to rubella are however unlikely to contact postnatal rubella during pregnancy hence vital access to the fetus is prevented. The actual role of passive maternal antibodies is therefore the prevention of infection of the newborn within the first few months of life.

Intrauterine antigenic challenge such as the presence of rubella in fetal blood stream stimulate an accelera*ed synthesis of fetal immunoglobulin M(IgM) evidenced by its high levels in cord blood. Crodock-Watson and fellow investigators (1980) demonstrated the varying levels of IgM in cord blood influenced by the actual period of maternal rubella infection during pregnancy. For infections occurring within 12-16 weeks after the last menstrual period specific IgM antibodies were detected in 56% of 50 infants. This proportion fell progressively to 12% after maternal rubella at 24-28 weeks, increased to 19% after rubella at 28-36 weeks and then to 58% when viraemia occurred during the last month of pregnancy. No evidence exist however to show that eith passive maternal antibody or endogenously synthesized fetal antibody play a role in fighting an already established intrauterine infection (Steihm, 1975).

In later life there is decreased maternal IgG and the infant has a higher probability to contact rubella thus acquiring a natural immunity. Immunity resulting from natural infection is in general more persistent than active immunity by vaccination. The ability of the HAI antibody titre to rubella persisting in protective levels for up to ten years in vaccinated subjects has been reported by Hermann and co-workers (1982). The authors postulated that potant rubella vaccine if properly administered

produces a high seroconversion rate and a high rate of antibody persistence (Hermann et al., 1982).

Reinfection in individuals with a naturally acquired immunity and those vaccinated have been reported after viral challenge (Wilkins et al., 1969; Landes et al 1980) or natural infection (Horstman et al., 1969; Partridge et al., 1981). A higher rate of reinfection in vaccine immune individuals than naturally immune individuals have also been reported (Chang et al., 1970; Horstmann et al., 1970; Evans et al., 1971). An antibody booster response is experienced in reinfected individuals without occurence of viraemia.

Immunity to rubella following congenital infection is different from that following postnatal rubella infection. There is generally a greater rate of decline of rubella specific HAI antibody in congenital rubella infants than in postnatally infected individuals. Serial antibody determination in a group of 20 patients with congenital rubella was followed from birth or shortly after birth to between 20-40 months of life. Seven of the children had no demonstratable antibody at the end of the period (Hardy et al., 1969). Cooper and coworkers (1971) compared antibody persistence patterns of children with congenital rubella and their mothers. The findings from this work show a loss of HAI antibody to undetectable levels in these children and also

demonstrated a failure of these children to exhibit an antibody booster response on challenge parenterally with attenuated rubella virus vaccines. It is also postulated that specific cell-mediated immunity response to rubella virus is defective in congenital rubella infants (Faccillo et al., 1974). This is supported by the isolation of high titres of the virus from the throat, urine and various tissues of children with the Congenital Rubella Syndrome (CRS) irrespective of the presence of fetal and maternal antibodies. Damage to lymphoid tissues including the thymus has also been reported in infants with congenital rubella (Singer et al., 1969). Using a specific microassay lymphocytotoxicity system Fuccillo and associates (1974) demonstrated an impaired cellular immune response in congenital rubella infected infants and further reported that this impaired cellular immunity can be altered on reinfection by rubella virus. The reason underlying this rapid decline in rubella specific antibody levels and the general unusual patterns of immunity in congenital rubella infants is yet to be fully understood.

EPIDEMIOLOGY

The control of a preventable infectious disease like Rubella becomes a realizable objective if its epidemiological pattern in the entire world population is well understood. It was based on this understanding

that the World Health Organization (WHO) in 1967 sponsored extensive collaborative surveys in various parts of the world to determine the sero-epidemiology of rubella in the entire world population. Areas studied in the first collaborative survey were Europe, Japan and Australia. Antibodies to the virus was present in 80-87% of women 17-22 years old. The virus was found to be endemic in these population (Rawls et al., 1967). Another collaborative survey was started in 1968 and the target population was Caribbean, Middle and South America. The result of the study show 80% immunity level in women of child-bearing age (Dowdle et al., 1970). Following these surveys other epidemiological studies were carried out in various parts of the world by various independent groups of scientists. Island population studies in Taiwan (Gale et al., 1972), Barbados (Evans et al., 1974), show that the virus was not endemic in the islands despite the large population and climatic factors. Recently, Rafnar (1982) undertook the screening of 730 Iceland school girls 12 years of age for rubella HAI antibodies. Fifty point nine percent of these girls were found to be seronegative and consequently immunized. Seroconversion was observed in 94.8% of the immunized girls one year after the immunization. Another sero-epidemiological survey was recently conducted in the State of Hawaii by Serdula and associates (1982) to determine rubella susceptibility

levels ten years after the introduction of the rubella vaccine in this island. Out of 3,852 serum specimens accepted for analysis from women for the period of two years, 23.8% were susceptible. Serdula and associates related the relatively low rubella susceptibility level to the positive effect of the rubella vaccination programme.

In Great Britain a National Congenital Rubella Surveillance Programme (NCRSP) was established in May 1971 in order to readily diagnose cases of congenital rubella defects or clinically suspected cases by standard Laboratory techniques. Two registries were established in two hospitals and out of the 1,630 children examined between July 1971 and June 1978, actual congenital Rubella confirmed or suspected cases total 523. An epidemic of rubella was reported in the Spring and Summer of 1978 in Britain leading to a high incidence of congenitally infected babies in the subsequent years (National Congenital Rubella Surveillance Programme, 1979).

The USSR has had no large rubella outbreaks and research on Congenital Rubella is still in its infant stage (Kantorovic et al., 1979). A comprehensive study of the congenital rubella problem in Moscow and other selected towns in USSR was carried out between 1971 and 1977 by Kantorovic and coworkers (1979). The findings from this investigation show that children

between the age of one to seven years have the highest incidence of rubella and about 20-25 rubella cases were recorded annually for every 100,000 adults. The teratogenic nature of rubella strains found in the USSR was confirmed by this study.

Rubella epidemics have been well studied in the United States of America. Rubella surveillance Report issued in November 1973 by The Centre for Disease Control (CDC) show that ten representative United States Communities were studied from 1928 to 1973. The result of this investigation show that large Rubella epidemics occur in the U. S. A. approximately every six to nine years with major ones occurring at intervals of up to 30 years (Horstmann, 1976). Rubella virus was found to be endemic in the United State and many states have developed various strategies to control their Congenital rubella problem. Short outbreaks of rubella are mainly experienced in institutions like Army Camps, hospitals and schools where people have very close contact with each other. Polk and coworkers (1980) reported an outbreak of rubella among hospital personnel in a large medical surgical hospital in Boston, Massachusetts. This resulted in the termination of a pregnancy. Epidemiological investigation conducted reveal that 12% of the women employers in this hospital were seronegative for rubella HAI antibodies at the time of the outbreak. Outbreak of rubella among hospital staff is of great

concern for two reasons; there are large numbers of women in the child-bearing age in this working population and the risk of exposure to rubella during an outbreak while in early gestation period is very high; secondly there is a high risk that the hospital personnel will transmit rubella to susceptible pregnant patients (Polk et al., 1980). Chappell and Taylor (1979) studied rubella susceptibility in a population of medical students in the Bowman Gray School of Medicine, North Carolina, U. S. A. in 1976 and 1977. Out of 457 students studied 18% were estimated at risk for rubella infection. No difference was found in the immunity level between the males and females studied and the students did not differ in immune status from the general population. An outbreak of rubella in Chicago in 1978 was recently reported by Lamprecht and coworkers (1982), following this outbreak an extensive survey of local health care personnel and hospital records was undertaken by the authors. Thirty one cases of infants with congenital rubella syndrome were identified. Isolates of rubella virus was made in eleven infants and persistent high rubella HAI titre was found in ten babies. The incidence of CRS in Chicago between July 1978 through June 1979 was found to be 48.9% for every 100,000 live births. Lamprecht and his fellow workers (1982) therefore called for an increased emphasis by physicians on detection and vaccination of women

susceptible to rubella infection. Preblude and associates (1980) noted however a great decrease in the incidence of postnatal rubella over the past decade (1969-1979) in the U. S. A. though there is a lack of concurrent reduction in the incidence of Congenital Rubella Syndrome. The need for increased immunization as a possible means of control of congenital rubella was therefore highly recommended.

In the African continent as in other developing world, research on rubella virus infection is still in its early stage (W. H. O. Report, 1976). There is generally a poor reporting of rubella cases by physicians due to a lack of emphasis on the disease resulting from its sublime clinical symptoms. The black skin also make the mild rubelliform rash difficult to observe (Bada and Odelola, 1978). The disease is however very prevalent throughout the African continent (Andre, 1978). A survey of rubella immunity in the Egyptian population was carried out by Khalifa and coworkers (1973). The finding from this survey show that the virus is endemic in the Egyptian population and the incidence of the rubella HAI antibodies increase with age. Out of 438 serun samples diagnosized using the HAI technique 70% of the children below two years of age were seropositive. This percentage increased by the age of ten years. The situation is similar to that found in a previous survey carried out in Tunisia.

A high level of rubella HAI antibodies was reported in a sero-epidemiological survey conducted in Tunisia by Nabli (1970). Out of 429 sera collected from both Urban and rural population 82.8% seropositive individuals were found in the urban population whereas 68.8% was found in the rural population.

In West Africa epidemiological surveys have been conducted in various countries. In Ghana Addy and Agbemadzor (1976) determined the immune status of 188 healthy urban females of the age group 0-19 years. The overall immunity level found in this study was 67.1% and immunity to rubella was acquired early in life and rises with increasing age of the girls. This result also agrees with the findings of Khalifa and associates (1973) for the Egyptian population though the studied population in Ghana were only urban females. The study also show that 75.3% of female of child-bearing age in Ghana were seropositive for rubella HAI antibodies. In Togo a total of 305 sera collected by the W. H. O. in 1976 wereanalysed for Rubella HAI antibodies among other sera collected from other parts of the world. Immunity was found to be age dependent. The percentage seropositive individuals rose from 14.6% for age group one to five to 76.2% for age group 17-29 and above. The W. H. O. (1976) reported in this survey that sero-immunity to rubella varied among the different countries of the world and even in different parts of

the same country. They therefore recommended that a comprehensive epidemiological picture of rubella virus infection and Congenital rubella syndrome could be obtained by combining results of various serological surveys. Clarke and associates (1980) studied rubella epidemiology in the Gambia. After a careful examination of clinical records in the hospital, no evidence of rubella malfermations in neonates was revealed. It was therefore concluded that it is likely that the strains of rubella circulating in Africa are potentially teratogenic but that the congenital rubella syndrome is infrequent due to the high level of immunity in adolescents and adults as was indicated by the survey. A survey of rubella immunity in the Nigerian population show an average of 68% seropositive individuals possessing rubella specific HAI antibodies (Odelola et al., 1977). One thousand eight hundred and forty seven serum specimens collected by these workers were analysed using the rubella HAI technique. In a recent survey conducted by Odelola and associates (1980) on rubella immunity in Nigerian children, immunity was found to rise with increasing age of the children. A sudden decrease in antibody titre was found for children at the age of 6-12 months. The high antibody level in cord blood from newborns was maternally acquired and can only persist during the first six months of life. The report of Okafor and Marshall

(1978) on the prevalence of antibodies to rubella virus in the sera of adult females in Enugu, Nigeria show that out of 40 serum specimens collected from female student nurses of average age 19.6 years in the University of Nigeria Teaching Hospital, 75% were seropositive. The authors also postulated that based on their findings a quarter of the pregancies in the Eastern Nigeria could result in congenital rubella. The sampled population was however small and only limited to female nurses of age range 18-25 years and may not be a reflection of the comprehensive picture of rubella virus infection in this part of Nigeria.

Rubella epidemiological surveys conducted in the Central and South Africa show a lower incidence of rubella HAI antibodies compared to the antibody level in North Afria (Andre, 1978). A study of 1033 sera collected from Kenya by the W. H. O. in 1968 show a marked difference in rubella immunity between the two Coastal areas namely; Kitui and Malindi. Fifty percent of the population were immuned at the age of 11 years in the Kitui area whereas in the Malindi district 50% of the population were already immune by the age of three years. Eighty percent of the female population in the Kitui area were only immune at the age of 31 years and above while in the Malindi this percentage is attained by the age of 21 years. Immunity to rubella virus was also found to be age-dependent in the

Kenya. The virus tends to circulate more freely in the urban areas of Nairobi than the rural areas (Hayden, 1971). The rate of immune response to rubella virus infection was determine - for the Angola population (Escudeiro et al., 1975). Out of 1552 sera collected from female children and teenagers living in Angola an average of 73% of the population were seropositive for rubella HAI antibodies. No significant difference in immunity was found out in this study between the several age groups and natural immunity was reported to be acquired before begining school in Angola (Escudeiro et al., 1975). This situation is in contrast with the findings in Europe where at the age of seven years only about 50% of the population is immuned to rubella virus (Escudeiro et al., 1975). Andre (1978) after a review of Rubella epidemiology in Africa called for continued epidemiological surveillance of the virus in the African continent in order to be able to adopt rational vaccination policies in the various countries of Africa.

LABORATORY DIAGNOSIS

Infections due to rubella virus can be easily confused with other diseases due to its uncharacteristic symptoms. Scarlet fever, mild measles, infectious mononucleosis, toxoplasmosis, erythema infectiousum all show symptoms very similar to rubella viruse disease therefore laboratory diagnosis of infection is of confirmatory importance. Routine laboratory diagnosis

will only give unsatisfactory results like Leukopenia and atypical lymphocytes, therefore serological investigation aimed at demonstrating the actual presence of specific viral antibodies is required. Isolation of the virus in tissue culture though laborious could also be undertaken. The type of cases requiring laboratory diagnosis are cases of congenital rubella in neonates, postnatal rubella in adults with a history of rubella or occurrence of rash, determination of the immune status of women during the child-bearing period. The laboratory diagnosis of postnatal rubella is of crucial importance for women within the first trimester of pregnancy and therapeutic abortion is usually administered for a confirmed case of rubella within this period.

The traditional methods for serological demonstration of rubella virus specific antibodies are, Viral neutralization (Parkman et al., 1964), Complement-fixation test (Sever et al., 1965) Flourescent antibody technique (Brown et al., 1964) and the Hemagglutination inhibition technique (Stewart et al., 1967). These techniques have associated problems which stimulated a lot of research for better alternatives. The viral neutralization technique is very complicated and time-consuming, it is therefore not suitable for epidemiological surveys (Stewart et al., 1967). The complement-fixation technique is useful for demonstrating recent

rubella infections. Complement-fixation antibodies become detectable about one week after the rash and reach maximum levels one to two weeks later (Rawls and Chernesky, 1980). However complement-fixation antibodies tend to disappear in many individual's serum over a few years. The absence of complement-fixation antibodies does not imply a lack of immunity to rubella virus infection. Acquired Immunity due to a long period of contact with the virus is not determined by the complement-fixation technique, therefore it is unsuitable for epidemiological survey work. The flourescent antibody technique though reliable is slow, expensive and complex. The technique of choice for most epidemiological surveys is the simple, rapid and reliable Hemagglutination-Inhibition technique (Stewart et al., 1967).

The major problems associated with the hemagglutination-inhibition (HAI) technique is the presence of non-immunoglobulin inhibitors of rubella hemagglutinin (HA) present in essentially all human sera. The methods of removal of these non-specific inhibitors and agglutinins vary with the Laboratories applying the test. Liebhaber (1970a and 1970b) recommended an alternative method for removal of these non-specific inhibitors after discrediting the routine Kaolin method and also the use of Dextrose gelatin Veronal buffer (DGV) as diluent for the test. These

inhibitors and agglutinins are Lipoproteins and the routine Kaolin method for their removal is not specific and therefore can lead to the removal of portions of the viral hemagglutinating antibodies. Liebhaber (1970a) therefore suggested the use of Dextran Sulfate and Calcium Chloride (DS-C) which selectively removes lipoproteins. The use of HSAG (HEPES-Saline-Albumin-Gelatin) diluent for the test was also recommended in place of DGV. In spite of these recommendations many Laboratories still rely very much on the routine Kaolin method. The recommended HEPES buffered saline was found to be unnecessary by Haukenes (1979). A method which circumvented the problem of non-specific inhibitors by allowing the hemagglutinin to react with erythrocytes first before addition of the serum was recommended (Haukenes, 1979). Another problem associated with the HAI test is the unreliability of its low titres. The HAI test may give false positive results at low titre levels (Harris et al., 1980) Incomplete removal of the non-specific inhibitors by the routine Kaolin method was implicated for this false positive results. The suggestion of Sever (1980) that all rubella sera for sero-diagnosis be saved by freezing for one year so that repeat testing may be conducted whenever clinically necessary is however very useful in overcoming this variability in results. These variations in results between laboratories led the Centre for Disease Control Atlanta, Georgia, U. S. A.

to standardize the rubella HAI technique and carry out supervision and workshops for clinical laboratories using the test in the United States. Most laboratories therefore use this standardized HAI test recommended by the Centre.

Recent serological techniques developed for demonstration of rubella specific antibodies include radioimmunoassay (RIA) (Smith et al., 1974), Enzyme-Linked Immunosorbent Assay (ELISA) (Van Loon et al., 1981), Solid-phase Immunosorbent Hemadsorption (SPIHAd) (Goldwater and Banatvala, 1981), Rapid Solid-phase Enzyme Immunoassay (Salonen and Vaheri, 1981) and Hemolysis in Gel technique (Sander and Niehaus, 1981). None of these new methods is free from problems therefore the standardized HAI technique remain very much in use for rubella serodiagnosis.

Isolation of rubella virus in tissue culture was first achieved by Parkman and his coworkers (1962) and Weller and Neva in 1962. Parkman and associates (1962) demonstrated the ability of rubella virus to interfere with the growth and cytopathic effect (CPE) of ECHO virus type II in primary cultures of African Green Monkey Kidney Cells (pGMK) whereas Weller and Neva (1962) demonstrated the Cytopathic Effect (CPE) of rubella virus in human amnion cultures. A wide variety of techniques and cell lines have since been

developed for isolation of the virus. These include, exclusion of Coxsackie Aq virus by rubella virus in vervet monkey-kidney tissue cultures and demonstration of CPE in human thyroid and continuous cell lines of rabbit-Kidney cells RK_{13} (McCarthy et al., 1963). Other methods are the detection of cytopathic effect in a continuous line of African Green Monkey Kidney Cells AGMK, AB-1 (Gunalp, 1965), Production of extensive CPE in rabbit-Kidney cell strain LLC-RK_1 (Hull and Butorac, 1965), cytopathic effect in primary cultures of rabbit embryo cells (Reddick and Roesel, 1966), growth and cytopathic effect in Rabbit-Cornea cell line (SRC) (Leerhroy, 1965), cytopathic effect in a continuous line of African Green monkey Kidney cells (Vero), (Rhim and Schell, 1967· Liebhaber et al., 1967). It has however been very difficult to apply any of these tissue culture systems with ease and advantage in different laboratories apart from where they were first demonstrated (McCarthy, 1969). Among the many factors responsible for this variability are, cytopathic effect (CPE) is very slow in onset therefore early recognition and differentiation from non-specific changes become very difficult for one who is not well trained in a particular system, the influence of culture media used on the development of CPE has been shown. Some batches of media 199 can support the growth and development of cytopathic effect of rubella virus

in rabbit-Kidney line LLC-RK_1 while others will not (Leerhroy, 1966), virus yield in high titre has not yet been acl.ieved in any of these cell systems except in the baby hamster kidney line (BHK-21) (McCarthy, 1969). As a result of this limited number of Laboratory assay systems for rubella virus based on recognition of CPE, the indirect interference system described by Parkman and coworkers (1962) is generally accepted.

Viral assay in tissue culture can be achieved by plaque assay (Rhim et al., 1967; Vaheri et al., 1967), by immunofluorescence (Hobbins and Smith, 1968), by hemagglutination (Sedwick and Sokol, 1970) or by positive or negative hemadsorption (Schmidt et al., 1969; Rawls et al., 1967).

Isolation of the virus can be made from nasopharyngeal washings by garglings, urine, central nervous system fluid, blood, human amnion, thyroid and the placenta. Specimens for Laboratory examination are normally collected in Hanks balanced salt solution, Medium 199 or Eagles Minimum essential medium (MEM). Possible contamination by fungi and bacteria is prevented by addition of 100 units per ml of penicillin, streptomycin and 50 units per ml of mycostatin. A 2%-10% heat inactivated foetal or newborn calf serum is also added as a nutritive supplement.

CONTROL

The successful isolation of rubella virus in cell culture systems in 1962 ushered in a new era of knowledge and hope for the control of rubella (Parkman et al., 1966). Various attempts to developing a rubella virus vaccine was undertaken by scientists following this break-through. A killed rubella virus vaccine was found immunologically incompetent because inactivation of the agent led to a loss of antigenicity. Attenuation of the virus by serial passage in tissue culture was therefore a more positive alternative and Parkman, Meyer and Panos in 1966 successfully developed and experimental live virus vaccine by making 77 serial passages of the virus in primary green monkey cells. The attenuated vaccine strain used by Parkman and associates was consequently named HPV_{77} (High Passage Virus 77). Five more serial passages were further made in duck embryo (DE) cell culture and an alternative production substrate was dog kidney cell culture in which 12 more serial passages were made. Two different rubella vaccine strains resulted; the $HPV_{77}DE_5$ and the $HPV_{77}DK_{12}$ respectively. The HPV_{77} vaccines were found to be able to stimulate the production in humans of serum neutralizing antibodies and the hemagglutination-inhibition antibodies following its administration. Vaccinated individuals were found to shed the virus from the throat for several days but no viraemia nor

infection in susceptible contacts were at that time associated with the vaccine hence its acceptability (Parkman et al., 1966). Following an extensive evaluation of these vaccine strains it was licensed in the United States of America in 1969. Huygelen and Coinvestigators (1967) developed another rubella vaccine strain, Cendehill, by making 51 passages in primary rabbit kidney cells. The vaccine was accepted for use and is manifactured by the SmithKline-RIT at Rixensart, Belgium. A third vaccine strain, RA27/3 was isolated by Plotkin (1969) in the Wister Institute, Philadelphia, U. S. A., by making 25 passages in human diploid cell cultures (WI-38), (Plotkin, 1969). The vaccine was licensed and used in Europe but gained a slow acceptance in the United States due to fears of its reactogenicity resulting from the use of human diploid cells as its production substrate. Lately a new Japanese vaccine strain, TO-336 have undergone comparative trails and found highly immunogenic and biologically acceptable for its non-placental transmission to the developing fetus (Best et al., 1974). These vaccines were all similar in their ability to stimulate the production of protective antibodies and lack of spread to susceptible contacts. The Plotkin vaccine strain RA27/3 however differ from the rest by its ability to infect by the nasal route and also induces a broader range of antibody response. LeBouvier and Plotkin (1971)

reported that the RA27/3 vaccine strain apart from inducing the production of serum neutralizing antibodies and the HAI antibodies also stimulates the production of local secretory IgA and precipitating antibodies comparable to those produced on infection by the wild virus.

Various clinical complications have recently been identified in subjects recieving these vaccine. The associated symptoms were found to be typical of a mild rubella and include; lymphadenopathy, mild fever with rash appearing after 10-20 days. Ogra and Herd (1971) identified arthritis in subjects receiving rubella vaccine. The presence of circulating immune complexes in post-vaccination sera of three of ten individuals vaccinated against rubella has been demonstrated. Arthralgia and other joint symptoms were observed in these individuals (Vergani et al., 1980). It has also been reported that on administration of rubella virus vaccine to a susceptible pregnant woman the vaccine virus can cross the placenta and infect the developing fetus with resultant congenital rubella disease in the newborn (Vaheri et al., 1972; Bolognese et al., 1973; Modlin et al., 1976; Hayden et al., 1980). The findings of Hinman and co-workers (1981) however contracdicts these earlier reports since no congenital malformations were observed in newborns whose mothers received any of the Cendehill, HPV_{77} or the RA27/3

vaccines before pregnancy. The continuous use of the rubella virus vaccines but with exclusion of pregnancy before administration to women in puerperium was recommended (Hinman et al., 1981). As a result of these complications associated with rubella virus vaccines and also the reported vaccine failure the $HPV_{77}DK_{12}$ licensed for use in 1969 was withdrawn from use in the United States in 1973 and Cendehill vaccines were also withdrawn from use in 1976. The only rubella vaccine currently in use in the United States is the Plotkin RA27/3.

Two widely different vaccination policies have been followed in the United States and in Great Britain. Whereas preschool age children are vaccinated in the United States in view to breaking the line of viral transmission to the older persons and mothers in Great Britian women between the age of 11-14 years are the vaccination targets. Recently the United States has adjusted her vaccination target to also complement women of child bearing age who are not pregnant nor hope to be pregnant three months after vaccination. Hospital workers have also been included as essential targets for vaccination both in Britain and in the United States.

In the African continent no universal vaccination policy is adapted rather during the Symposium held in Cairo, Egypt in 1979 on the'New Development in Vaccines' it was generally agreed that local prevalent situations should influence any vaccination policies adapted in African countries (Jurji, 1979).

The actual aim of vaccination is not prevention of postnatal rubella but rather the prevention of congenital rubella syndrome. Though the number of reported cases of CRS have been reduced in the United States and Great Britain the actual reduction of percentage of post-pubertal susceptible female have not been achieved (Pover et al., 1979). Moreover the reduction in incidence of CRS have been associated with an increased awareness of the disease and subsequent increase in therepeutic abortion among infected pregnant women.

Direct Chemotherapy have been attempted as a means of control of rubella virus infection. Schiff (1969) used high-titred lots of gamma globulin to prevent viraemia as well as clinical and serological infection in five subjects. Green and fellow workers (1965) demonstrated the ability of gamma globulin to prevent the disease but not the infection. Horstmann (1976) however pointed out that the effectiveness of gamma globulin as a prophylactic agent is highly

unpredictable and also unreliable and its use can only be accepted for a situation where for religious reasons or other reasons a rubella infected pregnant woman rejects therapeutic abortion. The use of interferon as a direct therapeutic agent for the congenital rubella syndrome have been reported. Though Desmyter and coworkers (1967) could not demonstrate inhibition of rubella virus by interferon in a rubella carrier culture established from an infected fetus Larsson and his colleagues (1976) were able to terminate rubella viraemia in a 14-month-old infant using $3x10^6$u/day dose of interferon for two weeks. Viral excretion was however not eradicated. More recently Arvin and associates (1982) have used human leukocyte alpha interferon at doses of $2x10^5$ to $7x10^5$u/kg per day for 10 days to decrease pharyngeal virus excretion in three infants. No significant side effects were associated with interferon use therefore the authors strongly pointed out that infants tolerate interferon well and further called for more scientific work on the therapeutic use of interferon at high dosage levels against congenital rubella disease.

MATERIALS AND METHODS

EPIDEMIOLOGICAL STUDIES

Alsever's Solution

This solution was prepared by dissolving the following in 250ml of distilled water.

Dextrose	20.5g
Sodium citrate	8.0g
Citric acid	0.55g
Sodium chloride	4.20g

The mixture was made up to 1 litre with sterile distilled water and sterilized by membrane filtration. The final solution at pH 6.2 was stored in 10ml volumes in refrigerator at $4^{o}C$.

Dextrose-Gelatin-Veronal Buffer

Gelatin (0.60g) was dissolved in 250ml of sterile distilled water in a stoppered 1 litre flask placed in a boiling-water bath. Barbital (5, 5-Diethylbarbituric acid (0.58g) was added. Fifty ml of 0.02g of Calcium chloride separately dissolved in distilled water in another flask was also added to the boiling barbital Gelatin mixture.

In another separate flask containing 500ml of distilled water the following salts were dissolved and added to the mixture:-

Sodium barbital	0.38g
Magnessium Sulphate ($M_gSO_4.7H_2O$)	0.12g

Sodium Chloride	8.5g
Dextrose	10.0g

The mixture was then made up to 1 litre. It was sterilized by membrane filtration, dispensed in 120ml volumes and stored at -20°C until required.

HSAG (HEPES-Saline-Albumin-Gelatin) Diluent

Preparation of this reagent was done in large quantities using methods recommended by Liedhaber (1970). In a 1 litre flask, 250ml of distilled water was added and the following dissolved in it.

HEPES (N-2-Hydroxyethypiperazine-N-2-ethanesulfonic acid)	5.960g
Sodium Chloride	8.190g
Calcium Chloride ($CaCl_2.2H_2O$	1.148g
Bovine Serum albumin	10.0g
Gelatin	0.0025g

The solution was then made up to 1 litre with sterile distilled water. The pH of this solution was adjusted to 6.5 by adding IN Sodium hydroxide. The final solution was sterilized by membrane filtration and stored at 4°C in a refrigerator.

25% Suspension of acid-washed Kaolin

The suspension was prepared by first straining 25g of acid-washed Kaolin through a fine sieve and dissolving this powder in 100ml of IN Sodium Chloride. The solution was then sterilized by autoclaving for 15 minutes at 1.05kg/cm^2 and 121^{o}C.

PREPARATION OF DUCK ERYTHROCYTE (RBC)

An initial sensitivity test was performed by titrating the Rubella HA antigen using red blood cells from Pigeon, Duck and Human group O to find out which was the most sensitive indicator for the titration of the antigen. The duck RBC gave a clearer agglutination pattern than the pigeon and human erythrocytes hence it was used for the test.

The red blood cells needed for the test was obtained by bleeding the duck from the wing vein. A 20Gxl needle on a 5ml syringe was used for the bleeding. Before each bleeding 2.0ml of the Alsever's solution was taken into the syringe. About 2.5ml-3.0ml of blood was collected from the duck each time and emptied into a universal bottle containing 9ml of alsever's solution. The cells collected were used for only one week from date of bleeding.

The erythrocytes were prepared for use by washing the cells three times in Dextrose-Gelatin-Veronal buffer (DGV). The cells were placed in a graduated conical centrifuge tube and four times its volume of DGV buffer was added and centrifuged at 900xG (2000 rpm) for 10 minutes. The buffy coat was carefully removed after each wash. The packed cell volume was read from the graduated conical centrifuge tube after the third wash and a 50% cell suspension was prepared by diluting the cells in an equal volume of DGV buffer. A 0.5% cell suspension was also prepared using HSAG diluent.

COLLECTION OF BLOOD SAMPLES

Blood samples were collected at random from healthy subjects from the four health zones in Imo State. The entire staff of the Medical Field Unit (M. F. U.) of the Imo State Ministry of Health were involved in this exercise as was directed by the Chief Health Officer of the State. Areas visited in the zones were Local Government headquarters, Schools, International Inoculation Units, Hospitals and Rural Health Centres.

Each subject was asked to complete a questionaire which demanded some personal information on the subject (See Appendix). 5-10ml of blood was collected in sterile MacCarthney bottles under sterile conditions by Venipuncture. The bottles were transported in a Coleman Brand Cooler packed with ice bags to maintain

a cold temperature of about 4°C and unusual aggitation of these bottles was minimized to avoid hemolysis of the blood. These bottles were kept at horizontal positions during clotting for maximum serum yield. The blood specimens were kept at 4°C in refrigerators at the Zonal Health Offices.

The serum was separated from the clot after an overnight stay in the refrigerator. After the retraction of the clot, separation of the serum from the clot was achieved by centrifugation for 10 minutes at 2000 r.p.m. and laboratory facilities were provided for this purpose by the major General Hospitals in each of these health zones. In Aba Health zone, the Microbiology Laboratory of the General Hospital, Aba, was used. In Umuahia Zone, the Pathology Laboratory of the Queen Elizabeth Specialist Hospital, Umuahia, was used. In Owerri Zone, the Haematology Laboratory, General Hospital, Owerri was used. In Okigwe Zone, Pathology Laboratory, General Hospital, Okigwe was used.

After centrifugation the separated sera were dispensed into sterile Bijou bottles and stored at -20°C in each of these Laboratories. The sera were transported after three weeks to Zaria by land in a Coleman Cooler packed with ice bags. The sera were immediately stored at -20°C on arrival in Zaria.

REMOVAL OF NON-SPECIFIC INHIBITORS IN TEST SERA

Each of the sera to be tested were first treated to remove the non-specific inhibitors and naturally occuring agglutinins present in all human sera. This was also done for the known positive and negative sera used as controls for the test. The methods of Clarke and Casals (1958), were used for removal of non-specific inhibitors and agglutinins with a few modifications. HSAG diluent was used instead of Borate Saline.

Point one ml. of the cold HSAG diluent was added to 0.1ml of the test serum and 0.6ml of a 25% suspension of the acid washed Kaolin was added. The suspension was mixed thoroughly by shaking and allowed to stand at room temperature for 20 minutes with frequent aggitation. At the end of the 20 minutes the Kaolin was sedimented by centrifugation. The supernatant liquid was put into a clean sterile centrifuge tube and 0.05ml of the 50% suspension of duck erythrocyte was added. It was mixed gently and the serum-erythrocyte mixture was incubated at 4°C for 1hr before centrifuging at 2000 r.p.m. (900xG) for 15 minutes. The supernatant liquid was removed and heated for 30 minutes at 56°C in order to inactivate the serum before use. This treated serum sample represent a 1:8 dilution.

TITRATION OF RUBELLA ANTIGEN

The rubella haemagglutinating (HA) antigen used for this test were obtained commercially from the Wellcome Reagents Limited, Wellcome Research Laboratories Beckenham England and Behring Institut, Behringwerke AG, Marburg, West Germany. The HA antigen was titrated each time the test was performed to verify the dilution giving complete agglutination of the 0.5% duck red blood cells.

The rubella antigen was available in a Lyophilized form and was reconstituted with 1ml distilled water and allowed to stand for 15 minutes at $4^{\circ}C$. A 1:4 dilution of the antigen was then prepared by adding 0.1ml of the antigen to 0.3ml of the Cold HSAG diluent. Using the dropping pipettes, purchased from Flow Laboratories Limited, Irvine, Scotland, 0.025ml of the HSAG diluent was added to wells 2 through 9 in each of three rows of wells in the V-shaped microtitration plate, obtained commercially from Flow Laboratories Limited, Irvine, Scotland. 0.05ml of the 1:4 dilution of the rubella HA antigen was added to the first well in each of the three rows. Using the diluting 'loop', purchased from Flow Laboratories Limited, Irvine, Scotland, 0.025ml was transfered from the first well into the second well in each row. This was thoroughly mixed and another transfer was made from the second well into the third

well in each of the three rows. After thorough mixing this transfer was continued until the ninth well and 0.025ml was discarded. The antigen dilution from well one through the ninth well now ranged from 1:4 in well one to 1:1024 in well nine with a two-fold difference between the various wells. 0.025ml of the Cold HSAG diluent was then added to each well of the diluted antigen. 0.05ml of the cold diluent was added in one additional well in each row for the erythrocyte control. The plate was then placed in the refrigerator for 15 minutes. At the end of the 15 minutes 0.05ml of cold 0.5% erythrocyte suspension was added to each well of the diluted antigen including the three wells containing 0.05ml of diluent only. The plate was gently tapped to ensure even distribution of the erythrocytes in each well, covered to avoid evaporation and incubated at $4^{\circ}C$ for 1½ hours. At the end of the incubation period the plate was removed from the refrigerator and allowed to stand for 15 minutes at room temperature before the agglutination pattern was read. The accepted antigen titre in each row was the highest dilution of the antigen causing complete agglutination of the duck red blood cell. This antigen titre which agreed in two of the rows was accepted for the test. The titre was multiplied by four to obtain 4 units of the antigen (4HAU) which was used for the test.

THE HEMAGGLUTINATION-INHIBITION TEST

(a) Titration of sera

Ten wells in a row of the microtitration plate were used for each serum sample to be tested. The first nine wells were used for the serum dilution and the tenth well was used for the serum control. Using the dropping pipettes, 0.025ml of the cold HSAG diluent was added to each well including the serum control omitting the first well in each row. 0.05ml of each treated 1:8 serum dilution was added to the first well and 0.025ml was added to the tenth well. Using the 0.025ml microtiter loops a serial two-fold dilution of the serum was made by transfering 0.025ml from the first well to the second and after thorough mixing, 0.025ml was transfered to the third well. It was continued in this fashion until the ninth well where 0.025ml was discarded. The dilution of each serum now range from 1:8 in the first well to 1:2048 in th ninth well. 0.025ml of the rubella antigen dilution which contain four hemagglutinating antigen unit (4HAU) was then added to each well omitting the tenth well. The serum-antigen-diluent mixtures were incubated at $4^{o}C$ for one hour and 0.05ml of the 0.5% suspension of the duck erythrocyte was added to each well. The plate was then gently shaken to ensure even distribution of cells. It was covered and placed for 1½ hours at $4^{o}C$. The plate was removed at the end of the 1½ hours

and placed at room temperature for 15 minutes before the agglutination patterns were read. The accepted serum titre was the reciproeal of the highest dilution of serum which completely inhibited hemagglutination. No agglutination was observed in the tenth well, used as the serum control. A serum titre of 1:8 or greater was accepted as an indication of previous exposure to rubella virus.

(b) Controls

Four other controls were included for each batch of sera tested, apart from the serum control, these were; Antigen Control, Positive and Negative serum controls and erythrocytes control.

Antigen Control:-

An antigen control was included in the test in order to check that 4HAU of the antigen was actually being used in the test. The process involved a back titration of the antigen used for the test as follows: 0.025ml of the cold HSAG diluent was added from wells two through five in each of three rows omitting the first well. 0.025ml of the antigen used for the particular test was then added to the first well and the second well in each row. The microtitre diluting loops were then used to transfer 0.025ml from the second well to the third well of each row. After a thorough mixing 0.025ml was transfered from the third well to the fourth.

This transfer was continued to the fifth well after which 0.025ml was discarded. The antigen units contained in the five wells were 4 units in the first, 2 units in the second, 1 unit in the third, ½ unit in the fourth and ¼ unit in the fifth. 0.025ml of the cold HSAG diluent was added to each well and after this 0.05ml of cold 0.5% erythrocyte suspension was added. The plate was covered after a gentle shake and incubated for 1½ hours at 4°C. At the end of this incubation period, the hemagglutinating pattern was read after allowing the plate to stand for 15 minutes at room temperature. Complete agglutination was observed to occur in wells one, two and three thus indicating that 4 HAU was used for the test.

Positive and Negative Serum Controls:-

In the same plate used for the antigen control, known positive and negative serum, pre-treated for removal of non-specific inhibitors and naturally occurring agglutinins, were also titrated using the same methods as was used for each sera tested. A 1:8 dilution was used for both the positive and the negative control serum. The negative control serum showed no inhibition of agglutination at any dilution while the titre of the known positive control serum remained positive at HAI titre 1:64.

Erythrocyte Control:-

For every test conducted an erythrocyte control was included. 0.025ml of the cold HSAG diluent was added to all the wells in one row of the control plate. 0.05ml of the 0.5% erythrocyte suspension was then added to each well in this row at the same time erythrocyte were being added to the test. No hemagglutination was observed in these wells after 1½ hours of incubation.

ISOLATION STUDIES

SOLUTIONS

Phosphate Buffered Saline (PBS)

The phosphate buffered saline required for cell washing was prepared by dissolving the following salts in 1000ml of glass distilled water,

Sodium Chloride	8g
Potassium Chloride	0.2g
Disodium Hydrogen phosphate	1.15g
Potassium dihydrogen phosphate	0.2g

The solution was sterilized by autoclaving at 121^{o}C for 20 minutes and stored at room temperature before use.

Hanks balanced Salt (BSS)

Two separate solutions (A and B) were prepared in two different flasks as follows:

Solution A

The following salts were dissolved in 100ml of distilled water:

Sodium Chloride	40.0g
Potassium chloride	2.0g
Magnessium sulphate ($MgSO_4.7H_2O$)	1.0g

Calcium chloride (0.70g) was dissolved in about 30ml of glass distilled water and slowly added, with constant stirring, to the solution of salts above. The total volume was made up to 250ml with glass distilled water.

Solution B

This was prepared by dissolving the following in 250ml of glass distilled water,

Disodium Hydrogen phosphate	0.30g
Potassium dihydrogen phosphate	0.30g
Glucose	5.0g
Phenol red	0.05g

The two solutions A and B were mixed by adding solution B to solution A with gentle stirring. The final 10X concentrated solution was stored at - 20°C. Before use a single strength solution was prepared and sterilized by membrane filtration (average pore size of 220mm) under negative pressure. The pH of the final single strength solution was adjusted to 7.4 by adding a few drops of sterile 8.8 percent sodium bicarbonate solution.

Sodium Bicarbonate (8.8%)

In 400ml of glass distilled water 42.05 grams of sodium bicarbonate was dissolved by gentle stirring at room temperature. The solution was then made up to 500ml and sterilized by membrane filtration (average pore size of 220mm) under negative pressure. The final sterile solution was dispensed in 50ml volumes in sterile 100ml hospital bottles.

STAINS

Trypan blue (0.2% Diamine Blue 3B)

Trypan blue	1.0g
Phosphate buffered saline	500ml

The above materials were mixed by stirring at room temperature for about 1 hour with a magnetic stirrer. The mixture was stored at room temperature.

Crystal violet

Crystal Violet	0.1g
Citric acid	1.92g
Glass distilled water	100ml

The citric acid was first dissolved in enough water to make 75.0ml. The crystal violet was then added and further diluted to 100ml with glass distilled water. The final solution was labelled and stored at room temperature.

SERA

Sterile Foetal Calf Serum and Newborn Calf Serum wereobtained from National Veterinary Research Institute, Virology Division, Vom, Plateau State and were dispensed in 20ml volumes and stored at - 20°C

TRYPSIN

The trypsin 5% (w/v) stock solution was prepared in phosphate buffered Saline by stirring trypsin 1:250 powder (Difco laboratories, West Moseley, Surrey) first at room temperature for several hours then at 4°C overnight. Insoluble materials were removed by centrifugation at 2000rev/min for 1 hour before sterilization by membrane (average pore size of 220mm) filtration and storage at -20°C in 5ml volumes.

Trypsinization Mixture

A 0.25% trypsin mixture was prepared asceptically from the 5% stock by mixing the following:

Trypsin 5% stock solution	5ml
Hank's Balanced Salt Solution	95ml
Penicillin and Streptomycin mixture	1ml
Fungizone	1ml

The mixture was stored at - 20°C until ready for use.

ANTIBIOTICS

A 600mg vial of Benzylpenicillin sodium BP and a vial of streptomycin sulphate (1g) both prepared by Glaxo laboratories Limited, Greenford, were obtained commercially and reconstituted asceptically in 100ml sterile glass distilled water. The mixture was dispensed in 10ml volumes and stored at - 20°C

MEDIA

Medium 199

The medium was modified with Earle's salts and glutamine without sodium bicarbonate. It was obtained from the Flow laboratories limited, Irvine in powder form and reconstituted in the Virology Division, National Veterinary Research Institute, Vom, Plateau State. The sterile reconstituted media containing sodium bicarbonate was obtained in 300ml volume bottles. It was stored at - 20°C.

Minimum Essential Medium (MEM)

This was also obtained from Flow laboratories, Irvine as a powder with glutamine and without sodium bicarbonate. It was made up at the National Veterinary Research Institute Vom, Plateau State with the addition of sodium bicarbonate. The sterile media were obtained in 300ml volumes and asceptically dispensed in 20ml volumes before storage at - 20°C.

CELLS AND TISSUE CULTURE

Vero Cells

Vero cells are an established cell line originally derived from the Kidneys of an African green monkey (Cercopithecus aethiops). These cells were obtained from the National Veterinary Research Institute, Vom, Plateau State at passage level 161. The confluent cells were obtained in a 60ml plastic tissue culture Falcon dish (sterilin Limited, Teddington, Middlesex, England) and further passaged six more times in the Department of Microbiology, Ahmadu Bello University, Zaria before use. The passaged cells were grown in the following growth medium.

Vero Cell Growth Medium

Constituents	Volume	Final Concentration
Medium 199 (or MEM)	85.5ml	1X
Foetal Calf Serum (or Newborn Calf Serum)	10ml	10%
Penicillin and streptomycin	1ml	1%
Fungizone	1ml	1%
Sodium Bicarbonate	2.5ml	4mM

The maintenance medium for the confluent Vero Cell monolayers was the same medium as above except that the concentration of the Foetal Calf Serum was reduced to 2%.

Culture of Continous Cell line (Vero)

These cells were cultured in 50ml plastic tissue culture Falcon dishes (sterilin Limited, Teddington Meddlesex, England).

The cells were subcultured by pouring-off old medium from a confluent culture and the monolayer washed three to four times in prewarmed phosphate buffered saline containing 1% Penicillin and streptomycin and 1% Fungizone. Trypsin mixture (0.25%) prewarmed at 37°C was added to just cover the cell sheet. After 60 seconds incubation at 37°C the trypsin mixture was poured off and the culture further incubated at 37°C until the cells started detaching gradually from the bottle in usually about 10 - 15 minutes. A small amount of growth medium was added and the cells resuspended by aspirating vigorously with a 5ml pipette against the inside of the bottle several times. A standard suspension of 50ml was made in growth medium and the cells counted. Fresh 50ml plastic tissue culture bottles were seeded with approximately 2 x 10^6 cells. 20ml of growth medium was added to a 50ml plastic tissue culture Falcon bottle. The cultures were incubated at 37°C and confluency of cells was obtained in 4 - 5 days.

Cell cultures for primary viral isolation were grown in 24 - well, 16mm well diameter, tissue culture dish (Tissue Culture Cluster 24, Costar, 205 Broadway Cambridge, Massachusetts, U.S.A.) Each well of the multiwell dish was seeded with approximately 2 x 10^5 cells in 2ml of growth medium. The cultures were then incubated at 37°C in a humid atmosphere of 5% CO_2 in air and confluent monolayers were obtained after overnight incubation.

Primary Monkey Kidney Cells

This primary cell line was obtained from the kidneys of a young monkey (Cereopithecus aethiops) of about 21 days old. The monkey was purchased from Alhaji Sani Bello, Kano Central Market, Kano, Kano State.

The young African green monkey was aneasthesized by putting it into a desicator flask containing cotton wool soaked in an Ether/Chloroform mixture (1:3). The kidneys were asceptically removed and the cells were derived from them.

A sterile forcep and scissors were used to hold and remove the kidney capsule and some fat. A stab was made with the sterile pair of scissors into the kidney and a slit was further made. The kidney was divided into two lobes and the outer deep red zone, the cortex, was removed and placed in a sterile petridish.

The sterile pair of scissors was use to mince the kidney cortex. The minced tissue was transfered into a trypsinization flask containing 0.25% trypsin in Hanks Balanced salt solution prewarmed at 37°C. A sterile magnetic stirring bar was placed in the mixture and the flask was stirred at 37°C on a stirring plate for 10 minutes at a speed relatively rapid without the formation of foam. The flask was removed and the supernatant fluid carefully poured off and discarded. Fresh trypsin mixture was added and stirring continued for 30 minutes. The supernatant fluid was removed and placed in a chilled

flask containing sterile growth nedium. Stirring was repeated with the addition of fresh trypsin and the cell suspension from the various trypsinizations were pooled. The remnants containing large tissue fragments were discarded. The cell suspension was filtered through sterile gauze pads to renove large tissue fragments. Sterile PBS was added to the filtrate and dispensed in equal volumes into sterile centrifuge tubes. The centrifuge tubes were filled with PBS, closed tightly with the aluminum foil covers and centrifuged at 2000 rev/min for 10 minutes. The supernatant fluid was discarded and the cell sediment was resuspended in fresh sterile PBS. The cells were washed 3 - 4 tines until the supernatant fluid became clear. The clear supernatant fluid was discarded and the cell sediment resuspended in the growth nedium. A viable cell count was nade as described below and the suspension diluted to contain 5 - 6 x 10^6 cells/ml. This was dispensed into 50ml plastic tissue culture Falcon dishes in 1ml volumes and growth nedium was added to make up to 10ml. It was incubated at 37°C. The cells attached to the inside surface of the plastic tissue culture dish after overnight incubation. A confluent nonolayer was obtained after about 4 - 5 days incubation. The growth nedium was then removed and replaced with the naintanance nedium.

Viable Cell Count

A known dilution of the cell suspension to be counted was made in 0.2% trypan blue stain prewarmed at 37°C. A cover glass was placed on the ruled area of an improved Neubauer Haemacytometer and the diluted cell suspension in 0.2% trypan blue stain was added. The viable cells which did not take up the blue stain were counted. The total amount of cells in the suspension was computed and the cells dispensed as required.

COLLECTION OF SPECIMEN

Specimen for Primary viral isolation were collected from the Out-patient Clinic, Department of Pediatric, Ahmadu Bello University Teaching Hospital, Zaria. Throat swabs were obtained from children between the age of 1 - 24 months with sterile cotton swabs. The swabs were then immersed in Cold 6ml MEM containing 1% bovine plasma albumin, Penicillin 100U/ml and streptomycin 100µg/ml and 10µg/ml of Fungizone. The specimens were transported to the Department of Microbiology, Ahmadu Bello University in Coleman Cooler packed with ice bags to maintain a temperature of about 4°C.

INOCULATION OF CULTURES

The Cold MEM containing each throat swab specimen was decanted and centrifuged at 2000 rev/min. for 15 minutes to remove mucous and extrenous materials. The maintenance medium was removed from the confluent cell culture monolayers using sterile pasteur pipettes and the wells were rinsed three times in PBS.

0.5ml volumes of each specimen was inoculated into separate wells in the 24 - well multiwell dish. After an adsorption period of 1 hour at room temperature the specimen fluid was removed and 2ml of fresh maintenance medium was added. The cultures were then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air and observed daily for cytopathic effect (CPE). Fluid from wells showing CPE were harvested and stored with 60% glycerol (1:1) at - 70°C in liquid Nitrogen for further investigation.

TITRATION OF ISOLATES

Titration of the isolates were performed in sterile 24-well plastic Tissue Culture dishes. Vero cells ($2x10^4$cells/ml) were seeded into these multiwells and confluency was attained after overnight incubation at 37°C in an atmosphere of 5% C^0_2 in air, The test was preformed with supernatant harvested from the vero cell monolayers showing CPE. The titre of the isolates was calculated by the Reed and Muench (1938) method.

NEUTRALIZATION OF ISOLATES

The neutralization assay was performed by a method described by Liebhaber and associates (1967) with a few modifications. Sterile plastic 24-well dishes (Tissue Culture Cluster 24, Costar, 205 Broadway Cambridge, Massachusetts, U.S.A.) were used for the test. Vero

cells ($2x10^4$cells/ml) were seeded into these wells and confluency was attained after overnight incubation at 37°C in an atmosphere of 5% carbon dioxide in air.

A standard rubella human antiserum obtained in a lyophilized form (Behring Institut, Behringwerke AG, Marburg, West Germany) was reconstituted with sterile distilled water. It was heat inactivated at 56°C for 30 minutes in a water bath before use.

Six two-fold serial dilutions of the serum were made in 0.5ml of Minimum essential medium. The viral isolate containing $100TCID_{50}$/ml was asceptically added in 0.5ml volumes into each serum dilution. The tubes were then incubated at 37°C for 1 hour after the incubation period, 0.2ml of the serum-virus mixture was inoculated in the Vero cell confluent monolayers. The test was performed in triplicate. Controls consisted of the virus, serum and diluent inoculated separately into the Vero cell confluent monolayers. All the plates were incubated at 37°C in an atmosphere of 5% carbon dioxide in air and observed for Cytopathic effect over a period of 12 days after inoculation.

RESULTS

Rubella antibody Distribution Pattern in the Health Zones

Haemagglutination-inhibition antibody distribution to Rubella virus infection varied significantly in the four health zones in Imo State. Table I shows that Okigwe health zone had the highest percentage seropositive individuals. Out of 202 serum samples analysed for Rubella Hemagglutination-inhibition antibodies, 82% were seropositive. Umuahia health zone which is as densely populated as Okigwe health zone had 74% seropositive individuals. Owerri and Aba zones both had low percentage seropositive individuals.

Rubella Haemagglutination-Inhibition Antibody Distribution Pattern in Relation to Age

Age distribution pattern of immunity show that HAI antibody titre to rubella virus infection increased with the increasing age of the individuals examined (Fig. I and Table 2). HAI antibody titre found to be low in the age group 5-9 years. Out of 449 serum samples analysed in this age group for Rubella HAI antibodies only 50.8% were seropositive. Adolescents and adults had the highest of immunity to rubella virus infection individuals of age group 10-14 years and 15-19 years had Rubella antibodies at percentage levels of 90% and 79% respectively.

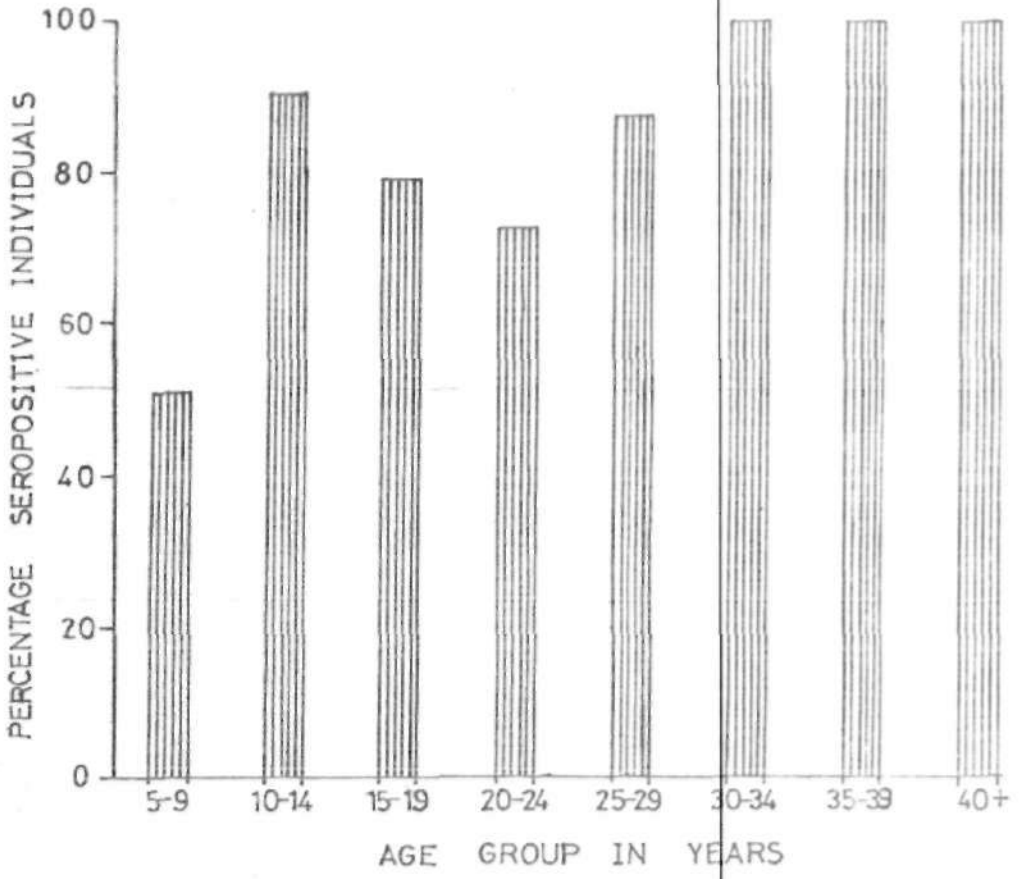


Fig. 1 Rubella HAI antibody distribution pattern in relation to age of people in Imo State of Nigeria.

Rubella Immunity in Relation to Socioeconomic Status

Rubella HAI antibody distribution was found to be influenced by socioeconomic status. Table 3 show that 70% of 486 serum samples collected from people of low socioeconomic status were seropositive. Samples collected from individuals of high socioeconomic status had 64% seropositives. Statistical analysis of results (Table 7) using the Chi-square distribution show that this difference in immunity was statistically significant at a probability level of 5%.

Rubella Immunity in the Urban and Rural Population

No difference in HAI antibodies was found between the rural and urban population as shown in Table 4. Analysis of the results with the Chi-square test (Table 7) show no significant difference between the two populations.

Rubella HAI antibody distribution Pattern among women of Child-bearing Age

Women in the age group 15-19 years and above were regarded as being in the child-bearing period. Samples collected from women in these age groups total 401. Out of this number 80.8% were seropositive. Table 5 and Fig. 2 show that the distribution pattern of immunity increased with age of the women. The age group 15-19 years had a high percentage level of immunity (79%) whereas the age group 20-24 years had only 57% seropositive individuals. A higher percentage level of

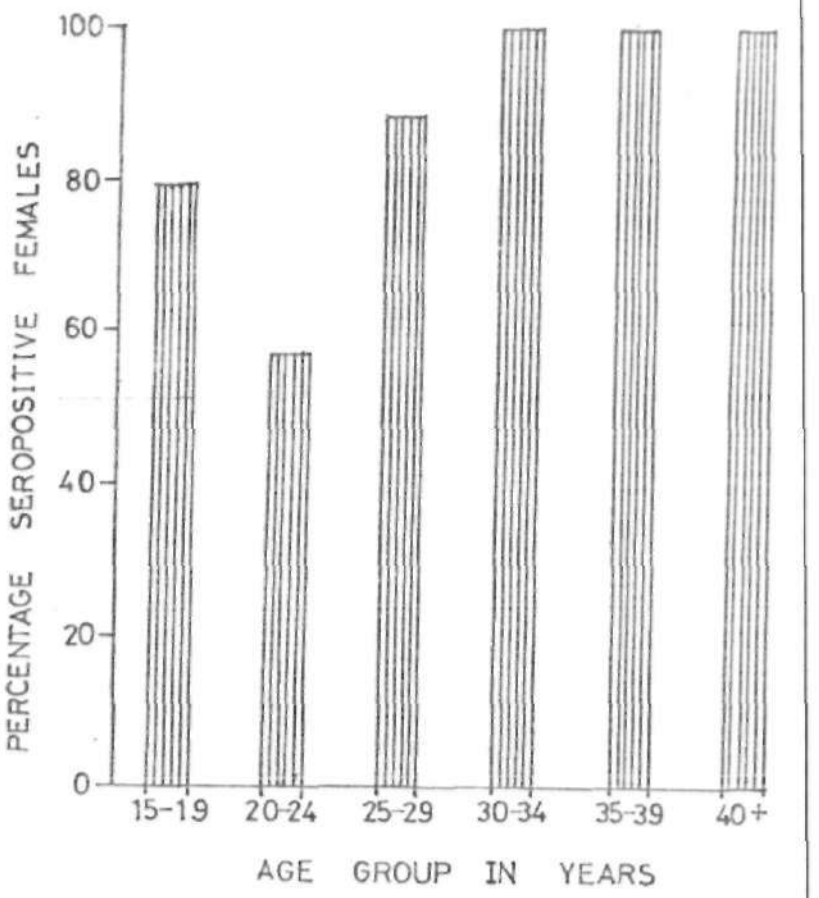


Fig. 2 Rubella HAI antibody distribution pattern among women of child-bearing age in Imo State of Nigeria.

immunity was found in the older age groups (Fig.2).

Incidence of Rubella Haemagglutination-Inhibition Antibodies in Relation to Sex

The results obtained from 687 serum samples collected from females in Imo State show that 74.5% were seropositive for Rubella HAI antibodies. Out of 244 serum samples collected from males only 46.3% were seropositive as shown in Table 6: Immunity to Rubella virus infection therefore varied with sex of the individuals tested. This differences in level of immunity between the male and female sex was found to be statistically significant by the Chi-square test (Table 7).

Determination of Prevalence rate of Rubella Infection in Imo State of Nigeria

The percentage prevalence rate of Infection to Rubella Virus was found to be 67% as shown in the calculations below:

Total number of sera tested for presence of Rubella HAI antibody = 931

Number of sera positive for Rubella HAI antibody = 625

Prevalence rate of Rubella Infection. $= \frac{\text{Number of sera Positive}}{\text{Total number of sera tested.}}$

$= \frac{625}{931}$

$= 0.67$

∴ % Prevalence rate of Rubella Infection = 67%

PRIMARY ISOLATION RESULTS

A total of 120 samples of throat swabs were collected from sick children at the Out-patient ward of the Pediatric clinic of Ahmadu Bello University Teaching Hospital, Zaria. Most of these children had evidence of a mild rash and a few others had posterior cervical auricular or occipital lymphadenopathy.

These specimen were inoculated into Vero cell confluent monolayers (Plate I) grown in a 24-well tissue culture dish (Tissue Culture Cluster 24, Costar, 205 Broadway, Cambridge, Massachusetts, U.S.A.). Cytopathic effect was observed in 48 out of the 120 monolayers inoculated. These cytopathic changes started as a gradual rounding of the cell foci after 2 - 4 days of incubation. This focal cell rounding was followed by an accumulation of the rounded cells within 4 - 7 days of incubation (Plate 2). After 2 - 4 days of further incubation there was pronounced degeneration of these cells leading to the destruction of the cell-sheet as shown in plate 3. The 48 isolates out of 120 specimen gave a 40% isolation rate.

Infectivity titration assay was performed to determine the titre of all the viral isolates and the results gave an average titre of $10^{5.2}TCID_{50}/ml$. In order to ensure that the observed CPE was due to the presence of Rubella virus, a neutralization test was conducted using human antiserum to rubella virus purchased from the

Behring Institute, Behringwerke AG, Marburg, West Germany. The rubella antiserum inhibited CPE at dilutions of up to 1:128 in all the isolates.

Health Zones	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
Owerri	210	56.2	43.8
Aba	217	53.9	46.1
Umuahia	302	74.2	25.8
Okigwe	202	82.2	17.8

Table 1: Rubella antibody distribution pattern in the Different Health Zones of Imo State.

Age-Group In Years	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
5 - 9	449	50.8	49.2
10 - 14	73	90.4	9.6
15 - 19	318	79.2	20.8
20 - 24	22	72.7	27.3
25 - 29	48	87.5	12.5
30 - 34	6	100	0
35 - 39	9	100	0
40^{+}	6	100	0
TOTAL	931	67.1	32.9

Table 2: Rubella Haemagglutination-Inhibition Antibody Distribution Pattern in Relation to Age of people in Imo State of Nigeria.

Nature of Community	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
Urban	390	63.6	36.4
Rural	541	69.7	30.3

Table 3: Rubella Immunity Status in Rural and Urban Populations of Imo State of Nigeria.

Sex	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
Male	244	46.3	53.7
Female	687	74.5	25.5

Table 4: Incidence of Rubella HAI antibodies in relation to sex of people in Imo State of Nigeria.

Socioeconomic Status	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
HIGH	445	63.8	36.2
LOW	486	70.2	29.8

Table 5: State of Rubella Immunity in relation to Socioeconomic Status of people in Imo State of Nigeria.

CRITERIA FOR PLACEMENT

(1) Low Socioeconomic Status:- Represents poor rural subsistent farmers, Civil servants with large families (family size > 6) earning salaries below GL 05, City dwellers on daily paid jobs and petty traders with large families (family size > 6).

(2) High Socioeconomic Status: Represents individuals not covered by the above Criteria.

Age-Group In Years	Number of Sera tested	Percentage (%) Positive Sera	Percentage (%) Negative Sera
15 - 19	316	79.4	20.6
20 - 24	14	57.1	42.9
25 - 29	52	88.5	11.5
30 - 34	4	100	0
35 - 39	9	100	0
40^{+}	6	100	0
TOTAL	401	80.8	19.2

Table 6: Rubella Immunity distribution among women of Child-bearing Age in Imo State of Nigeria.

Table 7: STATISTICAL ANALYSIS OF RESULTS

Variable Item	Chi-Square Value (X^2)		Degree of Freedom (df)	Probability Level (p)	Interpretation
	Computed Value $X_1^2 df = \frac{\sum(O-E)^2}{E}$	Table Value			
Health Zones	56.2	7.81	3	0.05	HS
Sex	64.96	3.84	1	0.05	HS
Community (Urban, Rural)	3.54	3.84	1	$0.05<p$ $(X_1^2>3.54)$ <0.1	NS
Socio-economic Status (High,Low)	3.9	3.84	1	$0.05<p$ $(X_1^2>3.9)$ <0.025	S

S = Significant Statistically

HS = Highly Significant Statistically

NS = Not Significant Statistically

O = Observed Value

E = Expected Value

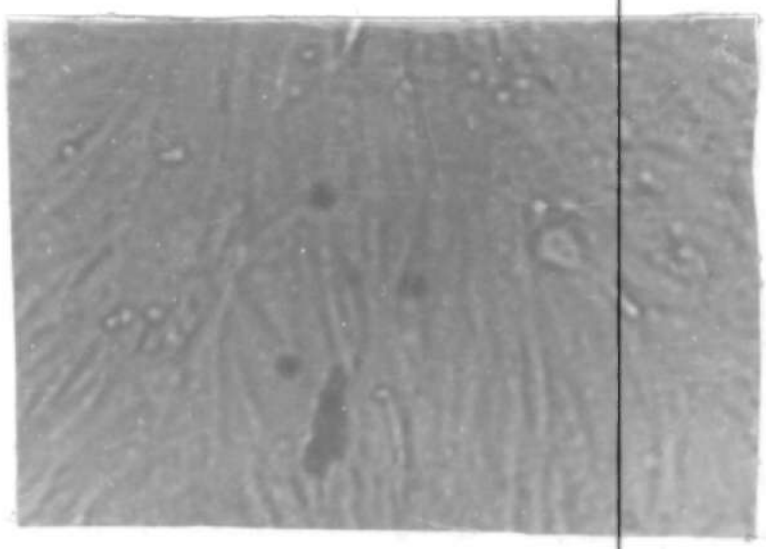


PLATE I: Photomicrograph of Uninoculated Vero cell confluent monolayers (Control) X1000

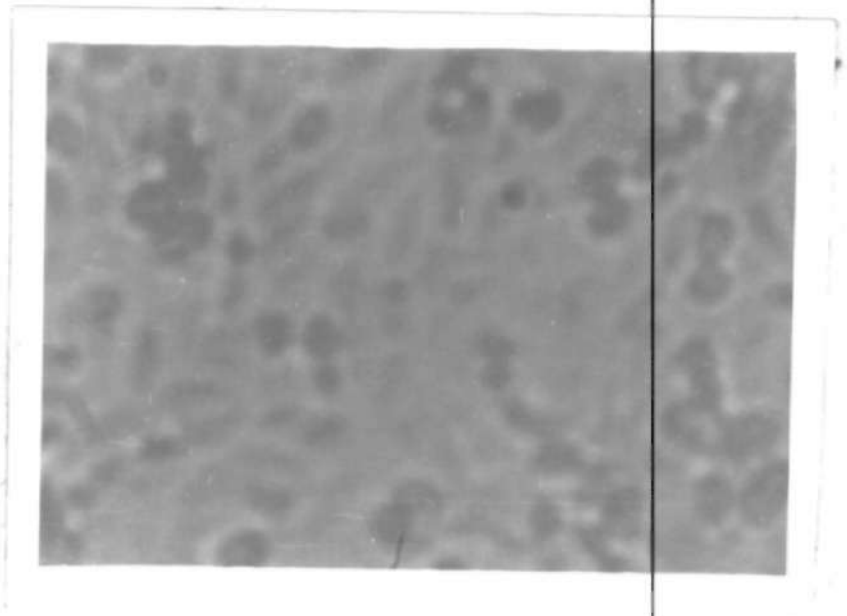


PLATE 2: Photomicrograph of CPE (accumulation of rounded cells) Observed after 4 days of infection X1000

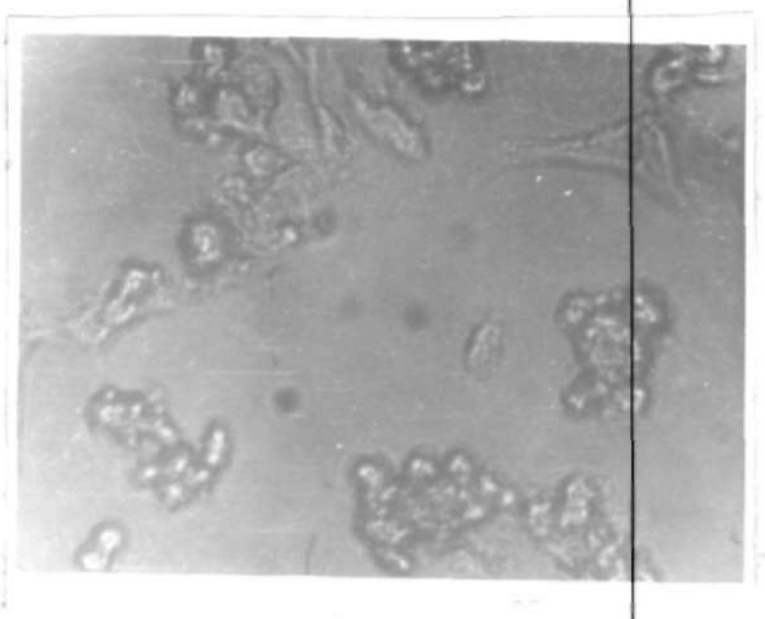


PLATE 3: Photomicrograph of CPE (destruction of the cell sheet) Observed after 21 days of infection X1000

D I S C U S S I O N

Rubella epidemiology has been poorly studied in the African continent. Only a few surveys have so far been conducted in Nigeria and the disease, though responsible for a number of babies with congenital defects, remain clinically and serologically undiagnosed (Bada and Odelola, 1978). This study reinforces the few existing information on the distribution pattern of rubella immunity in Nigeria. A hemagglutination-inhibition titre of 1:8 was regarded as evidence of pre-exposure to the virus and presence of rubella specific HAI antibodies. Out of 931 serum samples studied 625 were seropositive for rubella HAI antibodies. This figure gave an overall percentage HAI antibody titre of 67% which agree with the earlier findings of Odelola and Co-investigators (1977) who sowed a 68% HAI seropositive individuals in a survey of the Nigerian population. It is also in agreement with the findings in Ivory Coast where 68% of the population studied had rubella antibodies (Wurt$_z$ et al; 1970). Escudeiro and fellow workers (1975) however reported an average of 73% of the Angolan population studied possessing Rubella antibodies.

Age related pattern of immunity show that the level of rubella specific HAI antibodies increased with the increasing age of the individuals studied. Children in the age group 5-9 years had the lowest level of

rubella antibodies. Out of 449 children examined only 51% had rubella antibodies. The situation was different with adolescents and adults who possessed the highest level of rubella antibodies. The results show that individuals of the age group 10-14 years and 15-19 years who were born during the Civil war or who were minors (i.e. age / 5 years) at this period had rubella HAI antibodies at percentage levels of 90% and 79% respectively. General movement and high population density at the period could be implicated for this high immunity. Similar findings were made in Ghana though the studied population was only urban females (Addy et al, 1976). Khalifa and fellow workers (1973) also reported that 81% of Egyptian female of child-bearing age had rubella antibodies and immunity increased with increasing age in the Egyptian population. The findings of Escudiaro and co-incestigators (1975) however contrasts with the above findings. No significant difference was found between the various age groups studied in the Angolan population (Escudiaro et al, 1975). An increasing incidence with age of rubella antibodies was previously reported for the Swedish females by Ludstrom and associates (1967).

Results from this study also reveal that immunity to rubella virus infection varied significantly between the health zones in Imo State of Nigeria ($P<0.05$) Okigwe health zone had the highest percentage seropositive individuals (82%) for rubella HAI antibodies. Umuahia Health zone followed with 74% seropositives and Owerri and Aba zones had 56% and 54% respectively. A possible explanation for this variation in level of immunity could be due to the nature of settlement in the rural communities of these health zones and the geography of the areas. Okigwe and Umuahia health zones have rural communities with identical pattern of settlement. People tend to live together in large villages with very close contact between homes. This type of crowded condition facilitateseasy circulation of the virus in the population. The situation is different for Owerri and Aba health zones where large crowded rural communities with compact homes were generally absent. The tendency in these areas is for nuclear families to settle in compounds widely separated from other homes thus preventing easy spread of the virus. The nature of settlement in these zones is related to the topography of the zones. Aba and Owerri zones are generally flat while Okigwe and Umuahia zones are hilly causing overcrowding in the few flat areas. A similar finding was made in Taiwan where transmission of Rubella virus within and among different households

in rural communities were reported to be influenced by nature of settlement (Gale et al, 1972).

Immunity to rubella virus was found to vary with socioeconomic status. Out of 486 serum samples collected from people of low socioeconomic status 341 (70%) were seropositive for rubella HAI antibodies. There were 445 samples collected from people of high socioeconomic status out of which 284 (64%) were seropositive for rubella antibodies. This finding conform with that of Horstmann (1971) in a survey of the American population. The author reported that the proportion of susceptible women was higher in upper than in lower socioeconomic groups and further explained that this may be due to increased exposure in poor crowded homes.

Results from this study further reveal that no difference exist in immunity level between the urban and rural population studied. Immunity to rubella virus varied with sex of the individuals studied. A total of 687 serum samples were examined from females of various age groups and 512 (74.5%) had rubella antibodies. Serum samples collected from males totalled 244 and 113 (46%) had rubella antibodies. The findings contrast with that of Odelola and associates (1977) who found immunity to be independent of sex. The sampled male population in this study was however small. Earlier findings by other investigators however showed a general

high level of immunity to rubella virus among female populations (Khalifa et al, 1973; Escudiaro et al, 1975).

Primary isolation results show that 48 of the 120 samples of throat swabs showed cytopathologic changes in Vero cell confluent monolayers. The cytopathic effect observed started as a gradual rounding of the cell foci after 2-4 days of incubation. There was pronounced degeneration of cells leading to the destruction of the cell sheet after 9-14 days of incubation. The 40% isolation rate obtained in this study compares with the findings of Liebhaber and associates (1967) and McCarthy and co-workers (1963) achieving a 56% and 50% isolation rate respectively. The authors used Vero and Vervet Monkey Kidney cells and the study were undertaken in the United States of America and Britain respectively. The isolation rate of 40% is however higher than that of Weller and Neva (1962) who achieved only 25% isolation rate in primary human amnion cultures. The titre of the isolates was estimated to be $10^{5.2}TCID_{50}/ml$ and all the isolates were neutralized by a standard rubella human antiserum. A similar result was obtained by Liebhaber and fellow workers (1967) who also reported a general low titre of rubella virus isolates in cell culture.

The findings from this study emphasize the need for Rubella immunization in Nigeria. It further highlights the problem of adoption of an immunization policy based on our local conditions which was fully discussed by Jurji (1979). A survey of hospital records backed by continued research on perinatal infections as also here recommended.

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APPENDIX

AHMADU BELLO UNIVERSITY, ZARIA
DEPARTMENT OF MICROBIOLOGY

SERUM SURVEY OF IMO STATE
QUESTIONAIRE FORM

1. SAMPLE No. ..... DATE OF COLLECTION:...
2. NAME: .....
3. AGE: .....
4. LOCAL GOVERNMENT AREA: .....
5. SIZE OF COMMUNITY; URBAN ☐, RURAL ☐
6. ADDRESS: .....
   .....
   .....
   .....
7. SEX: ..... MARRITAL STATUS: .....
8. IF FAMALE, ARE YOU PREGNANT: .....
9. DURATION OF PREGNANCY: .....
10. TOTAL NUMBER OF PREGNANCIES:.....
11. NUMBER OF DELIVERIES: .....
12. NUMBER OF MISCARRIAGES, IF ANY: .....
13. OCCUPATION ..... (14) INCOME .....
15. SOCIOECONOMIC STATUS: ..... HIGH ☐ LOW ☐
16. IMMUNIZED AGAINST GERMAN MEASLES (RUBELLA) BEFORE
    INSERT YES ☐, NO. ☐ DON'T KNOW ☐
17. DATE IMMUNIZED ?, YEAR ..... MONTH .....
18. HAVE YOU HAD RUBELLA (GERMAN MEASLES) BEFORE
    INSERT YES ☐, NO. ☐, DON'T KNOW ☐

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-nic Status	Rubella Immuni-zation Status	HAI Ti-tre
1	Ow-1	18	F	Oratta	R	L	Nil	L8
2	Ow-2	12	F	Aboh/Mbaise	R	L	Nil	8
3	Ow-3	23	F	Aboh/Mbaise	R	L	Nil	L8
4.	Ow-4	26	F	Owerri	U	H	Nil	32
5.	Ow-5	34	F	Ikeduru	R	L	Nil	16
6.	Ow-6	17	F	Oratta	R	L	Nil	L8
7.	Ow-7	26	F	Owerri	U	H	Nil	L8
8	Ow-8	18	F	Owerri	U	L	Nil	L8
9.	Ow-9	28	F	Owerri	U	H	Nil	8
10.	Ow-10	29	F	Ahiara	R	L	Nil	8
11.	Ow-11	26	F	Ngor/Okpuala	R	L	Nil	16
12.	Ow-12	26	F	Mbaukwu	R	L	Nil	64
13.	Ow-13	36	F	Aboh/Mbaise	R	L	Nil	16
14.	Ow-14	17	F	Ahiazu/Mbais	R	L	Nil	L8
15.	Ow-15	9	M	Ngor/Okpuala	R	L	Nil	L8
16.	Ow-16	26	F	Ahiazu/Mbais	U	H	Nil	8
17.	Ow-17	15	F	Ahiazu/Mbais	R	L	Nil	8
18.	Ow-18	15	F	Oratta	R	L	Nil	16
19.	Ow-19	20	F	Aboh/Mbaise	R	L	Nil	64
20.	Ow-20	17	F	Ikeduru	R	L	Nil	L8
21.	Ow-21	19	F	Owerri	U	L	Nil	16
22.	Ow-22	17	F	Ide-ato	R	L	Nil	16

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
23.	Ow-23	32	F	Ido-ato	R	L	Nil	<6
24.	Ow-24	16	F	Ikeduru	R	L	Nil	16
25.	Ow-25	25	F	Ikeduru	U	H	Nil	8
26.	Ow-26	17	F	Ikeduru	R	L	Nil	8
27.	Ow-27	20	F	Ideato	R	L	Nil	64
28.	Ow-28	16	F	Ikeduru	R	L	Nil	16
29.	Ow-29	18	F	Ikeduru	R	L	Nil	16
30.	Ow-30	17	F	Mbieri	R	L	Nil	8
31.	Ow-31	16	F	Mbieri	U	L	Nil	<8
32.	Ow-32	12	F	Mbaukwu	R	L	Nil	16
33.	Ow-33	13	F	Ideato	R	L	Nil	8
34.	Ow-34	28	F	Aboh/Mbaise	U	H	Nil	32
35.	Ow-35	16	F	Ngor/Okpuala	R	L	Nil	16
36.	Ow-36	17	F	Ahiazu/Mbaise	R	L	Nil	<8
37.	Ow-37	10	M	Oratta	R	L	Nil	16
38.	Ow-38	6	F	Aboh/Mbaise	R	L	Nil	<8
39.	Ow-39	25	F	Aboh/Mbaise	U	H	Nil	<8
40.	Ow-40	17	F	Mbieri	R	L	Nil	8
41.	Ow-41	8	F	Oratta	R	H	Nil	32
42.	Ow-42	7	M	Ahiazu/Mbaise	R	H	Nil	<8
43.	Ow-43	8	M	Ikeduru	R	L	Nil	<8
44.	Ow-44	25	F	Ahiara	R	L	Nil	8
45.	Ow-45	6	M	Oratta	R	L	Nil	<8

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Tit-re
46.	Ow-46	12	M	Oratta	R	L	Nil	<8
47.	Ow-47	16	F	Oratta	R	L	Nil	16
48.	Ow-48	6	M	Oratta	R	L	Nil	<8
49.	Ow-49	9	M	Oratta	R	L	Nil	<8
50.	Ow-50	8	M	Ide-ato	R	L	Nil	<8
51.	Ow-51	7	M	Ahiazu/Mbaise	R	H	Nil	<8
52.	Ow-52	6	M	Oratta	R	L	Nil	<8
53.	Ow-53	8	F	Oratta	R	L	Nil	8
54.	Ow-54	42	F	Oratta	R	H	Nil	8
55.	Ow-55	6	M	Oratta	R	H	Nil	<8
56.	Ow-56	36	F	Ahiara	R	L	Nil	16
57.	Ow-57	7	M	Oratta	R	L	Nil	<8
58.	Ow-58	6	F	Oratta	R	L	Nil	<8
59.	Ow-59	19	F	Ahiazu/Mbaise	R	L	Nil	16
60.	Ow-60	8	M	Oratta	R	H	Nil	<8
61.	Ow-61	7	F	Oratta	R	L	Nil	<8
62.	Ow-62	40	F	Mbaukwu	R	H	Nil	8
63.	Ow-63	6	M	Ideato	R	L	Nil	<8
64.	Ow-64	20	F	Ahiazu/Mbaise	R	L	Nil	<8
65.	Ow-65	16	F	Ikeduru	U	L	Nil	16
66.	Ow-66	9	F	Mbaukwu	R	L	Nil	8
67.	Ow-67	9	M	Ahiazu/Mbaise	R	L	Nil	<8
68.	Ow-68	7	M	Ngor-Okpuala	R	L	Nil	<8

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
69	Ow-69	6	M	Ahiara	R	L	Nil	<8
70	Ow-70	26	F	Owerri	U	H	Nil	<8
71	Ow-71	15	M	Mbieri	R	L	Nil	<8
72	Ow-72	12	M	Aboh/Mbaiwe	R	L	Nil	16
73.	Ow-73	7	F	Mbaukwu	R	H	Nil	32
74.	Ow-74	7	F	Ngor/Okpuala	R	L	Nil	<8
75.	Ow-75	36	F	Ngor/Okpuala	R	L	Nil	16
76.	Ow76	6	F	Mbaukwu	R.	L	Nil	<8
77.	Ow-77	6	M	Ahiazu/Mbaise	R	L	Nil	<8
78.	Ow-78	33	F	Ahiazu/Mbaise	R	L	Nil	16
79.	Ow-79	8	M	Mbaukwu	R	H	Nil	<8
80.	Ow-80	29	F	Owerri	U	H	Nil	32
81.	Ow-81	5	M	Ahiazu/Mbaise	R	L	Nil	<8
82.	Ow-82	24	F	Oboh/Mbaise	U	H	Nil	<8
83.	Ow-83	29	F	Ahiazu/Mbaise	R	L	Nil	16
84	Ow-84	18	F	Mbaukwu	R	L	Nil	16
85	Ow-85	27	F	Ahiazu/Mbaise	R	L	Nil	64
86	Ow-86	16	F	Ngor/Okpuala	R	L	Nil	16
87	Ow-87	8	F	Ahiara	R	L	Nil	8
88.	Ow-88	7	M	Keduru	R	H	Nil	<8
89.	Ow-89	40	F	Mbieri	R	H	Nil	8
90.	Ow-90	15	F	Ideato	R	L	Nil	<8
91.	Ow-91	18	F	Ideato	R	L	Nil	8

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
92.	Ow-92	?	M.	Ngor/Okpuala	R	L	Nil	<8
93.	Ow-93	19	F	Mbieri	R	L	Nil	16
94.	Ow-94	14	F	Ahiara	R	L	Nil	8
95.	Ow-95	28	F	Aboh/Mbaise	R	L	Nil	8
96.	Ow-96	17	F	Ahiazu/Mbaise	R	L	Nil	16
97.	Ow-97	15	F	Aboh/Mbaise	U	L	Nil	<8
98.	Ow-98	19	F	Ikeduru	U	L	Nil	16
99.	Ow-99	23	F	Ngor/Okpuala	R	L	Nil	<8
100.	Ow-100	5	M	Ahiara	R	L	Nil	<8
101.	Ow-101	7	F	Mbaukwu	R	L	Nil	8
102	Ow-102	39	F	Ideato	R	H	Nil	8
103.	Ow-103	7	F	Ideato	R	H	Nil	32
104.	Ow-104	9	M	Ideato	R	H	Nil	<8
105.	Ow-105	28	F	Mbieri	R	L	Nil	16
106.	Ow-106	7	F	Mbieri	R	L	Nil	<8
107.	Ow-107	17	F	Aboh/Mbaise	R	L	Nil	<8
108.	Ow-108	15	F	Oratta	R	L	Nil	16
109.	Ow-109	16	F	Oratta	R	L	Nil	8
110.	Ow-110	8	M	Aboh/Mbaise	R	L	Nil	<8
111.	Ow-111	7	M	Oratta	R	H	Nil	<8
112.	Ow-112	18	F	Oratta	R	L	Nil	16
113.	Ow-113	16	F	Mbieri	R	L	Nil	16
114.	Ow-114	5	M	Aboh/Mbaise	R	L	Nil	<8

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
115.	Ow-115	5	F	Ikeduru	R	L	Nil	<8
116.	Ow-116	16	F	Aboh/Mbaise	U	L	Nil	16
117.	Ow-117	19	F	Oratta	R	L	Nil	<8
118.	Ow-118	12	M	Ahiara	R	L	Nil	16
119.	Ow-119	13	M	Ikeduru	R.	L.	Nil	<8
120.	Ow-120	28	F	Ideato	R	L.	Nil	64
121.	Ow-121	5	M	Ikeduru	R	L	Nil	<8
122.	Ow-122	6	M	Ideato	R	L	Nil	<8
123.	Ow-123	24	F	Ahiara	R	L	Nil	<8
124.	Ow-124	29	F	Aboh/Mbaise	U	H	Nil	32
125.	Ow-125	14	F	Aboh/Mbaise	R	L	Nil	8
126.	Ow-126	17	F	Mbieri	U	L	Nil	<8
127.	Ow-127	28	F	Mbieri	U	H	Nil	<8
128.	Ow-128	18	F	Ikeduru	R	L	Nil	8
129.	Ow-129	21	F	Oratta	R	L	Nil	64
130.	Ow-130	26	F	Ikeduru	R	L	Nil	8
131.	Ow-131	39	F	Mbieri	R	L	Nil	16
132.	Ow-132	42	F	Ahiara	R	L	Nil	16
133.	Ow-133	36	F	Ahiazu/Mbaise	R	L	Nil	16
134.	Ow-134	16	F	Owerri Urban	U	L	Nil	<8
135.	Ow-135	38	F	Ahiara	R	L	Nil	16
136.	Ow-136	7	F	Ngor/Okpuala	R	L	Nil	<8
137.	Ow-137	24	F	Ikeduru	U	H	Nil	32

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
138.	Ow-138	5	F	Mbaukwu	R	H	Nil	32
139.	Ow-139	9	M	Ideato	R	L	Nil	<8
140.	Ow-140	27	F	Owerri	U	H	Nil	<8
141.	Ow-141	32	F	Ngor/Okpuala	R	L	Nil	8
142.	Ow-142	26	F	Oratta	R	L	Nil	<8
143.	Ow-143	7	M	Ahiazu/Mbaise	R	H	Nil	<8
144.	Ow-144	8	M	Mbaukwu	R	L	Nil	<8
145.	Ow-145	28	F	Ikeduru	R	L	Nil	64
146.	Ow-146	16	F	Ideato	R	L	Nil	<8
147.	Ow-147	10	F	Ahiara	R	L	Nil	8
148.	Ow-148	17	F	Oratta	R	L	Nil	8
149.	Ow-149	25	F	Ikeduru	R	L	Nil	16
150.	Ow-150	23	F	Ngor/Okpuala	R	L	Nil	64
151.	Ow-151	19	F	Mbaukwu	R	L	Nil	<8
152.	Ow-152	45	F	Aboh/Mbaise	R	H	Nil	8
153.	Ow-153	16	F	Owerri	U	L	Nil	16
154.	Ow-154	6	F	Ngor/Okpuala	R	L	Nil	<8
155.	Ow-155	15	F	Mbaukwu	R	L	Nil	16
156.	Ow-156	11	M	Mbieri	R	L	Nil	<8
157.	Ow-157	9	M	Abiara	R	L	Nil	<8
158.	Ow-158	18	F	Ahiazu/Mbaise	R	L	Nil	16
159.	Ow-159	15	F	Ahiara	R	L	Nil	8
160.	Ow-160	13	M	Ideato	R	L	Nil	16

RESULTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
161.	Ow-161	1y	M	Mbieri	R	L	Nil	<8
162.	Ow-162	16	F	Ngor/Okpuala	R	L	Nil	16
163.	Ow-163	5	M	Mbieri	R	L	Nil	<8
164.	Ow-164	17	F	Mbaukwu	R	L	Nil	16
165.	Ow-165	9	F	Ngor/Okpuala	R	L	Nil	8
166.	Ow-166	6	F	Mbieri	R	L	Nil	<8
167.	Ow-167	14	M	Mbieri	R	L	Nil	16
168.	Ow-168	10	M	Mbaukwu	R	L	Nil	<8
169.	Ow-169	8	M	Ahiara	R	L	Nil	<8
170.	Ow-170	5	M	Ngor/Okpuala	R	L	Nil	<8
171.	Ow-171	29	F	Ikeduru	R	L	Nil	16
172.	Ow-172	28	F	Ideato	R	L	Nil	64
173.	Ow-173	12	F	Ahiara	R	L	Nil	8
174.	Ow-174	16	F	Ahiara	R	L	Nil	<8
175.	Ow-175	20	F	Ideato	R	L	Nil	<8
176.	Ow-176	8	M	Ahiara	R	H	Nil	<8
177.	Ow-177	29	F	Ikeduru	R	L	Nil	8
178.	Ow-178	43	F	Ngor/Okpuala	R	H	Nil	8
179.	Ow-179	27	F	Mbieri	U	H	Nil	<8
180.	Ow-180	6	F	Mbieri	R	L	Nil	8
181.	Ow-181	26	F	Ikeduru	U	H	Nil	32
182.	Ow-182	6	M	Ngor/Okpuala	R	L	Nil	<8
183.	Ow-183	8	F	Mbaukwu	R	L	Nil	16

RESUTS (OWERRI HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
184.	Ow-184	39	F	Mbaukwu	R	L	Nil	16
185.	Ow-185	7	M	Mbaukwu	R	L	Nil	<8
186.	Ow-186	10	M	Ngor/Okpuala	R	L	Nil	16
187.	Ow-187	14	M	Abo'i/Mbaise	R	L	Nil	<8
188.	Ow-188	39	F	Ahiara	R	L	Nil	16
189.	Ow-189	18	F	Mbieri	U	L	Nil	<8
190	Ow-190	27	F	Mbieri	U	H	Nil	8
191.	Ow-191	16	F	Ahiara	R	L	Nil	8
192.	Ow-192	20	F	Ngor/Okpuala	R	L	Nil	64
193.	Ow-193	18	F	Ideato	R	L	Nil	16
194.	Ow-194	7	F	Ahiazu/Mbaise	R	L	Nil	<8
195.	Ow-195	15	F	Ikeduru	R	L	Nil	16
196	Ow-196	6	F	Ahiara	R	H	Nil	32
197.	Ow-197	5	M	Mbaukwu	R	L	Nil	<8
198.	Ow-198	7	M	Ahiara	R	L	Nil	<8
199.	Ow-199	24	F	Mbieri	R	L	Nil	64
200.	Ow-200	18	F	Ideato	R	L	Nil	8
201.	Ow-201	8	M	Ngor-Okpuala	R	L	Nil	<8
202.	Ow-202	7	M	Ahiazu/Mbaise	R	L	Nil	<8
203.	Ow-203	25	F	Ideato	R	L	Nil	16
204.	Ow-204	28	F	Mbaukwu	R	L	Nil	64
205.	Ow-205	27	F	Mbieri	U	L	Nil	8
206.	Ow-206	17	F	Ideato	R	L	Nil	16
207.	Ow-207	6	F	Ahiara	R	H	Nil	32
208.	Ow-208	8	F	Ahiara	R	L	Nil	<8
209.	Ow-209	13	M	Mbaukwu	R	L	Nil	<8
210.	Ow-210	16	F	Ahiara	R	H	Nil	16

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
211.	Ok-1	16	F	Okigwe	U	H	Nil	<8
212.	Ok-2	6	F	Afikpo-Road	U	H	Nil	<8
213.	Ok-3	16	F	Okigwe	U	H	Nil	32
214.	Ok-4	17	F	Isuochi	U	H	Nil	64
215.	Ok-5	18	F	Isuochi	U	L	Nil	16
216.	Ok-6	18	F	Okigwe	U	H	Nil	8
217.	Ok-7	22	F	Okigwe	U	L	Nil	32
218.	Ok-8	16	F	Isuochi	U	H	Nil	64
219.	Ok-9	8	M	Okposi	R	L	Nil	16
220.	Ok-10	8	F	Okposi	R	H	Nil	16
221.	Ok-11	16	F	Okigwe	U	H	Nil	64
222.	Ok-12	16	F	Okigwe	U	H	Nil	32
223.	Ok-13	16	F	Okigwe	U	L	Nil	<8
224.	Ok-14	15	F	Okigwe	U	H	Nil	64
225.	Ok-15	18	F	Okigwe	U	L	Nil	16
226.	Ok-16	24	F	Isuochi	U	L	Nil	32
227.	Ok-17	18	F	Okigwe	U	L	Nil	16
228.	Ok-18	18	F	Okigwe	U	H	Nil	8
229.	Ok-19	17	F	Okigwe	U	H	Nil	64
230.	Ok-20	16	F	Isuochi	U	L	Nil	16
231.	Ok-21	9	M	Okposi	R	L	Nil	64
232.	Ok-22	12	F	Okposi	R	H	Nil	32
233.	Ok-23	16	F	Isuochi	U	H	Nil	8

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
234.	Ok-24	3	M	Okigwe	U	H	Nil	16
235.	Ok-25	11	F	Okposi	R	H	Nil	64
236.	Ok-26	8	F	Okigwe	U	H	Nil	<8
237.	Ok-27	8	F	Isuochi	U	H	Nil	<8
238.	Ok-28	8	F	Isuochi	U	H	Nil	<8
239.	Ok-29	16	F	Isuochi	U	H	Nil	32
240.	Ok-30	6	F	Onuimo	R	H	Nil	32
241.	Ok-31	16	F	Okposi	R	L	Nil	16
242.	Ok-32	18	F	Isuochi	U	H	Nil	<8
243.	Ok-33	6	F	Isuochi	U	L	Nil	32
244.	Ok-34	16	F	Isuochi	U	L	Nil	<8
245.	Ok-35	8	F	Isuochi	U	H	Nil	32
246.	Ok-36	6	M	Okigwe	U	H	Nil	32
247.	Ok-37	8	F	Onuimo	R	H	Nil	16
248.	Ok-38	17	F	Isuochi	U	H	Nil	8
249.	Ok-39	16	F	Isuochi	U	H	NIl	32
250.	Ok-40	20	F	Isuochi	U	L	Nil	32
251.	Ok-41	8	F	Okigwe	U	H	Nil	32
252.	Ok-42	7	M	Okposi	R	L	Nil	64
253.	Ok-43	13	F	Okposi	R	H	Nil	64
254.	Ok-44	11	F	Okposi	R	H	Nil	32
255.	Ok-45	7	M	Isuochi	U	H	Nil	<8
256.	Ok-46	7	M	Isuochi	U	H	Nil	32

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
257.	Ok-47	7	F	Okigwe	U	L	Nil	32
258.	Ok-48	6	F	Okigwe	U	H	Nil	64
259.	Ok-49	7	M	Okigwe	U	H	Nil	<8
260.	Ok-50	15	F	Isuochi	U	H	Nil	64
261.	Ok-51	6	F	Okigwe	U	H	Nil	<8
262.	Ok-52	5	F	Isuochi	U	H	Nil	64
263.	Ok-53	8	M	Onuimo	R	L	Nil	16
264.	Ok-54	15	F	Okposi	R	L	Nil	64
265.	Ok-55	16	F	Okposi	R	L	Nil	128
266.	Ok-56	13	M	Okposi	R	L	Nil	32
267.	Ok-57	7	M	Onuimo	R	L	Nil	64
268.	Ok-58	14	F	Okposi	R	L	Nil	32
269.	Ok-59	9	M	Okposi	R	H	Nil	<8
270.	Ok-60	10	M	Onuimo	R	L	Nil	32
271.	Ok-61	15	F	Isi-ukwuato	R	L	Nil	16
272.	Ok-62	17	F	Mbano	U	H	Nil	<8
273.	Ok-63	7	F	Mbano	U	L	Nil	32
274.	Ok-64	9	M	Onuimo	R	H	Nil	<8
275.	Ok-65	6	F	Mbano	U	H	Nil	<8
276.	Ok-66	10	F	Onuimo	R	H	Nil	32
277.	Ok-67	12	F	Onuimo	R	H	Nil	64
278.	Ok-68	14	F	Onuimo	R	L	Nil	16
279.	Ok-69	14	F	Onuimo	R	L	Nil	8

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
280.	Ok-70	15	F	Onuimo	R	L	Nil	64
281	Ok-71	15	F	Onuimo	R	L	Nil	128
282	Ok-72	14	F	Onuimo	R	L	Nil	32
283	Ok-73	15	F	Onuimo	R	L	Nil	16
284.	Ok-74	23	F	Mbano	U	L	Nil	32
285.	Ok-75	16	F	Mbano	U	H	Nil	32
286.	Ok-76	7	M	Isiukwuato	R	L	Nil	64
287.	Ok-77	12	F	Isiukwuato	R	H	Nil	32
288.	Ok-78	14	F	Isiukwuato	R	H	Nil	64
289.	Ok-79	19	F	Mbano	U	H	Nil	<8
290.	Ok-80	17	F	Mbano	U	H	Nil	8
291.	Ok-81	8	M	Mbano	U	H	Nil	<8
292.	Ok-82	9	M	Mbano	U	H	Nil	16
293.	Ok-83	15	F	Mbano	U	H	Nil	64
294.	Ok-84	16	F	Mbano	U	L	Nil	<8
295.	Ok-85	6	F	Mbano	U	H	Nil	64
296	Ok-86	17	F	Mnano	U	H	Nil	32
297.	Ok-87	6	M	Mbano	U	H	Nil	32
298.	Ok-88	8	F	Mbano	U	H	Nil	32
299.	Ok-89	7	F	Mbano	U	H	Nil	<8
300.	Ok-90	15	F	Isiukwuato	R	L	Nil	64
301.	Ok-91	16	F	Isiukwuato	R	L	Nil	128
302.	Ok-92	16	F	Mbano	U	H	Nil	64

RESULTS OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-ter
303.	Ok-93	19	F	Mbano	U	L	Nil	16
304.	Ok-94	13	F	Isi-ukwuato	R	L	Nil	32
305.	Ok-95	10	M	Isi-ukwuato	R	L	Nil	32
306.	Ok-96	16	F	Mbano	U	H	Nil	64
307.	Ok-97	18	F	Isuochi	U	L	Nil	16
308.	Ok-98	9	M	Okposi	R	H	Nil	<8
309.	Ok-99	8	F	Okposi	R	H	Nil	16
310.	Ok-100	6	M	Okposi	R	L	Nil	16
311.	Ok-101	8	F	Ohazara	U	H	Nil	<8
312.	Ok-102	17	F	Ohazara	U	H	Nil	32
313.	Ok-103	16	F	Ohazara	U	H	Nil	<8
314.	Ok-104	14	F	Ohazara	R	L	Nil	16
315.	Ok-105	8	F	Ohazara	U	L	Nil	32
316.	Ok-106	7	F	Ohazara	U	H	Nil	<8
317.	Ok-107	15	F	Ohazara	R	L	Nil	64
318.	Ok-108	14	F	Ohazara	R	L	Nil	128
319.	Ok-109	15	F	Ohazara	R	L	Nil	32
320.	Ok-110	11	M	Ohazara	R	L	Nil	32
321.	Ok-111	9	M	Ohazara	R	H	Nil	<8
322.	Ok-112	17	F	Ohazara	U	H	Nil	8
323.	Ok-113	8	M	Ohazara	U	H	Nil	16

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
324.	Ok-114	16	F	Ohazara	U	L	Nil	<8
325.	Ok-115	16	F	Ohagara	U	H	Nil	32
326.	Ok-116	9	F	Ohazara	U	H	Nil	32
327.	Ok-117	14	F	Uturu	R	L	Nil	16
328.	Ok-118	13	F	Uturu	R	L	Nil	8
329.	Ok-119	6	F	Uturu	R	H	Nil	32
330.	Ok-120	9	M	Uturu	R	L	Nil	16
331.	Ok-121	8	F	Uturu	R	H	Nil	16
332.	Ok-122	23	F	Afikpo	U	L	Nil	32
333.	Ok-123	8	M	Uturu	R	L	Nil	64
334.	Ok-124	10	F	Uturu	R	H	Nil	32
335.	Ok-125	13	F	Uturu	R	H	Nil	64
336.	Ok-126	18	F	Uturu	U	H	Nil	8
337.	Ok-127	8	M	Uturu	U	H	Nil	<8
338.	Ok-128	15	F	Uturu	U	H	Nil	64
339.	Ok-129	7	F	Uturu	U	H	Nil	64
340.	Ok-130	7	M	Afikpo	U	H	Nil	32
341.	Ok-131	19	F	Uturu	U	L	Nil	16
342.	Ok-132	17	F	Uturu	U	H	Nil	64
343.	Ok-133	18	F	Uturu	U	L	Nil	16
344.	Ok-134	8	F	Okposi	R	H	Nil	32
345.	Ok-135	14	F	Okposi	R	L	Nil	8
346.	Ok-136	12	F	Uturu	R	H	Nil	64

RESULT3 (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
347.	Ok-137	11	F	Ihube	R	H	Nil	32
348.	Ok-138	8	M	Ihube	R	L	Nil	64
349.	Ok-139	21	F	Afikpo	U	L	Nil	32
350.	Ok-140	9	F	Ihube	R	H	Nil	16
351.	Ok-141	8	M	Ihube	R	L	Nil	16
352.	Ok-142	6	F	Ihube	R	H	Nil	32
353.	Ok-143	14	F	Ihube	R	L	Nil	8
354.	Ok-144	13	F	Ihube	R	L	Nil	16
355.	Ok-145	9	F	Afikpo	U	H	Nil	32
356.	Ok-146	17	F	Afikpo	U	H	Nil	32
357.	Ok-147	16	F	Afikpo	U	L	Nil	<8
358.	Ok-148	9	M	Afikpo	U	H	Nil	16
359.	Ok-149	19	F	Afikpo	U	H	Nil	8
360.	Ok-150	16	F	Afikpo	U	H	Nil	64
361.	Ok-151	19	F	Afikpo	U	L	Nil	16
362.	Ok-152	17	F	Afikpo	U	H	Nil	64
363.	Ok-153	18	F	Afikpo	U	L	Nil	16
364.	Ok-154	17	F	Afikpo	U	H	Nil	8
365.	Ok-155	8	F	Afikpo	U	H	Nil	<8
366.	Ok-156	7	M	Afikpo	U	H	Nil	32
367.	Ok-157	6	F	Afikpo	U	H	Nil	64
368.	Ok-158	15	F	Afikpo	U	H	Nil	64
369.	Ok-159	8	M	Afikpo	U	H	Nil	<8

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
370.	Ok-160	9	M	Ihube	R	H	Nil	<8
371.	Ok-161	11	M	Ihube	R	L	Nil	32
372.	Ok-162	13	F	Ihube	R	L	Nil	32
373.	Ok-163	15	F	Ihube	R	L	Nil	128
374.	Ok-164	15	F	Ihube	R	L	Nil	64
375.	Ok-165	16	F	Afikpo Road	U	H	Nil	32
376.	Ok-166	17	F	Afikpo Road	U	H	Nil	<8
377.	Ok-167	15	F	Ihube	R	L	Nil	16
378.	Ok-168	8	F	Afikpo	U	L	Nil	32
379.	Ok-169	8	F	Afikpo Road	U	H	Nil	64
380.	Ok-170	17	F	Afikpo Road	U	H	Nil	32
381.	Ok-171	17	F	Afikpo Road	U	H	Nil	<8
382.	Ok-172	15	F	Ihube	R	L	Nil	16
383.	Ok-173	7	F	Afikpo Road	U	L	Nil	32
384.	Ok-174	6	F	Afikpo Road	U	H	Nil	<8
385.	Ok-175	10	M	Ihube	R	L	Nil	32
386.	Ok-176	13	F	Ihube	R	L	Nil	32
387.	Ok-177	15	F	Ihube	R	L	Nil	64
388.	Ok-178	16	F	Ihube	R	L	Nil	128
389.	Ok-179	19	F	Afikpo Road	U	H	Nil	8
390	Ok-180	18	F	Afikpo Road	U	L	Nil	16
391.	Ok-181	18	F	Afikpo Road	U	H	Nil	64
392.	Ok-182	17	F	Afikpo Road	U	L	Nil	16

RESULTS (OKIGWE HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
393.	Ok-183	15	F	Afikpo Road	U	H	Nil	64
394.	Ok-184	7	M	Afikpo Road	U	H	Nil	32
395.	Ok-185	6	F	Afikpo Road	U	H	Nil	64
396.	Ok-186	19	F	Afikpo Road	U	H	Nil	64
397.	Ok-187	8	M	Afikpo Road	U	H	Nil	<8
398.	Ok-188	13	F	Ihube	R	H	Nil	64
399.	Ok-189	11	F	Ihube	R	H	Nil	32
400.	Ok-190	8	M	Isiukwuato	R	L	Nil	64
401.	Ok-191	24	F	Afikpo Road	U	L	Nil	32
402.	Ok-192	8	F	Isiukwuato	R	H	Nil	16
403.	Ok-193	19	F	Afikpo Road	U	H	Nil	8
404.	Ok-194	8	M	Mbano	U	H	Nil	16
405.	Ok-195	16	F	Mbano	U	L	Nil	<8
406.	Ok-196	17	F	Mbano	U	H	Nil	32
407.	Ok-197	8	F	Mbano	U	H	Nil	<8
408.	Ok-198	9	M	Isiukwuato	R	H	Nil	<8
409.	Ok-199	13	F	Isiukwuato	R	L	Nil	16
410.	Ok-200	14	F	Isiukwuato	R	L	Nil	8
411.	Ok-201	6	F	Isiukwuato	R	H	Nil	32
412.	Ok-202	9	M	Isiukwuato	R	L	Nil	16

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
413.	A-1	26	F	Aba	U	L	Nil	16
414.	A-2	26	F	Aba	U	L	Nil	32
415.	A-3	27	M	Aba	U	H	Nil	16
416.	A-4	8	M	Aba	U	H	Nil	<8
417.	A-5	6	F	Aba	U	H	Nil	32
418.	A-6	7	F	Aba	U	H	Nil	<8
419.	A-7	7	F	Aba	U	H	Nil	256
420.	A-8	7	F	Aba	U	H	Nil	<8
421.	A-9	7	F	Aba	U	H	Nil	<8
422.	A-10	8	F	Aba	U	H	Nil	64
423.	A-11	6	F	Aba	U	H	Nil	<8
424.	A-12	7	F	Aba	U	H	Nil	<8
425.	A-13	9	F	Obioma-Ngwa	U	H	Nil	<8
426.	A-14	7	F	Aba	U	H	Nil	16
427.	A-15	7	M	Obioma-Ngwa	U	H	Nil	<8
428.	A-16	7	M	Aba	U	L	Nil	<8
429.	A-17	7	M	Aba	U	L	Nil	<8
430.	A-18	8	M	Obioma-Ngwa	U	H	Nil	32
431.	A-19	6	F	Aba	U	H	Nil	32
432.	A-20	6	F	Aba	U	L	Nil	128
433.	A-21	8	F	Aba	U	H	Nil	<8
434.	A-22	8	F	Aba	U	H	Nil	64
435.	A-23	7	F	Ukwa	U	H	Nil	64*

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
436.	A-24	7	M	Aba	U	H	Nil	<8
437.	A-25	8	F	Aba	U	H	Nil	<8
438.	A-26	5	M	Ukwa	U	H	Nil	<8
439.	A-27	3	M	Aba	U	H	Nil	128
440.	A-28	8	F	Ukwa	U	H	Nil	32
441.	A-29	8	M	Aba	U	H	Nil	32
442.	A-30	7	M	Aba	U	H	Nil	<8
443.	A-31	7	F	Etiti-Ngwa	U	L	Nil	<8
444.	A-32	6	M	Aba	U	L	Nil	128
445.	A-33	7	M	Aba	U	L	Nil	<8
446.	A-34	9	M	Etiti-Ngwa	U	H	Nil	<8
447.	A-35	6	M	Aba	U	H	Nil	<8
448.	A-36	8	F	Etiti-Ngwa	U	L	Nil	128
449.	A-37	7	F	Obi-Ngwa	U	H	Nil	16
450.	A-38	7	M	Aba	U	H	Nkl	8
451.	A-39	6	F	Obi-Ngwa	U	H	Nil	64
452.	A-40	7	F	Aba	U	H	Nil	32
453.	A-41	8	F	Aba	U	H	Nil	32
454.	A-42	9	F	Aba	U	H	Nil	128
455.	A-43	7	F	Aba	U	H	Nil	64
456.	A-44	6	F	Aba	U	H	Nil	<8
457.	A-45	6	F	Aba	U	H	Nil	8
458.	A-46	12	M	Aba	U	H	Nil	8

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
459.	A-47	7	F	Aba	U	L	Nil	<8
460.	A-48	9	F	Obi-Ngwa	U	H	Nil	16
461.	A-49	6	M	Aba	U	H	Nil	<8
462.	A-50	9	F	Asa	U	H	Nil	64
463.	A-51	6	M	Aba	U	H	Nil	<8
464.	A-52	8	F	Aba	U	H	Nil	64
465.	A-53	7	F	Asa	U	H	Nil	32
466.	A-54	6	M	Aba	U	H	Nil	<8
467.	A-55	8	F	Asa	U	H	Nil	32
468.	A-56	7	F	Asa	U	H	Nil	<8
469.	A-57	9	F	Asa	U	H	Nil	64
470.	A-58	6	M	Asa	U	L	Nil	<8
471.	A-59	24	F	Asa	U	L	Nil	16
472.	A-60	8	M	Asa	U	H	Nil	<8
473.	A-61	7	F	Asa	U	H	Nil	128
474.	A-62	6	M	Asa	U	H	Nil	<8
475.	A-63	12	M	Asa	U	H	Nil	32
476.	A-64	9	M	Asa	U	H	Nil	<8
477.	A-65	5	F	Asa	U	H	Nil	64
478.	A-66	6	F	Asa	U	H	Nil	<8
479.	A-67	5	F	Isiala-Ngwa	U	H	Nil	32
480.	A-68	8	M	Isiala-Ngwa	U	H	Nil	<8
481.	A-69	9	M	Ike-ala-Ngwa	U	H	Nil	8

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
482.	A-70	6	M	Isiala-Ngwa	U	H	Nil	<8
483.	A-71	7	F	Isiala-Ngwa	U	H	Nil	<8
484.	A-72	8	F	Asa	U	H	Nil	16
485.	A-73	7	M	Ukwa	U	H	Nil	<8
486.	A-74	8	F	Asa	U	H	Nil	64
487.	A-75	9	F	Asa	U	H	Nil	32
488.	A-76	5	F	Ikeala-Ngwa	U	H	Nil	64
489.	A-77	10	F	Ikeala-Ngwa	U	H	Nil	128
490.	A-78	6	F	Ikeala-Ngwa	U	H	Nil	<8
491.	A-79	9	F	Ikeala-Ngwa	U	L	Nil	128
492.	A-80	28	F	Asa	U	L	Nil	32
493.	A-81	6	M	Isiala-Ngwa	U	L	Nil	<8
494.	A-82	8	M	Ukwa	U	L	Nil	<8
495.	A-83	8	F	Ukwa	U	H	Nil	256
496.	A-84	7	M	Ukwa	U	L	Nil	128
497.	A-85	7	M	Ukwa	U	L	Nil	<8
498.	A-86	8	F	Ukwa	U	H	Nil	64
499.	A-87	6	M	Ukwa	U	H	Nil	<8
500.	A-88	29	F	Ukwa	U	H	Nil	32
501.	A-89	8	F	Asa	U	L	Nil	<8
502.	A-90	7	F	Asa	U	L	Nil	32
503.	A-91	6	F	Isiala-Ngwa	U	H	Nil	<8
504.	A-92	6	F	Isiala-Ngwa	U	H	Nil	<8

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
505.	A-93	25	M	Isiala-Ngwa	U	H	Nil	32
506.	A-94	7	F	Isiala-Ngwa	U	H	Nil	<8
507.	A-95	5	F	Isiala-Ngwa	U	H	Nil	<8
508.	A-96	6	M	Isiala-Ngwa	U	H	Nil	128
509.	A-97	7	F	Isiala-Ngwa	U	H	Nil	<8
510.	A-98	6	F	Isiala-Ngwa	U	H	Nil	8
511.	A-99	15	M	Isiala-Ngwa	U	H	Nil	8
512.	A-100	10	M	Etiti-Ngwa	U	H	Nil	8
513.	A-101	8	F	Isiala-Ngwa	U	L	Nil	128
514.	A-102	[illegible]	F	Isiala-Ngwa	U	H	Nil	8
515.	A-103	6	F	Isiala-Ngwa	U	H	Nil	<8
516.	A-104	30	M	Isiala-Ngwa	U	L	Nil	16
517.	A-105	8	M	Etiti-Ngwa	U	H	Nil	32
518.	A-106	6	F	Etiti-Ngwa	U	H	Nil	<8
519.	A-107	9	M	Ukwa	U	H	Nil	<8
520.	A-108	7	F	Ukwa	U	H	Nil	<8
521.	A-109	8	F	Ukwa	U	H	Nil	32
522.	A-110	8	F	Ukwa	U	H	Nil	<8
523.	A-111	7	F	Ukwa	U	H	Nil	<8
524.	A-112	8	M	Obi-Ngwa	U	L	Nil	<8
525.	A-113	8	F	Obi-Ngwa	U	H	Nil	32
526.	A-114	5	M	Obi-Ngwa.	U	H	Nil	<8
527.	A-115	7	F	Obi-Ngwa	U	H	Nil	<8

RESULTS (ABA HEALTH ZONE)

Serial	Sample	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
528.	A-116	27	F	Obi-Ngwa	U	L	Nil	16
529.	A-117	8	F	Obi-Ngwa	U	L	Nil	<8
530.	A-118	7	F	Obi-Ngwa	U	H	Nil	<8
531	A-119	8	F	Obi-Ngwa	U	H	Nil	64
532.	A-120	9	F	Etiti-Ngwa	U	H	Nil	<8
533.	A-121	28	F	Etiti-Ngwa	U	L	Nil	64
534.	A-122	6	M	Obi-Ngwa	U	H	Nil	128
535.	A-123	7	F	Obi-Ngwa	U	H	Nil	<8
536.	A-124	8	M	Obi-Ngwa	U	H	Nil	<8
537.	A-125	9	F	Etiti-Ngwa	U	H	Nil	256
538.	A-126	9	F	Ukwa	U	H	Nil	128
539.	A-127	8	M	Ukwa	U	L	Nil	<8
540.	A-128	7	M	Ikeala-Ngwa	U	L	Nil	128
541.	A-129	8	F	Ikeala-Ngwa	U	H	Nil	64
542.	A-130	6	M	Ikeala-Ngwa	U	H	Nil	8
543.	A-131	7	F	Ikeala-Ngwa	U	H	Nil	64
544.	A-132	7	F	Ikeala-Ngwa	U	H	Nil	16
545.	A-133	9	M	Etiti-Ngwa	U	H	Nil	<8
546.	A-134	7	F	Etiti-Ngwa	U	H	Nil	<8
547.	A-135	9	M	Etiti-Ngwa	U	H	Nil	<8
548.	A-136	8	F	Etiti-Ngwa	U	H	Nil	32
549.	A-137	12	M	Etiti-Ngwa	U	H	Nil	32
550.	A-138	12	M	Etiti-Ngwa	U	H	Nil	8

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
551.	A-139	7	F	Etiti-Ngwa	U	H	Nil	<8
552.	A-140	6	F	Etiti-Ngwa	U	H	Nil	8
553.	A-141	12	F	Obi-Ngwa	U	L	Nil	128
554.	A-142	21	M	Ikeala-Ngwa	U	H	Nil	16
555.	A-143	5	M	Ikeala-Ngwa	U	L	Nil	<8
556.	A-144	7	F	Obi-Ngwa	U	H	Nil	<8
557.	A-145	6	M	Obi-Ngwa	U	L	Nil	<8
558.	A-146	9	F	Etiti-Ngwa	U	H	Nil	<8
559.	A-147	12	M	Etiti-Ngwa	U	L	Nil	128
560.	A-148	7	F	Osisi-Oma Ngwa	U	H	Nil	<8
561.	A-149	6	M	Osisi-Oma Ngwa	U	L	Nil	<8
562.	A-150	29	F	Ukwa	U	L	Nil	32
563.	A-151	5	F	Ukwa	U	L	Nil	<8
564.	A-152	5	F	Ukwa	U	H	Nil	<8
565.	A-153	9	F	Ukwa	U	H	Nil	64
566.	A-154	8	F	Ukwa	U	H	Nil	<8
567.	A-155	27	F	Osisi-Oma Ngwa	U	L	Nil	16
568.	A-156	12	M	Osisi-Oma Ngwa	U	H	Nil	128
569.	A-157	6	F	Ikeala-Ngwa	U	H	Nil	256
570.	A-158	7	M	Ikeala-Ngwa	U	H	Nil	<8
571.	A-159	9	M	Ikeala-Ngwa	U	H	Nil	<8
572.	A-160	8	F	Ikeala-Ngwa	U	H	Nil	128
573.	A-161	7	F	Osisi-Oma Ngwa	U	H	Nil	<8

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
574.	A-162	10	M	Osisi-Oma Ngwa	U	H	Nil	8
575.	A-163	8	F	Osisioma Ngwa	U	H	Nil	64
576.	A-164	6	F	Osisioma Ngwa	U	H	Nil	<8
577.	A-165	9	F	Osisioma Ngwa	U	H	Nil	32
578.	A-166	7	F	Osisioma Ngwa	U	H	Nil	64
579.	A-167	5	M	Isiala-Ngwa	U	H	Nil	8
580.	A-168	7	M	Isiala-Ngwa	U	H	Nil	128
581.	A-169	6	M	Osisioma Ngwa	U	L	Nil	128
582.	A-170	9	F	Osisioma Ngwa	U	H	Nil	128
583.	A-171	8	F	Osisioma Ngwa	U	H	Nil	64
584.	A-172	7	F	Isiala-Ngwa	U	H	Nil	16
585.	A-173	6	F	Osisioma Ngwa	U	H	Nil	32
586.	A-174	8	F	Etiti-Ngwa	U	H	Nil	<8
587.	A-175	9	F	Etiti-Ngwa	U	L	Nil	256
588.	A-176	9	F	Etiti-Ngwa	U	H	Nil	<8
589.	A-177	28	F	Etiti-Ngwa	U	L	Nil	32
590.	A-178	8	M	Obi-Ngwa	U	H	Nil	<8
591.	A-179	6	F	Obi-Ngwa	U	H	Nil	<8
592.	A-180	25	F	Obi-Ngwa	U	L	Nil	64
593.	A-181	5	F	Obi-Ngwa	U	L	Nil	<8
594.	A-182	7	F	Isiala-Ngwa	U	H	Nil	64
595.	A-183	8	M	Obi-Ngwa	U	H	Nil	<8
596.	A-184	5	F	Osisioma Ngwa	U	H	Nil	32

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
597.	A-185	7	F	Osisioma Ngwa	U	H	Nil	<8
598.	A-186	9	M	Osisioma Ngwa	U	H	Nil	8
599.	A-187	5	F	Ikeala-Ngwa	U	H	Nil	<8
600.	A-188	9	M	Ikeala-Ngwa	U	H	Nil	<8
601.	A-189	10	M	Ikeala-Ngwa	U	H	Nil	8
602.	A-190	9	F	Ukwa	U	H	Nil	32
603.	A-191	9	F	Etiti-Ngwa	U	H	Nil	8
604.	A-192	7	M	Osisioma Ngwa	U	L	Nil	<8
605.	A-193	30	M	Osisioma Ngwa	U	H	Nil	16
606.	A-194	6	M	Obi-Ngwa	U	L	Nil	<8
607.	A-195	8	F	Obi-Ngwa	U	H	Nil	<8
608.	A-196	9	M	Obi-Ngwa	U	H	Nil	32
609.	A-197	7	F	Obi-Ngwa	U	H	Nil	<8
610.	A-198	7	M	Obi-Ngwa	U	H	Nil	<8
611.	A-199	8	F	Ikeala-Ngwa	U	H	Nil	16
612.	A-200	7	F	Ikeala-Ngwa	U	H	Nil	<8*
613.	A-201	9	M	Etiti-Ngwa	U	H	Nil	<8
614.	A-202	9	F	Osisioma Ngwa	U	H	Nil	512
615.	A-203	8	F	Osisioma Ngwa	U	H	Nil	8
616.	A-204	28	M	Osisioma Ngwa	U	H	Nil	16
617.	A-205	8	M	Osisioma Ngwa	U	L	Nil	<8
618.	A-206	9	M	Isiala-Ngwa	U	L	Nil	128
619.	A-207	25	F	Isiala-Ngwa	U	L	Nil	32

RESULTS (ABA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
620.	A-208.	7	F	Ikeala-Ngwa	U	H	Nil	<8
621.	A-209	8	F	IkealaNgwa	U	L	Nil	<8
622.	A-210	6	F	Obi-Ngwa	U	H	Nil	<8
623.	A-211	7	F	Etiti-Ngwa	U	L	Nil	128
624.	A-212	9	F	Ukwa	U	H	Nil	<8
625.	A-213	13	M	Ukwa	U	H	Nil	8
626.	A-214.	9	M	Ukwa	U	H	Nil	128
627.	A-215.	7	M	Asa	U	H	Nil	<8
628.	A-216	8	M	Asa	U	H	Nil	8
629.	A-217	6	F	Ukwa	U	H	Nil	16
				RESULTS (UMUAHIA HEALTH ZONE)				
630.	U-1	18	F	Ikwuano/Umu.	R	L	Nil	<8
631.	U-2	15	F	Ihitte	R	L	Nil	<8
632.	U-3	19	F	Arochukwu/Oh.	R	L	Nil	64
633.	U-4	16	F	Ihitte	R	L	Nil	16
634.	U-5	13	M	Ikwuano/Umu.	U	L	Nil	16
635.	U-6	16	F	Agugiri	R	L	Nil	512
636.	U-7	9	F	Ikwuano/Umu.	U	H	Nil	32
637.	U-8	19	F	Etiti	R	L	Nil	256
638.	U-9	8	F	Afugiri	R	L	Nil	<8
639.	U-10	6	M	Umunna	R	H	Nil	16
640.	U-11	18	F	Bende	R	L	Nil	64
641.	U-12	15	F	Bende	R	H	Nil	64

RESULTS UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
642.	U-13	17	F	Umunna	R	L	Nil	32
643.	U-14	15	F	Etiti	R	L	Nil	<8
644.	U-15	18	F	Arochukwa/Oh.	R	L	Nil	32
645.	U-16	6	F	Bende	R	H	Nil	<8
646.	U-17	19	F	Obowo	R	L	Nil	32
647.	U-18	7	F	Arochukwu/Oh.	R	H	Nil	<8
648.	U-19	6	M	Obowo	R	H	Nil	<8
649.	U-20	18	F	Ikwuano/Umu.	U	H	Nil	32
650.	U-21	5	M	Arochuckwu/Oh.	R	H	Nil	16
651.	U-22	16	F	Ihitte	R	L	Nil	128
652.	U-23.	9	M	Obowo	R	H	Nil	<8
653.	U-24	18	F	Obowo	R	L	Nil	32
654.	U-25.	5	F	Ihitte	R	H	Nil	<8
655.	U-26	17	F	Umuneato	R	L	Nil	16
656.	U-27	8	M	Ihitte	R	H	Nil	<8
657.	U-28	8	M	Obowo	R	L	Nil	16
658.	U-29	9	M	Odidanyanwu	R	L	Nil	64
659.	U-30	15	F	Ikwuano/Umu.	U	L	Nil	256
660.	U-31	18	F	Bendo	R	L	Nil	64
661.	U-32	6	F	Umuneato	R	L	Nil	<8
662.	U-33	17	F	Odidanyawu	R	L	Nil	16
663.	U-34	9	M	Bendo	R	H	Nil	16
664.	U-35	17	F	Odidanyawu	R	L	Nil	256

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
665.	U-36	16	F	Umuneato	R	L	Nil	64
666.	U-37	16	F	Odidayanwu	R	L	Nil	<8
667.	U-38	18	F	Umuneato	R	L	Nil	32
668.	U-39	18	F	Bende	R	H	Nil	64
669.	U-40	18	F	Ikwuano/Umu.	R	L	Nil	<8
670.	U-41	15	F	Umuneato	R	H	Nil	64
671.	U-42	7	M	Ikwuano/Umu.	R	L	Nil	64
672.	U-43	18	F	Odidanyanwu	R	L	Nil	<8
673.	U-44	16	F	Bende	R	H	Nil	32
674.	U-45	17	F	Odidanyanwu	R	L	Nil	32
675.	U-46	18	F	Bende	R	H	Nil	32
676.	U-47	8	F	Ikwuano/Umu.	U	H	Nil	8
677.	U-48	8	M	Ikwuano/Umu.	R	H	Nil	<8
678.	U-49	18	F	Etiti	R	L	Nil	128
679.	U-50	17	F	Ikwuano/Umu.	R	L	Nil	128
680.	U-51	6	M	Odidanyanwu	R	H	Nil	<8
681.	U-52	17	F	Etiti	R	L	Nil	64
682.	U-53	17	F	Ihitte	R	L	Nil	32
683.	U-54	17	F	Ihitte	R	L	Nil	8
684.	U-55.	17	F	Bende	R	L	Nil	16
685.	U-56.	16	F	Ihitte	R	L	Nil	<8
686.	U-57.	15	F	Ihitte	R	L	Nil	256
687.	U-58	17	F	Ititte	R	L	Nil	16

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
688.	U-59	15	F	Ihitte	R	L	Nil	32
689.	U-60	17	F	Obowo	R	L	Nil	128
690.	U-61	18	F	Etiti	R	L	Nil	64
691.	U-62	16	F	Obowo	R	L	Nil	<8
692.	U-63	18	F	Etiti	R	L	Nil	32
693.	U-64	8	M	Afugiri	R	H	Nil	<8
694.	U-65.	18	F	Ihitte	R	L	Nil	<8
695.	U-66.	6	F	Obowo	R	H	Nil	32
696.	U-67.	6	F	Obowo	R	L	Nil	64
697.	U-68	7	M	Obowo	R	H	Nil	8
698.	U-69	7	M	Obowo	R	H	Nil	<8
699.	U-70	19	F	Odidanyanwu	R	L	Nil	32
700.	U-71	7	F	Obowo	R	H	Nil	32
701.	U-72	6	M	Obowo	R	L	Nil	64*
702.	U-73	15	F	Umuneato	R	L	Nil	16
703.	U-74	6	F	Obowo	R	H	Nil	<8
704.	U-75	7	F	Obowo	R	L	Nil	32
705.	U-76	9	F	Afugiri	R	H	Nil	<8
706.	U-77	6	M	Obowo	R	L	Nil	64
707.	U-78	5	M	Agugiri	R	L	Nil	16
708.	U-79	6	M	Obowo	R	H	Nil	<8
709.	U-80	6	M	Obowo	R	H	Nil	<8
710.	U-81	8	F	Obowo	R	L	Nil	64

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commu-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
711.	U-82	8	M	Obowo	R	L	Nil	16
712.	U-83	16	F	Bende	R	L	Nil	<8
713.	U-84	16	F	Bende	R	L	Nil	<8
714.	U-85	16	F	Bende	R	L	Nil	64
715.	U-86	18	F	Afugiri	R	L	Nil	512
716.	U-87	18	F	Umuneato	R	L	Nil	32
717.	U-88	17	F	Bende	R	L	Nil	8
718.	U-89	18	F	Bende	R	L	Nil	32
719.	U-90	17	F	Bende	R	L	Nil	16
720.	U-91	18	F	Bende	R	L	Nil	256
721.	U-92	6	M	Bende	R	H	Nil	16
722.	U-93	16	F	Bende	R	L	Nil	<8
723.	U-94	7	F	Bende	R	L	Nil	<8
724.	U-95	7	M	Bende	R	H	Nil	<8
725.	U-96	7	M	Bende	R	H	Nil	8
726.	U-97	7	F	Bende	R	L	Nil	512
727.	U-98	7	F	Bende	R	L	Nil	<8
728.	U-99	7	M	Bende	R	H	Nil	256
729.	U-100	7	M	Umuneato	R	H	Nil	<8
730.	U-101	6	M	Umuneato	R	H	Nil	8
731.	U-102	18	F	Umunna	R	H	Nil	16
732.	U-103	16	F	Umunna	R	H	Nil	16
733.	U-104	18	F	Umunna	R	H	Nil	128

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
734.	U-105	18	F	Umunna	R	H	Nil	<8
735.	U-106	18	F	Umunna	R	L	Nil	64
736.	U-107	13	F	Umunna	R R	L	Nil	32
737.	U-108	6	F	Umuneato	R	H	Nil	<8
738.	U-109	18	F	Umunna	R	H	Nil	64
739.	U-110	17	F	Umunna	R	L	Nil	64
740.	U-111	18	F	Umunna	R	L	Nil	512
741.	U-112	16	F	Etiti	R	L	Nil	<8
742.	U-113	19	F	Ikwuano/Umu.	R	L	Nil	32
743.	U-114	5	M	Ihitte	R	H	Nil	<8
744.	U-115	5	F	Obowo	R	H	Nil	<8
745.	U-116	6	M	Obowo	R	H	Nil	<8
746.	U-117	15	F	Umuneato	R	L	Nil	512
747.	U-118	8	M	Ikwuano/Umu.	R	H	Nil	8
748.	U-119	7	M	Ihitte	R	H	Nil	<8
749.	U-120	16	F	Umuneato	R	L	Nil	16
750.	U-121	5	M	Etiti	R	L	Nil	64
751.	U-122	19	F	Obowo	R	L	Nil	<8
752.	U-123	18	F	Ikwuano/Umu.	R	L	Nil	256
753.	U-124	17	F	Umuneato	R	L	Nil	64
754.	U-125	6	M	Umuneato	R	H	Nil	16
755.	U-126	5	F	Umuneato	R	L	Nil	<8
756.	U-127	19	F	Ikwuano/Umu.	R	L	Nil	16

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
757.	U-128	19	F	Obowo	R	L	Nil	<8
758.	U-129	17	F	Umuneato	R	L	Nil	64
759.	U-130	18	F	Ikwuano/Umu.	R	L	Nil	32
760.	U-131	16	F	Ikwuano/Umu.	R	H	Nil	64
761.	U-132	19	F	Etiti	R	L	Nil	128
762.	U-133	18	F	Etiti	R	H	Nil	32
763.	U-134	8	F	Etiti	R	L	Nil	64
764.	U-135	9	F	Bende	R	H	Nil	32
765.	U-136	6	F	Bende	RR	L	Nil	64
766.	U-137	8	F	Umunna	R	H	Nil	32
767.	U-138	18	F	Arochukwu/Oh.	U	H	Nil	32
768.	U-139	17	F	Bende	U	H	Nil	16
769.	U-140	7	F	Etiti	R	L	Nil	<8
770.	U-141	18	F	Bende	R	L	Nil	64
771.	U-142	16	F	Bende	R	H	Nil	128
772.	U-143	18	F	Etiti	R	L	Nil	8
773.	U-144	17	F	Umunna	R	H	Nil	16
774.	U-145	19	F	Obowo	R	H	Nil	<8
775.	U-146	8	M	Umunna	R	H	Nil	256
776.	U-147	8	F	Umunna	R	L	Nil	512
777.	U-148	6	F	Bende	R	L	Nil	32
778.	U-149	9	F	Umunna	U	H	Nil	32
779.	U-150	18	F	Bende	R	L	Nil	8

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Ti-
780.	U-151	16	F	Umunna	R	L	Nil	64
781.	U-152	16	F	Umunna	U	L	Nil	256
782.	U-153	19	F	Bende	R	L	Nil	64
783.	U-154	17	F	Bende	R	L	Nil	<8
784.	U-155	16	F	Obowo	R	L	Nil	<8
785.	U-156	17	F	Umunna	R	L	Nil	256
785	U-157	7	M	Etiti	R	H	Nil	<8
787.	U-158	15	F	Etiti	R	L	Nil	32
788.	U-159	8	M	Bende	R	L	Nil	64
789.	U-160	7	F	Obowo	R	H	Nil	32
790.	U-161	8	M	Umunna	R	H	Nil	8
791.	U-162	16	F	Obowo	R	H	Nil	32
792.	U-163	19	F	Obowo	R	H	Nil	64
793.	U-164	12	M	Arochukwu/Oh.	U	L	Nil	16
794.	U-165	18	F	Umunna	R	L	Nil	128
795.	U-166	5	F	Afugiri	R	L	Nil	512
796.	U-167	17	F	Agugiri	R	L	Nil	8
797.	U-168	18	F	Ikwuano/Umu.	R	L	Nil	256
798.	U-169	18	F	Arochukwu/Oh.	R	L	Nil	64
799.	U-170.	16	F	Arochukwu/Oh.	R	H	Nil	128
800.	U-171	6	F	Afugiri	R	L	Nil	<8
801.	U-172	17	F	Arochukwu/Oh.	R	H	Nil	16
802.	U-173	18	F	Afugiri	U	H	Nil	32

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Commn-nity	Socio-Econo-mic Status	Rubella Immuni-zation Status	HAI Ti-tre
803.	U-174	16	F	Obowo	R	L	Nil	32
804.	U-175	7	M	Ihitte	R	H	Nil	8
805.	U-176	16	F	Obowo	R	L	Nil	64
806.	U-177	6	M	Ihitte	R	L	Nil	64
807.	U-178	19	F	Obowo	R	L	Nil	64
808.	U-179	5	F	Etiti	R	H	Nil	32
809.	U-180	7	F	Afugiri	U	H	Nil	32
810.	U-181	18	F	Odidanyanwu	R	L	Nil	8
811.	U-182	6	F	Etiti	R	L	Nil	32
812.	U-183	18	F	Afugiri	R	H	Nil	32
813.	U-184	16	F	Arochukwu/Oh.	R	L	Nil	<8
814.	U-185	17	F	Afugiri	U	L	Nil	256
815.	U-186	6	F	Odidanyanwu	R	L	Nil	64
816.	U-187	8	F	Etiti	R	H	Nil	32
817.	U-188	5	F	Odidanyanwu	R	L	Nil	64
818.	U-189	7	M	Arochukwu/Oh.	R	H	Nil	256
819.	U-190	18	F	Arochukwu/Oh.	R	H	Nil	16
820.	U-191	16	F	Afugiri	R	H	Nil	<8
821.	U-192	15	F	Arochukwu/Oh.	R	L	Nil	<8
822.	U-193	8	F	Afugiri	U	H	Nil	8
823.	U-194	16	F	Odidanyanwu	R	L	Nil	128
824.	U-195	16	F	Arochukwu/Oh.	R	L	Nil	<8
825.	U-196	5	M	Arochukwu/Oh.	R	H	Nil	<8

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
826.	U-197	14	M	Odidanyanwu	U	L	Nil	16
827.	U-198	19	F	Arochukwu/Oh.	R	H	Nil	64
828.	U-199	15	F	Afugiri	R	H	Nil	32
829.	U-200	7	F	Odidanyanwu	R	L	Nil	64
830.	U-201	5	F	Etiti	R	H	Nil	32
831.	U-202	8	F	Etiti	R	L	Nil	32
832.	U-203	9	F	Afugiri	R	L	Nil	512
833.	U-204	8	F	Etiti	R	L	Nil	64
834.	U-205	15	F	Odidanyanwu	R	L	Nil	<8
835.	U-206	18	F	Afugiri	R	H	Nil	32
836.	U-207	17	F	Odidanyanwu	R	L	Nil	64
837.	U-208	15	F	Ikwano/Umu.	R	L	Nil	8
838.	U-209	18	F	Ikwuano/Umu.	R	H	Nil	32
839.	U-210	19	F	Afugiri	R	H	Nil	64
840.	U-211.	9	F	Afugiri	U	H	Nil	32
841.	U-212	9	M	Ihitte	R	H	Nil	8
842.	U-213	14	M	Afugiri	U	L	Nil	16
843.	U-214	15	F	Ihitte	R	L	Nil	128
844.	U-215	9	M	Odidanyanwu	R	L	Nil	64
845.	U-216	6	F	Ikwuano/Umu.	R	H	Nil	32
846.	U-217	6	F	Afugiri	U	H	Nil	8
847.	U-218	19	F	Odidanyanwu	U	L	Nil	256
848.	U-219	16	F	Afugiri	U	H	Nil	32

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
849.	U-220	19	F	Ikwuano/Umu.	R	L	Nil	32
850.	U-221	9	M	Afugiri	R	H	Nil	256
851.	U-222	17	F	Arochukwu/Oh.	R	H	Nil	16
852.	U-223	19	F	Afugiri	R	H	Nil	<8
853.	U-224	7	M	Ikwuano/Umu.	R	H	Nil	<8
854.	U-225.	19	F	Afugiri	R	L	Nil	256
855.	U-226	15	F	Ikwuano/Umu.	R	L	Nil	<8
856.	U-227	6	F	Ododanyanwu	R	L	Nil	32
857.	U-228	17	F	Ikwuano/Umu.	R	L	Nil	8
858.	U-229	17	F	Afugiri	R	L	Nil	<8
859.	U-230	19	F	Ikwuano/Umu.	R	H	Nil	16
860.	U-231	19	F	Ododanyanwu	R	L	Nil	64
861.	U-232.	7	F	Ikwuano/Umu.	R	L	Nil	<8
862.	U-233.	19	F	Afugiri	R	H	Nil	128
863.	U-234	18	F	Ododanyanwu	R	L	Nil	64
864.	U-235	18	F	Ihitte	R	L	Nil	256
865.	U-236	7	F	Ihitte	R	L	Nil	32
866.	U-237	17	F	Arochukwu/Oh.	R	L	Nil	<8
867.	U-238	18	F	Arochukwu/Oh.	R	L	Nil	64
868.	U-239	7	M	Umunna	R	H	Nil	<8*
869.	U-240	9	F	Umunna	U	H	Nil	32
870.	U-241	6	M	Ihitte	R	H	Nil	<8
871.	U-242	18	F	Bende	R	L	Nil	8

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
872.	U-243	16	F	Arochukwu/Oh.	R	L	Nil	<8
873.	U-244	17	F	Umunna	R	L	Nil	64
874.	U-245	8	F	Ihitte	R	L	Nil	64
875.	U-246	17	F	Ihitte	R	L	Nil	<8
876.	U-247	19	F	Bende	R	H	Nil	32
877.	U-248	17	F	Arochukwu/Oh.	U	L	Nil	256
878.	U-249	15	F	Ihitte	R	H	Nil	32
879.	U-250	18	F	Umunna	R	H	Nil	64
880.	U-251	11	M	Umunna	U	L	Nil	16
881.	U-252	19	F	Ihitte	R	L	Nil	128
882.	U-253	9	F	Arochukwu/Oh.	U	H	Nil	8
883.	U-254	5	M	Afugiri	R	H	Nil	256
884.	U-255	17	F	Afugiri	R	H	Nil	16
885.	U-256	7	F	Ihitte	R	H	Nil	32
886.	U-257	6	F	Ihitte	R	L	Nil	64
887.	U-258	19	F	Umunna	R	H	Nil	<8
888.	U-259	6	F	Arochukwu/Oh.	R	L	Nil	512
889.	U-260	18	F	Bende	R	L	Nil	64
890.	U-261	7	M	Etiti	R	H	Nil	8
891.	U-262	7	F	Obowo	R	H	Nil	32
892.	U-263	6	M	Ihitte	R	L	Nil	64
893.	U-264	16	F	Odidanyanwu	R	L	Nil	32
894.	U-265	15	F	Arochukwu/Oh.	U	H	Nil	32

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
895.	U-266	19	F	Umunna	R	H	Nil	128
896.	U-267	18	F	Arochukwu/Oh.	R	H	Nil	16
897.	U-268	17	F	Ihitte	R	L	Nil	8
898.	U-269	7	F	Obowo	R	L	Nil	<8
899.	U-270	17	F	Umunna	R	L	Nil	16
900.	U-271.	6	M	Ihitte	R	L	Nil	16
901.	U-272	16	F	Etiti	R	L	Nil	<8
902	U-273	9	M	Etiti	R	L	Nil	64
903.	U-274	18	F	Ikwuano/Umu.	R	L	Nil	256
904.	U-275	15	F	Obowo	R	L	Nil	<8
905.	U-276	17	F	Umunna	R	L	Nil	64
906.	U-277	18	F	Etiti	R	L	Nil	<8
907.	U-278	9	F	Umunna	R	L	Nil	<8
908.	U-279	18	F	Umuneato	R	L	Nil	64
909.	U-280	9	M	Arochukwu/Oh.	R	H	Nil	16
910.	U-281	18	F	Obowo	R	L	Nil	16
911.	U-282	18	F	Arochukwu/Oh.	R	L	Nil	32
912.	U-283	15	F	Etiti	R	L	Nil	<8
913.	U-284	17	F	Etiti	R	L	Nil	32
914.	U-285	17	F	Ikwuano/Umu.	R	L	Nil	128
915.	U-286	19	F	Arochukwu/Oh.	R	L	Nil	512
916.	U-287	6	M	Etiti	R	H	Nil	<8
917.	U-288	18	F	Arochukwu/Oh.	R	L	Nil	16

RESULTS (UMUAHIA HEALTH ZONE)

Serial No.	Sample No.	Age	Sex	L.G.A.	Nature of Community	Socio-Economic Status	Rubella Immunization Status	HAI Titre
918.	U-289	15	F	Ununeato	R	H	Nil	64
919.	U-290	9	M	Arochukwu/Oh.	R	H	Nil	8
920.	U-291	6	F	Ununeato	R	H	Nil	<8
921.	U-292	15	F	ArochukwuEOh.	R	L	Nil	32
922.	U-293	8	M	Ihitte	R	H	Nil	<8
923.	U-294	6	F	Etiti	R	H	Nil	<8
924.	U-295	17	F	Obowo	R	L	Nil	32
925.	U-296	19	F	Ihitte	R	L	Nil	<8
926.	U-297	5	M	Obowo	R	H	Nil	<8
927.	U-298	15	F	Ikwuano/Umu.	R	L	Nil	32
928.	U-299.	9	F	Ununeato	R	H	Nil	<8
929.	U-300.	19	F	Obowo	R	L	Nil	32
930.	U-301	18	F	Ikwuano/Umu.	R	L	Nil	16
931.	U-302	5	M	Ihotte	R	L	Nil	16

Abbreviations

M = Male

F = Female

R = Rural Community

U = Urban Community

L = Low Socioeconomic Status

H = High Socioeconomic Status.