Carriage rate of Neisseria meningitidis and some observations on the persistence of immunity in school children in parts of northern Nigeria.

BY

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A project report submitted to the school of postgraduate studies, Ahmadu Bello University Zaria, Nigeria, in partial fulfillment of the requirements for the degree of Master of Science in Microbiology.

DECLARATION

Carriage rate of *Neisseria meningitidis* and some observations on the persistence of immunity in school children in parts of northern Nigeria.

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I declare that this survey was conducted solely by the author under the close supervision of Professor S. O. Emejuaiwe of the Department of Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Onaiwu I. Enabulele

DEDICATION

This thesis is dedicated
solely to the memory of
my late Parents, Mr. & Mrs.
E. Idahosa Idehen, for gearing
my interests in early life
towards academic pursuits.

THESIS APPROVAL

This thesis meets the regulations governing the degree of Master of Science of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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ABSTRACT

Asymptomatic carriage of Neisseria meningitidis was studied in a total of 502 healthy school childrer in Zaria 214 in Shika and 332 in parts of Kano State, all in Nigeria between the months of August and December, 1982.
The prevalence of N. meningitidis averaged 2I4.68% in Zaria 12.60% in Shika and 7.88% in Kano. The prevalence of N. meningitidis averaged 17.67% in children aged 5 - 7 years and increased to 36.67% in children aged 14 - 16 years old among those investigated in Zaria. In Shika, it averaged 6.91% in children aged 5 - 7 years and increased to 16.67% in those aged 14 - 16 years while in Kano, it average 4. 33% in the former set of children and increased to 14.28% in latter. There was no sex influence on the rate of meningococcal carriage in the groups studied.

Analysis of the duration of immunity in school children vaccinated against groups A, meningococci was carried out three years after the children had received a bivalent (A + C) meningococcal polysaccharide vaccine. Sera were obtained from 69 of such immunized children in parts of Kano State. Additional 53 sera specimens were collected from non-vaccinated school children from the same area and used as controls.

The duration of immunity to group A meningococci was determined by measuring meningococcal antibody levels in serum samples using the complement-dependent bactericidal antibody assay technique. It was observed that three

years after vaccination, the group A meningococcal antibody level was much higher in the immunized children than in the control group of similar age range.

Furthermore, it was observed that the rate of meningococcal carriage was much lower in the immunized children than in the non-immunized group. Meningococcal carriage amongst the groups investigated, showed that only group C meningococci was present in very low number amongst the vaccinated school children (5 carriers among 69 children), and group A meningococci was completely absent in these children. In general, carriers for meningococci groups A, D and X were absert in Kano and Shika whereas these groups were prominently present in Zaria. In addition, carriage rate for group C was identically low in Kano and Shika and significantly higher in Zaria.

The persistence of elevated level of bactericidal antibodies in both immunized and non-immunized children was found to be markedly age dependent.

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ABBREVIATIONS

Auto Ag; AAg.....	Auto-agglutinable
Multiple Ag;	Multiple - agglutinable
Non Aggl; NA	Non - agglutinable
Poly Ag; PAg	Poly-agglutinable
S D	Standard deviation
+	Positive
S+	Slightly positive
-	Negative
C NS	Central nervous system
C S F	Cerebrospinal fluid.

INTRODUCTION

Neisseria meningitidis continues to be an important aetiological agent of bacteremia and meningitis in humans. Since about 1880, the northern savannah region of tropical Africa has been regularly affected by epidemics of meningococcal disease (Greenwood et al, 1979). Epidemics have been recorded in Nigeria as far back as 1948 - 50 (Horn, 1951) and more recently in 1977 (Greenwood et al, 1979). Besides, sporadic cases have also been recorded in both Northern and Southern Nigeria, for instance, between January 1976 and March 1977, Neisseria meningitidis accounted for 7.7% of pyogenic meningitis in Ibadan, Nigeria (Montefiore et al. 1978). N. Meningitidis Serogroups A, B, and C (Marks et al 1979) have been reported to be responsible for most of the out-breaks and children below 5 years old and young adults (Marks et al, 1979) are known to be mostly at risk of contracting the disease, though reports from developing countries do not fully confirm this observation (Galazka, 1982).

Neisseria meningitidis can be carried in the nasopharynx of apparently healthy individuals and such carriers could serve as a reservoir of potentially infectious organisms (Bhatti et al 1982). Studies of Sippel and Girgis (1978) showed that meningococcal disease patients are also important in its transmission particularly when these patients are also nasopharygeal carriers.

There is the belief by some authors that carriage rate in the population increases markedly just before an outbreak (Leedom et al 1967, Foster et al, 1971), but this belief cannot yet be fully sustained through available data. The factors responsible for initiation of major epidemics is still unknown. Carrier state with meningococci, groupable or non-groupable give rise to bactericidal antibodies (Frasch and Chapman, 1973).

However, carriage rate has been little studied, especially in the African Meningitis belt. A few studies of the carriage rate in the general population during an epidemic has been undertaken (Riding and Corkhill, 1932; Hassan-King et al, 1979). Njoku-Obi and Agbo (1976), also studied the carriage rate of N. Meningitidis in adults and children in parts of Eastern Nigeria.

This work aims at finding out the carriage rate of Neisseria meningitidis in school age children in parts of Northern Nigeria and the distribution of the prevalent serogroups. Such studies are necessary since they expose those at risk to whom preventive measures could be applied. Besides, such prevalence studies aid in selection of appropriate strains of vaccines for use in the areas under study.

The effectiveness of group A and group C meningococcal polysaccharide vaccines in controlling outbreaks of meningococcal disease has been clearly demonstrated in many parts of the world. However, there are still some uncertainties about how these vaccines can be used most effectively (Anon, 1978). It is essential to know the duration of protective immunity after vaccination before a rational vaccination programme can be devised. Clinical protection against group A meningococcal disease has been observed to last for two to three years after vaccination (Wahdan et al 1973; Peltola, 1978), however, in a second trial (Wahdan et al, (1977) observed that protection lasted for only one year. Continued trials are necessary to provide further information on the duration of clinical protection conferred on individuals by vaccination. Studies of Goldschneider et al (1969) indicated a strong correlation between meningococcal antibody levels and protection against infection. Lepow et al (1977) estimated the protective level of meningococcal antibody to be about 2 µg/ml which is the mean antibody level in adult populations. Goldschneider et al (1969) estimated the mean meningococcal bactericidal antibody titre in adults to be about 1: 16. Thus indirect evidence for the duration of clinical protection conferred on individuals by vaccination can be obtained by serial measurements of the bactericidal antibody titres at varying periods after vaccination.

The antibody response of children in the developed parts of the world to groups A and C meningococcal vaccines have been well documented (Lepow et al 1977; Kaythy et al 1980) but there have been comparatively few reports on the antibody response and duration in children within the African meningitis belt (Greenwood et al, 1980 (b). Besides, the antibody response and duration have been shown to be dependent on the prevaccination antibody level, the initial rise in titre of meningococcal antibody after vaccination (Kaythy et al, 1980), hemoglobin genotype, level of malnutrition and attack of malaria (Greenwood et al, 1980(a). More information is therefore necessary on the nature of the response to immunisation

and the probable duration of the antibody response to immunisation within the African meningitis belt. Since the highest incidence of meningococcal disease is not among adults but among children, aged 5 - 15 years old (Mohammed, 1983), vaccine evaluation within this age group is very necessary. Such information is presented as a part of this report. We measured the bactericidal titre of meningococcal antibodies in school age Nigerian children immunised three years before the initiation of these studies with serogroups A and C meningococcal polysaccharide vaccines.

LITERATURE SURVEY

1. The ORGANISM:

A. Colonial Morphology:

On enriched media, after 24 - 48 hours, meningococci form convex glistening, elevated, creamy, mucoid colonies 1 - 5 mm in diameter. The colonies are trasparent, non-pigmented or yellowish and non-hemolytic. Typical organisms are diplococci with their adjacent sides either flattened or convex (Leannette et al, 1974).

B. Structure:

The neisseriae possess a typical gram-negative cell envelope in thin sections (Goldschneder, et al 1969; Devoe et al, 1973). The envelope consists of two trilaminar membrane-like layers - the inner plasma membrane and the outer endotoxin - containing membrane (Anderseen et al, 1979), separated by a periplasmic space and an electron-dense peptidoglycan layer. The outer membrane of gram-negative cells have been extensively studied. Generally, they consist of a mosaic of lipoprotein, lipopolysaccharide, and protein. The hydrophobic portions of the lipoprotein and lipopolysaccharide form a basic layer and the protein molecules lie mainly on the outside.

Proteins found on the surface of the outer membrane are responsible for serotype specificity and are termed serotype Antigens (Frasch and Gotschlich, 1974).

Many carrier isolates and nearly all cases isolates of meningococci have chemically and immunologically distinct capsules composed of linear polymers of mono- and dissaccharides (Gotschlich et al, 1969).
These capsular polysaccharides provide a scheme for classifying meningococcal strains into serogroups.
Their nature has been extensively studies in some groups.
Group A polysaccharide has been shown to be a polymer of a phosphorylated hexosamine (Gotschlich et al, 1969) which has now been characterised as D-mannosamine.
It is a pure polymer of mannosamine phosphate which is partially N - and O - acetylated (Liu et al, 1971).
Groups B and C polysaccharides are homopolymers of sialic acid, the latter being partially O-acetylated (Liu et al, 1971) and exhibiting protective properties in man (Gotschlich et al, 1969; Bundle et al, 1974).

These polysaccharides are free of mucopeptide i.e. they are not covalently linked to the mucopeptide of the meningococcus and their exact location is still unknown (Liu et al, 1971). Not much is known about the composition of the capsular polysaccharides of the other serogroups.

The presence of pili has also been reported on some strains of the meningococcus (Jephcott et al,1971) although these pili could be lost during serial subculture (Devoe and Gilchrist, 1973).

C. Antigenic Classification:

In 1954, the Neisseriaceae subcommittee of the Nomenclature committee of the International Association of Microbiological Societies now the International Union of Microbiological Societies, recommended that meningococci should be classified into serogroups A,B,C, and D based on their polysaccharide antigens. Three new groups X, Y and Z were discovered by Slaterus in 1961, which were not agglutinated by antisera to the former set of meningococci. Evans et al, (1968) described three additional serogroups - ^{B}O, 29E and W135 but ^{B}O and 29E corresponded to Slaterus groups Y and Z. Today, meningococcal strains can be separated into at least nine serogroups - namely, A,B,C,D,X,Y,Z, 29E and W135 (Gold and Wyle, 1970), based on antigenic specificity of their capsular polysaccharides (Graven et al, 1978).

Meningococcal strains can be further sub-divided into distinct serotypes (Frasch and Chapman, 1972; Frasch and Chapman, 1973). This subdivision is based on the immunological specificity of certain distinct outer membrane proteins known as serotype antigens.

Studies of Frasch and Chapman in 1972 and 1973 respectively indicate that serotype antigens are most likely high molecular weight complexes containing protein, which could be extracted by mild acid or saline at 100°C. That of group B proved to be a very high molecular weight lipoprotein - lipopolysaccharide complex (Frasch & Gotschlich, 1974).

The fact that antibodies to serotype antigens are able to initiate a complement-mediated bactericidal effect strongly suggest that it is located on the surface of of the meningococci, most likely associated with the outer membrane of the cell envelope. Proof of this was evident from the fact that the outer membrane and serotype antigens had almost identical sodium dodecyl sulphate - polyacrylamide gel electrophoresis patterns (Frasch and Chapman, 1974). Today, it is known that the dominant major outer membrane protein of most meningococcal strains determine their serotypic classification. Group B meningococci for instance are divided into over 15 protein serotypes (Frasch, 1977). About 6-7 serotypes have also been identified within group C (Gold and Wyle, 1970). Group A serotype antigens have been indistinguishable from one another so far (Sippel and Quann, 1977). However, differences in bactericidal reactions, suggest that other antigens may play a role in antibody response to group A strains (Sippel and Quann, 1977). Not much has been done on serotype antigens of the other serogroups.

THE DISEASE

1. Epidemiology:

Meningococcal meningitis and sepsis have been reported in all the continents. Groups A, B, C, are responsible for most epidemics and incidents (Marks et al, 1979). The minor serogroups of Neiserria meningitidis (X, Y, Z, W135 and 29E) were initially considered to be of low virulence and were thought to produce very mild clinical disease. Recent literature (Kleiman et al, 1978), however, indicates that meningococcal disease does occur with groups Y and W135. Ryan et al (1980) also reported two cases of severe meningococcemia and meningitis with disseminated intravascular coagulation caused by serogroups X and Z meningococci.

Since about 1880, the Northern Savannah region of tropical Africa, referred to as the African meningitis belt, has been regularly affected by epidemics of meningococcal disease (Greenwood et al, 1979). Group A meningococci have been the cause of most African epidemics although outbreaks of group B infection have also been recorded in the Sudan, (Evans-Jones et al, 1977). Epidemics due to group C has also been recorded in Northern Nigeria (Whittle et al, 1975). Sporadic cases are often caused by group B meningococci (Peltola, 1983).

Some evidence suggests that certain serotypes may cause disease more often than others for instance, serotype 2 organisms were much higher during group B and C epidemics (Gold et al, 1971), and for most cases of groups Y and W135 (Frasch & Friedmann, 1972) while serotypes 4 and 6 have been isolated only from carriers (Frasch and Chapman, 1972).

Recorded meningococcal disease epidemics in Nigeria dates back to 1948 (Horn, 1951) and one of the most recent recorded case was in 1977 (Greenwood et al, 1979). This was due predominantly to group A meningococci. Few unrecorded sporadic cases occur in both the Northern and Southern Nigeria. For instance, between January, 1976 and March 1977, Neisseria meningitidis accounted for 7.7% of pyogenic meningitis in Ibadan, Nigeria (Montefiore et al, 1978).

Endemic meningococcal disease in civilian populations occur most frequently in children with peak incidence being between the ages of 6 months and 2 years (Goldschneider et al, 1969) presumably because of the absence of serum bactericidal antibodies (Marks et al, 1979), and young adults (Hassan King et al, 1979), between the ages of 5 - 15 years in the African Meningitis belt. (Mohammed, 1983).
Epidemics affect all age groups.

Man is the reservoir of meningococci. *Meningococci are spread by nasopharyngeal carriers.* Such carriers form about 5 - 20% of the population and they are the only important reservoirs of the organism in nature and the source of the vast majority of new infections (Sanders and Deal, 1970). Reports of carriage rates in Nigeria and other parts of the World have been rather conflicting. Fraser et al,(1973) reported 2.4% carriage rate in a mixed population consisting of civilian children, adults, naval and nursing students in the United States. This could rise to about 60% in closed populations like a military enclave (Altmann et al, 1973).

Studies of Sanborn et al, (1972) in Northern Nigeria showed a carriage rate of group A meningococci in the general population of 3½% during the middle of the dry season and 7% at the onset of the rains. Patterns of nasopharyngeal carriage among household contacts of African patients with the disease studied by Hassan-King et al (1979) during an epidemic showed a carriage rate of 3% in the general population of the epidemic strain group A. However, the relationship of carriage rates to disease outbreaks is still uncertain. (Sanders and Deal, 1970).

Apart from nasopharyngeal carriers there are reports of isolation of meningococci from urogenital sites (Chapel et al, 1977: Faur et al 1975). The role of isolates from these seemingly abnormal sites, in meningococcal infections is unknown.

Results of Sippel and Girgis (1978) also show that meningococcal patients are also important in meningococcal disease transmission particularly when they are also nasopharyngeal carriers. They found that pharyngeal isolation rate in family members of meningococcal meningitis patients who were not pharyngeal carriers was 23% while that in those of patients who were also carriers was 40%. Isolates from both family members and patients had the same sulfadiazine sensitivity pattern. These suggest strongly that the patients are transmitting these organisms and are therefore very significant sources of virulent meningococci,

Meningococcal disease and colonisation is known to result from aerosol dispersion (Rybkina et al 1976), though only very little is known about the factors that facilitate person to person transmission (Herman and Keynan, 1980). Increased catarrh, coughing, sneezing and expectoration that accompany upper respiratory tract infections result in increased dispersal of aerosol droplets containing the organism (Herman and Keynan, 1980).

The risk of contracting the disease increases when factors associated with poverty are present (Fraser et al, 1973). Sleeping proximity is suggested as a probable factor in the contagion of meningococcal disease (Herman and Keynan, 1980). This could explain why household and dormitory contacts are at increased risk of acquiring infection whereas schoolmates and hospital personnel are less so (Jacobson et al, 1977). Epidemics normally start towards the end of the dry season when it is hot, dry and dusty and finishes at the onset of the rains in the African meningitis belt (Hassan-King et al, 1979). The factors responsible for this trend is still not well understood. Monlineux (1969) suggested that low absolute humidity is likely to be the most important factor since epidemics start when absolute humidity is low in both tropical and temperate areas. Lapeysonnie (1965) had earlier suggested that a dependence on low absolute humidity could also account for the clearly defined southern border of the African meningitis belt.

Monlineux (1969) suggested that a low absolute humidity could favour the survival of meningococci in droplets of respiratory secretions. Results of Greenwood et al (1969) tend to suggest that a low absolute humidity could predispose to meningococcal disease by damaging the local defense mechanisms of the nasopharynx, thus increasing the likelihood that infection with a meningococcus would lead to clinical disease rather that to an asymptomatic carrier state. The factors responsible for initiating the regular appearance of major epidemics within the African meningitis belt is still elusive.

Pathogenesis

The development and course of meningococcal infection are governed by a complex of many variables including virulence factors such as encapsulation (Moxon and Murphy, 1978) and toxigenicity. Briefly, a respiratory focus of infection, presumably nasopharyngeal, could either be asymptomatic (carrier state) or could result in bacteremia, which may then lead to metastatic infection of the meninges, joints, pericardium and other sites (Kleiman et al, 1978).

What accounts for the peculiar susceptibility of the few individuals who develop meningococcal disease rather than asymptomatic infection is still unknown. Lack of immunity usually facilitate the development of bacteremia which can lead to an acute purulent meningitis (Bhatti et al, 1982). Artenstein et al, (1970) suggested that other factors like anatomic abnormalities, deficiency of secretory immunoglobulins and antecedent or coincident viral infections could facilitate the egress of Neisseria meningitidis from the respiratory tract to the bloodstream or meninges.

The possession of a capsule is thought to be one of the virulence factors of meningococci. Encapsulated bacteria are known to resist phagocytosis (Melly et al 1974).

Certain bacteria are able to evade the normal phagocytic defense mechanisms and this resistance to phagocytosis often appears to be related to the production of a capsule by such organisms (Wilkinson et al, 1979). Encapsulation is thought to interfere with effective opsonization (Peterson et al, 1978). A precise mechanism for this inhibition of opsonization is not known but one possibility is that this capsular substance physically obscures receptor sites for immunoglobulins or complement on the cell wall (Weinstein and Young, 1978).

The possession of pili by some strains of meningococci could also help in its pathogenetic mechanisms. There is increasing recognition that in some infection processes, pili may enhance bacterial virulence by facilitating adherence to and colonisation of mucosal surfaces (Silverblatt et al, 1979). Though this has not been demonstrated with meningococci, but with the gonococci, a closely related species, pili have been found to promote attachment to several kinds of Leukaryotic cells and these organelles are thought to influence virulence by anchoring gonococci to mucosal sites (King et al, 1978).

Meningococcal endotoxin has been implicated in the course of disease caused by the meningococcus (Ducker and Simmons, 1968). Similarities have been found between the general clinical findings in animals treated with meningococcal endotoxin and the clinical features of meningococcal disease in humans (Gaskins and Dalldorf, 1976).

Devoe and Gilchrist (1973) have proposed that the endotoxin resides in the outer membrane of the meningococcus and that an oversynthesis of this endotoxin-containing outer membrane, resulting in a continuous release of endotoxin is a characteristic feature of the meningococcus during cell division. Hill and Weiss (1974) reported that open segmented trilaminar structures from the cell wall enriched fractions contained most of the endotoxin. The endotoxin is thought to be a part of the lipoplysaccharide moiety of the outer membrane, as meningococcal lipopolysaccharide has been shown to be potent in the induction of the generalised and dermal Schwartzman reactions (Margaretten et al, 1967; Davis and Arnold, 1974), the features of which are almost identical with those of fulminant meningococcemia.

Devoe et al,(1977), have further shown that a combination of degraded meningococcal material egested from polymorphonuclear leucocytes and relatively small amounts of endotoxin released from rapidly dividing cells are sufficient to produce disseminated intravascular coagulation (a complication of both meningococcemia and meningitis).

The mechanism of endotoxin release has been studied by several workers. Devoe and Gilchrist (1973) proposed a mechanism whereby rapidly growing meningococci released

substantial amounts of endotoxin-containing cell wall into the medium without lysis. Such cells evaginate outer cell walls to form blebs which are subsequently released into the medium both in the stationary phase of growth (Anderseen et al, 1979) and during the log phase of growth (Devoe and Gilchrist, 1973).

C. CLINICAL MANIFESTATIONS AND COMPLICATIONS:

Neisseria meningitidis may cause a mild nasopharyngitis and in some cases, may invade the bloodstream and find their way into the meninges or may give rise to overwhelming septicemia. In rare instances however, the meningococci remain in the blood for a long time, continuously or intermittently causing a mild protracted variant of meningococcal septicemia termed chronic meningococcemia. (Niklasson and Svanborn, 1973). Though rare, cases of meningococcal pneumonia have also been described by some authors (Putsch et al, 1970). These authors suggested that the meningococcus should be regarded as a potential respiratory tract pathogen. There's very little or no information on this type of meningococcal infection.

Rarer diseases caused by meningococci are arthritis (Schaad, 1980), opthalmitis (Mason et al, 1979), Urethritis (Carpenter and Charles, 1942), vaginitis (Gregory and Abramson, 1971) and peritonitis (Page et al, 1974).

a. Meningococcemia: A diagnosis of meningococcemia is made in a patient with fever, petechiae, clear cerebrospinal fluid and a positive blood culture.

Benign Meningococcemia:-

This is characterised by fever, skin manifestations and arthralgia, in a relatively healthy looking individual. The fever is continuous or intermittent and the skin manifestations consist of recurring maculopalpular, nodular and/or petechial eruptions without pus. The natural course can be protracted for weeks or months and meningitis may supervene at any time (Olcein et al, 1978). This disorder has been described mostly in adults with mean age 27 - 28 years. It is a relatively rare disease with no signs of shock, disturbed ventilation, heart affection, renal damages, and/or meningitis. (Olcein et al, 1978).

Fulminant meningococcemia:-

The fulminant form of meningococcal sepsis was earlier named Waterhouse-Friderichsen syndrome with reference to the adrenal glands (Niklasson et al, 1971). This is usually accompanied by purpuric skin lesions and renal cortical necrosis. Disseminated intravascular coagulation usually depicts the peak of meningococcal septicemia and is manifested by thrombosis in many organs (Minna et al, 1974).

Disseminated intravascular coagulation is a dynamic pathologic process triggered by activation of the clotting cascade, with the resultant generation of excess thrombin within the vascular system, that leads to further activation of the coagulation system, shortened survival of certain hemostatic elements, deposition of fibrin in the microcirculation and activation of the fibrinolytic system (Feinstein, 1982). Lowered levels of hemostatic factors and secondary fibrinolysis may result in clinically excessive bleeding, particularly when the patients blood vessels have been damaged by vascular puncture, trauma or surgery. In contrast, fibrin deposits may cause micro-or macro-thrombosis (Feinstein, 1982). Deykin (1970) sees the central pathologic process in disseminated intravascular coagulation, as a response to an underlying illness, that provokes a generalised activation of the hemostatic mechanism beyond that normally confined to areas of local vascular injury. He considers the syndrome as a reflection of the interplay of two basic processes, the first being a complex sequence of events leading to the evolution of thrombin and the second being a series of protective mechanisms that the body employs to defend itself against the generalised clotting that would occur in the absence of unchecked thrombin. Clinical lesions in disseminated intravascular coagulation include a variety of cutaneous alterations which when recognised can lead to its prompt diagnosis such lesions include

pretechiae, purpura, palpable purpura, hemorrhagic bullae, purpurae fulminans and acral necrosis (Robboy et al, 1973).

Circulatory failure or shock could result from disseminated intravascular coagulation. Pallor, Cyanosis, unconsciousness, cold extremeties, rapid and feeble pulsations, loud cardiac sounds, collapsed veins and reduced arterial pressure are the characteristic features of shock. To these sings of circulatory failure, add purpuric lesions on the skin, leukopenia, hyperglycemia and the syndrome of fulminant meningococcal infection is complete (May, 1960).

Histopathologic findings in disseminated intravascular coagulation include deposition of fibrin thrombi in vessels of varying sizes and necrosis of vessel walls (Robboy et al, 1973). In purpura or petechiae present for less than 24 hours, fibrin thrombi were observed in capillaries of the superficial vascular plexus. The lightly woven eosinophilic fibrin aggregates were usually centrally located in the vascular lumina. In older lesions, biopsies revealed epidermal necrosis, formation of subepidermal bullae and patchy necrosis of the acrine gland.
In palpable purpurae, fibrin thrombi in small vessels and extensive haemorrhage are observed (Robboy et al, 1973).

Mechanism of Initiating Disseminated Intravascular Coagulation in Meningococcal Disease:-

The mechanism of disseminated intravascular coagulation is not yet fully understood but bacterial endotoxins are known to induce hypercoagulability, manifested by an increase in certain blood factor activities (Lapinski and Jeljaszewicz, 1969). Lesions in meningococcal disease are histologically indistinguishable from the dermal Shwartzmann reaction which is produced experimentally by Neisseria meningitidis endotoxin (Margaretten et al, 1967).

In vitro, endotoxin is capable of activating the Hageman factor (Rodriguez-Erdmann, 1965) and either directly or through the Hagemann factor, causes release of platelet factor III (PF3) from thrombocytes. Activated Hageman factor and PF3 would then activate prothrombin via the intrinsic clotting system and initiate clotting (Evans et al, 1969).

Deykin (1970) describes the intrinsic clotting system as follows: the first step is the conversion of the Hageman factor or factor XII from its inert or precursor form to its active or enzymatic form. Activated factor XII, in turn converts factor XI or plasma thromboplastin antecedent (PTA) from its inert form to its activated or enzymatic form. Activated factor IX, in the presence of platelet phospholipids, forms a stable

macromolecular complex with factor VIII or the antihemophilic factor. This complex, composed of activated factor IX, factor VIII and phospholipid converts factor X or the Stuart factor into its active form. Although activated factor X itself, can slowly convert prothrombin to thrombin, the rate of prothrombin conversion is dramatically increased by the presence of platelet phospholipids and an accessory clotting factor; factor V. The complex formed by activated factor X, factor V and platelet phospholipid is the prothrombin converting principle which rapidly and completely cleaves prothrombin, liberating thrombin.

Fibrinogen is known to be composed of a dimer of three chains - Alpha, beta and gamma chains. Thrombin cleaves off the alpha chains releasing fibrinopeptide A and next the beta chains releasing fibrinopeptide B. The remaining molecule is now termed fibrin monomer which rapidly polymerises to form a macromolecular structure that precipitates out from plasma, forming a gel. Factor XIII, an enzyme activated by thrombin, then forms covalent links within the interstices of the clot so that it is no longer disociable. (Deykin, 1970).

In addition, as a protective mechanism, the activation of factor XII leads to the production of kallikrein, which in turn, results in the conversion of plasminogen to plasmin, (Colman, et al, 1979) a potent proteolytic enzyme capable of digesting fibrin.

Thus fibrinolysis almost invariably accompanies thrombin evolution. The balance between these two processes determine the degree of hemorrhage and/or thrombosis seen clinically (Colman et al, 1979). However, the overall importance of fibrinolysis in disseminated intravascular coagulation is disputed (Feinstein, 1982).

Evans et al (1969) proposed the following sequence of events in fulminating meningococcemia:- Favoureble growth conditions in the body lead to rapid bacterial multiplication causing vasculitis , thrombosis and hemorrhage by direct bacterial invasion possibly supplemented by release of endotoxin. Circulating endotoxin lead to widespread endothelial degeneration in many organs. Whether endothelial cell necrosis is a direct result of endotoxin or occurs secondary to intravascular clot formation and subsequent vascular thrombosis is not known. Margeretten et al, (1967) however, proposed that the necrotic skin lesions, including bilateral adrenal necrosis are mediated by the blood clotting system, and are not due to the direct effect of the toxin on parenchymal cell. Sections of affected tissues prepared by this author show disseminated fibrin thrombi in capillaries, and small blood vessels to a degree significant for obstruction of the microcirculation. Accelerated intravascular clotting presumably follows because of direct effects of endotoxin on the clotting mechanism. So Evans and co-workers

believe that severe injury to the vascular endothelium and connective tissue serve to initiate hypercoagulable state with intravascular clotting and consumption of coagulation factors.

Meningococcal Meningitis:

The clinical symptoms of this disease include intense headache, lethargy or confusion, vomitting, irritability and fever. Nuchal rigidity appears in over 80% of cases (Swartz and Dodge, 1965).

The development and course of meningeal infection are governed by the complex interaction of many variables (Scheld, 1981), including microbial virulence factors such as encapsulation (Robbins et al, 1974), clearance of bacteria from the blood (Moxon and Ostrow, 1977), antibacterial mechanisms in the subarachnoid space (Scheld et al, 1979) anatomic and functional changes resulting from inflammation (McAllister et al, 1975) and antibiotic therapy (Scheld, 1981).

The source of seeding of the central Nervous system (CNS) is not yet understood but the occurence of bacteremia reflect either a primary hematogenous seeding of the CNS, a seeding of the bloodstream from a CNS source or a simultaneous spread from an infected source to both the CNS and bloodstream (Scheld et al, 1979). Studies of Moxon et al, 1979 on the infant rate model of meningitis have suggested that bacteremia is primary and of central importance in the pathogenesis of meningitis but studies

on bacterial concentrations in the cerebrospinal fluid of children with meningitis have suggested that secondary bacteremia is common and dependent on bacterial multiplication within the cerebrospinal fluid and the subarachnoid space (Feldman. 1976) Studies of Scheld et al (1979) demonstrates secondary bacteremia in dogs 2 hours after intracisternal inoculation. Organisms were detected in the superior saggital sinus before peripheral arterial blood.

Petersdorf and Luttrel (1962) describes the pathological features of meningitis as follows:- Grossly purulent exudate was present over the convexities of the cerebral hemispheres as well as at the base of the brain. Histological lesions of the brain could be divided into 5 types:

(1) exudate of the convexity and basal subarachnoid spaces of the brain and spinal cord

(2) exudate of the ventricular system and central canal of the spinal cord

(3) Vascular congestion and inflammation of the choroid plexus

(4) Major sinus and superficial and deep venous thrombophlebitis with subarachnoid bleeding

(5) Cortical granulomata and necrosis of neural tissue.

Exudates consist primarily of granulocytes and often extend from the olfactory lobe to the medulla (McAllister et al, 1975). Once initiated, bacterial meningitis initiates inflammatory reactions in the central nervous system that result in the generation of chemotactic factors and in the appearance of various classes of immunoglobulins, polymorphonuclear leucocytes and complement in bactericidal amounts, all of which are expected to improve the local host defence mechanisms (Scheld, 1981). Scheld (1981) concluded that mortality or mechanism of death in meningitis seemed to be related to subarachnoid hemorrhage in a few animals but in the majority, interference with cerebrospinal fluid circulation subsequent to obstruction of the ventricular system by the inflammatory exudate, must have resulted in functional hydrocephalus. Pus in the central canal of the spinal cord led to severe inflammatory necrosis of the surrounding grey matter.

Hydrocephalus is one of the major complications of meningitis. The exact mechanism responsible for increased intracranial pressure and hydrocephalus in bacterial meningitis is unknown but it is usually ascribed to obstruction by purulent material, in the acqueduct of the foramina of the 4th ventricle, in the subarachnoid space or in the arachnoid villi. The latter would then cause alteration in cerebrospinal fluid absorption with an

increase in the resistance of cerebrospinal fluid outflow from the subarachnoid space back into the venous system (Scheld, 1981). Cerebral edema of both vasogenic and cytotoxic types may contribute to the raised intracranial pressure in some cases. Cerebral edema may be secondary to pial vessel invasion by microorganisms with resultant vessel thrombosis or cerebral infarction or the direct cytotoxicity of leucocyte products within the subarachnoid space (Scheld et al, 1980).

Immunity and Control:

Immunity to meningococcal disease has been shown to be antibody-mediated (Goldschneider et al, 1969). These protective antibodies may be directed against

(a) the group - specific capsular polysaccharride antigens,

(b) the protein serotype antigens which are shared among many strains of pathogenic meningococci (Kasper et al, 1973) and

(c) other cross-reactive antigens.

The mechanism has been shown to be induction of serum anticapsular antibody with complement-dependent bactericidal activity (Gotschlieh et al, 1969 ; (Nicholson & Lepow, 1979). All normal sera contain precipitins against some Neisseria meningitidis (Hoff and Hoiby, 1978).

Immunity to systemic meningococcal infection among infants in the neonatal period is associated with passive transfer of IgG antibodies from mother to the fetus (Goldschneider et al, 1969). In children and adults, carriage of groupable or non-groupable meningococci induces antibodies to meningococci (Frasch and Chapman, 1973). Though carrier strains are generally of low virulence, they contain antigens which cross-react with those of pathogenic strains (Goldschneider et al, 1969). Besides, natural immunity to meningococcal disease is initiated, reinforced and broadened by intermittent carriage of different strains of meningococci throughout life (Goldschneider et al, 1969).

Carriage of other Neisseria species could also induce antibodies against some meningococci, since meningococcal antigens show cross-reactivity with antigens from these other Neisseria species and these species are part of the normal flora of the human body (Hoff and Hoiby, 1978). For instance, carriage of Neisseria lactamica may be a frequent contribution to the development of natural immunity to Neisseria meningitidis in infants and young children (Gold et al, 1978).

Antibodies against Neisseria meningitidis polysaccharides may also be induced by other bacteria, since some widely occuring bacteria have been found to possess capsules which cross-react with Neisseria meningitidis polysaccharides antigens e.g. E. coli K1

has been found to be structurally related to group B capsular polysaccharide (Hoff and Hoiby 1978).

Groups A and C capsular polysaccharide antigens have been shown to produce protective antibodies in humans Artestein et al, 1970, Wadhan et al, 1973) but that of group B does not (Wyle et al, 1972; Gotschlich et al, 1975). Within group B, it appears to be primarily the serotype antigens that elicit bactericidal antibodies (Frasch and Gotschlic, 1974). Group B meningococcus is highly heterologous and as such, group B antibody would only be protective against strains of the homologous protein serotype. (Sippel and Quan, 1977). There is very little or no information on the protective ability of antibodies from the other serogroups.

Infants in the neonatal period have passive immunity acquired from their mothers. After birth, serum antibody content diminishes, reaching its lowest level at 5 - 7 months of age. Then with exposure to meningococci and bacteria with similar antigens, the antibody level gradually increases, although its concentration may still be low up to 18 months of age. Between 2 and 12 years of age, there is a further progressive increase. Children aged 13 - 19 years may have antibody levels equal to those seen in adults. 60% of unimmunized adults show antibody levels of 2 rg/ml or more and 87% of them have more than rg/ml (Galazka, 1982).

Two promising areas at which control measures should be directed are mass vaccination of those at risk,

especially case contacts during epidemics and eradication of carriers.

Carriers are the only important reservoir of the organisms in nature and the source of a vast majority of new infections but unfortunately, most antimeningococcal drugs are useless in eradicating the carrier state (Sanders and Deal, 1970). Though a rapidly increasing carrier rate does not necessarily portend the occurence of meningococcal disease, when symptomatic disease appears in a confined population, elimination of meningococci from all carriers will abort the outbreak (Feldmann, 1966).

Among available antimicrobial agents each of the following has been demonstrated to be of little or no use in the elimination of Neisseria meningitidis from a symptomatic carriers

(a) Penicillin G, administered orally or parenterally despite its proven value at higher dosage in the treatment of meningitis, and meningococcemia,
(b) Benzathine penicillin,
(c) ampicillin,
(d) erythromycin,
(e) tetracycline,
(f) chloramphenicol and
(g) Cephalexin (Artenstein et al, 1970).

However, rifampin, a new synthetic macrolide antibiotic has been shown to be highly effective in eradicating the carriage status. Though resistant mutants have been isolated in the laboratory, it has not yet been found naturally (Artenstein et al, 1970). Besides rifampin, monocycline is the only other drug known to be capable of eradicating Neisseria meningitidis from the nasopharynx of carriers. Studies of Sivonen et al, (1978) shows 78% success for Rifampin and 62% for minocycline. The dosage described by these workers is 600 mg per day for four days as a single dose before breakfast for rifampin, and for minocycline, a starting dose of 200 mg followed by 100 mg every 12 hours for 5 days. However the effect of these drugs are disappointingly short i.e. new acquisition easily follows due to reinfection. Vaccination also decreases carriage to some extent but it is not very effective (Gotschlich et al, 1969).

USE OF VACCINE

The Walter Reed Army Institute of Medical Research has succeeded in extracting and purifying the group specific polysaccharides of groups A and C meningococci to date. Three meningococcal vaccines prepared from these are now available:- monovalent vaccines composed of either group A or C purified capsular polysaccharide and a bivalent vaccine of groups A and C polysaccharide. A polyvalent meningococcal vaccine that includes groups A, C and Y has also been developed and shown to be effective in clinical trials (Farquhar et al, 1978). It has not been possible to isolate an immunogenic capsular polysaccharide from group B so far. Progress is also being made in the isolation and characterisation of specific polysaccharides from groups Y and W135 organisms (Galazka, 1982). Galazka (1982) believes that immunisation against Neisseria meningitidis disease should not be included within routine immunization programmes in developing countries because of problems like irregularity of epidemics, the changing serogroups of meningococci, the variation in age distribution of cases in different areas, the need for a series of doses of group A vaccine in infants and young children to maintain a long-lasting anti - A immunity, the low efficacy of group C vaccine in children below 2 years of age, the scarce information on the effect of simultaneous administration of Neisseria meningitidis

vaccines with other vaccines and the still high cost of the current meningococcal vaccines.

Inspite of these problems, highlighted by Galazka (1982), Ukeje (1980) considers the present day knowledge of the behaviour of the disease in the face of a well-planned immunization programme, reasonable enough to warrant the inclusion of seasonal, well-timed mass immunization against cerebrospinal meningitis as a routine aspect of primary health care, since Neisseria meningitidis infections have caused untold hardship, suffering and death to many in the African meningitis belt (Njoku-Obi, 1980). Okere (1980) strongly supports this latter recommendation for the following reasons.

a) Once established that a particular meningococcal group is the causative agent in a particular place, vaccination programmes can be embarked upon without waiting for an epidemic to occur.
b) Vaccination not only stops the illness but also confers immunity.
c) The emergence of antibiotic-resistant strains, as a result of chemoprophylaxis will be more disastrous than the possibility of another meningococci serogroup emerging among the vaccinated population.
d) Vaccination takes care of the real possibility of the continued presence of the organisms in the environment since most of the people in Africa are unable to look after their health and are resentful of modern medical methods.

At the moment, groups A and C vaccines are recommended for use in the control of cerebrospinal meningitis due to these serogroups during epidemics, so as to prevent the spread of the infection (Galazka, 1982).

The protective efficacy of meningococcal vaccines is dependent upon the induction and persistence of bactericidal antibodies (Glode et al, 1979). Immunity following immunization with groups A and C vaccines is group-specific and protection is achieved in one week (Galazka, 1982). Immunization of adults and children over 2 years of age with group C capsular polysaccharides confers protection against these organisms (Artenstein et al, 1970; Tauney et al, 1974). The concentration of antibody induced in younger children and infants with group specific vaccines is very low. This problem could be overcome by administering a second dose 3 - 4 months later (Galazka, 1982). Efficacy of group A vaccine has also been proved in adults and older children but there are still doubts as to efficacy in younger children and infants. The level of antibodies induced in infants less than 1 year old is poor (Galazka, 1982).

The duration of post-immunization immunity is not known with certainty. The available data for group A vaccine suggest that a single dose of vaccine administered to persons over 2 years of age provides protection for 1 - 3 years. The protective efficacy of group C

polysaccharide vaccine seems to be of short duration, probably 1 year or less (Lepow et al, 1977; Kaythy et al, 1980).

Adverse reactions to groups A and C vaccines are generally mild and infrequent in school age children and adults, following primary and booster immunizations made by conventional subcutaneous or by the injector routes (Artenstein et al, 1970). No serious local or systemic reactions have been reported although transient local erythema, at the site of injection and mild irritability are infrequently noted. In infants and young children local reactions were also mild and included erythema, oedema and tenderness at the site of injection.

Vaccines at present, are used mainly for preventing and controlling epidemics (Galazka, 1982).

MATERIALS AND METHODS

1. Sample Collection:

Samples were collected transorally from pupils aged 3 - 16 years in Islamic and primary schools in Zaria, and Shika in Kaduna State and Barbura, Dambatta and Wusansan in Kano State. School-age pupils sampled in Kano State were mainly those that were previously immunized against groups A and C meningococci. Cotton-tipped wooden applicators were thrusted into the oral cavities, down to behind the uvula and rotated as described by Hoeffler in 1974. Swabs were immediately immersed in Stuart's transport medium (Bacto) and transported to the laboratories where they were immediately cultured.

2. Cultivation of the Organisms:

Nasopharygngeal cultures were swabbed into fresh Thayer-Martin plates (Twine and Henley, 1982), prepared as follows. Bacto- G.C. medium base and Bacto hemoglobin were prepared and aucoclaved separately at 121^{o}C for 15 minutes as direct by the manufacturer. They were allowed to cool to about 50^{o}C, mixed and Bacto yeast supplement B was added as directed by the manufacturers. The antibiotics Vancomycin, colisti sulphate and Nystatin were added as directed by the manufacturer (Difco) to inhibit other throat microflora.

Beeded plates were then incubated at 37°C under 5% carbondioxide and 80% humidity in a carbondioxide incubator. Plates were examined after 24 hours for convex glistening elevated, butyrous, transparent and mucoid colonies 1 - 3 mm in diameter. Two of such colonies were picked from each plate and sub-cultured onto fresh Thayer-Martin medium, for another 24 hours for further identification. Plates lacking such colonies were subcultured for another 24 hours and discarded if there was still no growth.

3. Tests for Identification of the Organisms

A. Gram Stain

Heat-fixed smears of organisms from each colony was covered with the basic dye, crystal violet for one minute and washed with water. A solution of gram's iodine was poured on to the smear for another one minute and then washed off with water. All bacteria are stained deep blue at this point in the proceedure. Cells were then treated with acetone for about 30 seconds to effect decolorisation of gram-negative cells and then washed with a jet of water. Smears were then covered with safranin for about 30 seconds, washed with water, and blotted dry for examination under oil immersion lens. Gram positive bacteria retain the deep blue colour of crystal violet while gram-negative oneswere counterstained red by safranin. Colonies showing gram-negative diplococci were subjected to other tests for further identification.

B. Oxidase Test:

Kovacs (1956) modification of the oxidase test was used on organisms from colonies found to consist of gram-negative diplococci. A piece of Whatman's No. 1 filter paper of about 6 cm square was laid in a petridish. Two to three drops of freshly prepared 1% tetramethyl-para-phenylenediamine dihydrochloride solution was dropped unto the center of the filter paper. Part of a suspected colony was picked with a platinum wire needle and thoroughly smeared unto the reagent-impregnated filter paper, in a line 3-6 mm long. Oxidase positive colonies which turn dark-purple in 5-10 seconds are subjected to further tests.

C. Test For B-D-galactosidase activity:

This test distinguishes Neisseria meningitidis from Neisseria lactamicus, both of which are culturally very similar. Bacto O-nitrophenyl-B-D-galactopyranoside (ONPG) disks were used. 0.2 ml of 0.85% sodium chloride solution were dispersed in Kahn tubes. A loopful of suspected culture was suspended in each solution and an ONPG disk were asceptically introduced into the tube. Each tube was then incubated at 37°C for 20 minutes. The development of a yellow colour indicates the presence of the enzyme galactosidase. Negative tubes are held for another 4 hours before making a final observation. Neisseria meningitidis are ONPG-negative.

D. Sugar degradation tests:

Bacto cystine tryptic Agar was prepared and sterilised by autoclaving at 121°C for 15 minutes according to the manufacturers directions. Solutions of maltose, dextrose and sucrose were prepared and sterilised through membrane filters (0.22 Microns millipore filters) on a filteration unit to which negative pressure was applied. Each solution was asceptically added to the sterile cystine tryptic agar to a concentration of 1% and poured into screw-cap test tubes.

A very heavy inoculum of suspected culture was inoculated unto the surface of each tube, each of which was tightly capped and incubated for 24 hours at 37°C. Tubes with clear-cut fermentation reactions were read after 24 hours while those with doubtful or negative reactions were incubated for another 48 - 100 hours before making final observations. Positive fermentation tubes change from red to yellow, while negative ones remain red. Neisseria meningitidis are known to ferment dextrose and maltose but not sucrose.

E. Determination of Serogroups

All isolates that are gram-negative, oxidase positive and ONPG-negative, irrespective of the sugar fermentation reactions were serogrouped by macroscopic slide agglutination, using commercially

available rabbit antisera obtained from wellcome laboratories. An inoculum of suspected cultures was emulsified in a drop of normal saline on a clean slide. A drop of polyvalent meningococcal antisera (Polyvalents A-D and X-Z, W135) was added unto it and the slide was rocked gently from side to side for 30 seconds to 2 minutes. The appearance of floculations on any of the slides indicate that theorganism belongs to that polyvalent group. Emusions of the organism were then treated with each of the monovalent antisera. (A,B,C,D,X,Y,Z & W135) in the same manner as described above to determine the actual group to which it belongs. To reduce the problem of autoagglutinations, a major problem encountered in slide agglutinations, overnight cultures of the organism in tryptic soy broth was used instead of emulsions in saline. Cultures that agglutinated spontaneously either in saline or broth were regarded as autoagglutinable, those that agglutinated the two polyvalent antisera were regarded as polyagglutinable, those that agglutinated more than one monovalent antiserum were regarded as multiple agglutinable, while those that were not agglutinated by any of the antisera; whether polyvalent or monovalent, were regarded as non-agglutinable.

Collection of blood

Blood was collected from some of the pupils, from parts of Kano State, some of whom were known to have been previously immunized against groups A and C meningococci. The upper limb of such pupils was tied with a turnique and the inner surface of the elbow joint was cleaned with cotton wool moistened with methylated spirit. A 5 ml sterile syringe with needle was inserted into a vein at this joint and about 4 - 5 ml of blood was withdrawn and poured into sterile universal bottles. Samples were taken to the lab, centrifuged asceptically at 1,500 rpm and the sera were separated and stored in the deep freezer.

5. Bactericidal Antibody Assay:

The method recommended by the World Health Organisation expert committee on biological standardization (1976) was used for the bactericidal antibody assay. Sera diluted 1: 2 with normal saline were inactivated at 56°C for 30 minutes. Serial two-fold dilutions of 0.2 ml of the serum in Dulbecos phosphate buffered saline were performed in sterile plastic microtitration plates with 24 wells per plate, using sterile 1 ml disposable pipettes.

A lyophilized meningococcal culture was inoculated on to Thayer-Martin medium and incubated at 37°C in air containing 5% carbondioxide overnight.

A 25 ml quantity of tryptic Soy broth was added asceptically to a side-arm flask and a 5 ml quantity to a sterile culture tube. A loopful of the over-night culture was transferred to the 5 ml of broth in the culture tube, which was then throughly mixed and poured into the side-arm flask. The culture was then incubated at 37°C on a shaker for approximately 5 hours. The turbidity was diluted to match Marcfarland's nephelometer standard tube No. 1 containing about 300 million cells per ml using a spectronic 20. Serial tenfold dilutions were made to bring it to about 300 cells per ml.

0.2 ml of the adjusted suspension was added to each well, followed by 0.2 ml of pre-screened complement (Bacto) using sterile 1 - ml disposable pipettes. The following control samples were included in each test: a cell growth control containing 0.2 ml saline, 0.1 ml bacteria and 0.1 ml heat-inactivated complement, a complement control containing 0.2 ml saline, 0.1 ml bacteria and 0.1 ml active complement without serum, and a serum control containing 0.2 ml serum, 0.1 ml bacteria and 0.1 ml saline. The plates were incubated for 30 minutes in 5% carbondioxide in a carbondioxide incubator.

Subsequently, a mixture of tryptic soy broth with 1.2% agarose and 1% heat-inactivated horse serum was brought to a temperature of about 45°C and 1 ml of the medium is added to each well using a warm 1-ml automatic syringe. The trays were then gently swirled and the medium is allowed to set at room temperature. The trays are covered and incubated at 37°C for 18-24 hours in 5% carbondioxide.

The antibody titre was expressed as the highest serum dillution that gave 50% or greater killing as compared with the cell control.

City	No. of children Sampled	No. of Carriers detected	Mean Carriage rate (%)	Standard deviation
Zaria	502	124	24.68	1.33
Shika	214	27	12.60	0.31
Kano	332	26	7.88	4.62

Table 1: Meningococcal Carriage rates in parts of Northern Nigeria

Category	No of pupils examined	No of carriers detected	Carriage rate %
Immunized	212	12	5.66
Non-Immunized	120	14	11.66

Table 1b carriage rate of Neisseria meningitidis in immunized and non-immunized pupils in parts of Kano State.

City	No. of children sampled		No. of Carriers detected		Mean Carriage rate (%)		Standard deviation	
	Males	Females	Males	Females	Males	Females	M	F
Zaria	329	173	80	44	24.02	26.61	3.04	4.04
Shika	143	71	20	7	14.43	10.39	2.73	1.014
Kano	243	98	20	6	8.01	7.82	4.15	6.24

Table 2: Sex distribution of carriage rates of N. meningitidis in parts of Northern Nigeria

Age-group (Years)	No. of Children sampled	No. of Carriers detected	Mean Carriage rates (%)	Standard deviation
5 - 7	185	33	17.67	5.0
8 - 10	205	55	26.39	6.80
11 - 13	92	29	31.70	7.34
14 - 16	20	7	36.67	10.79

Table 3: Age distribution of carriers in Zaria

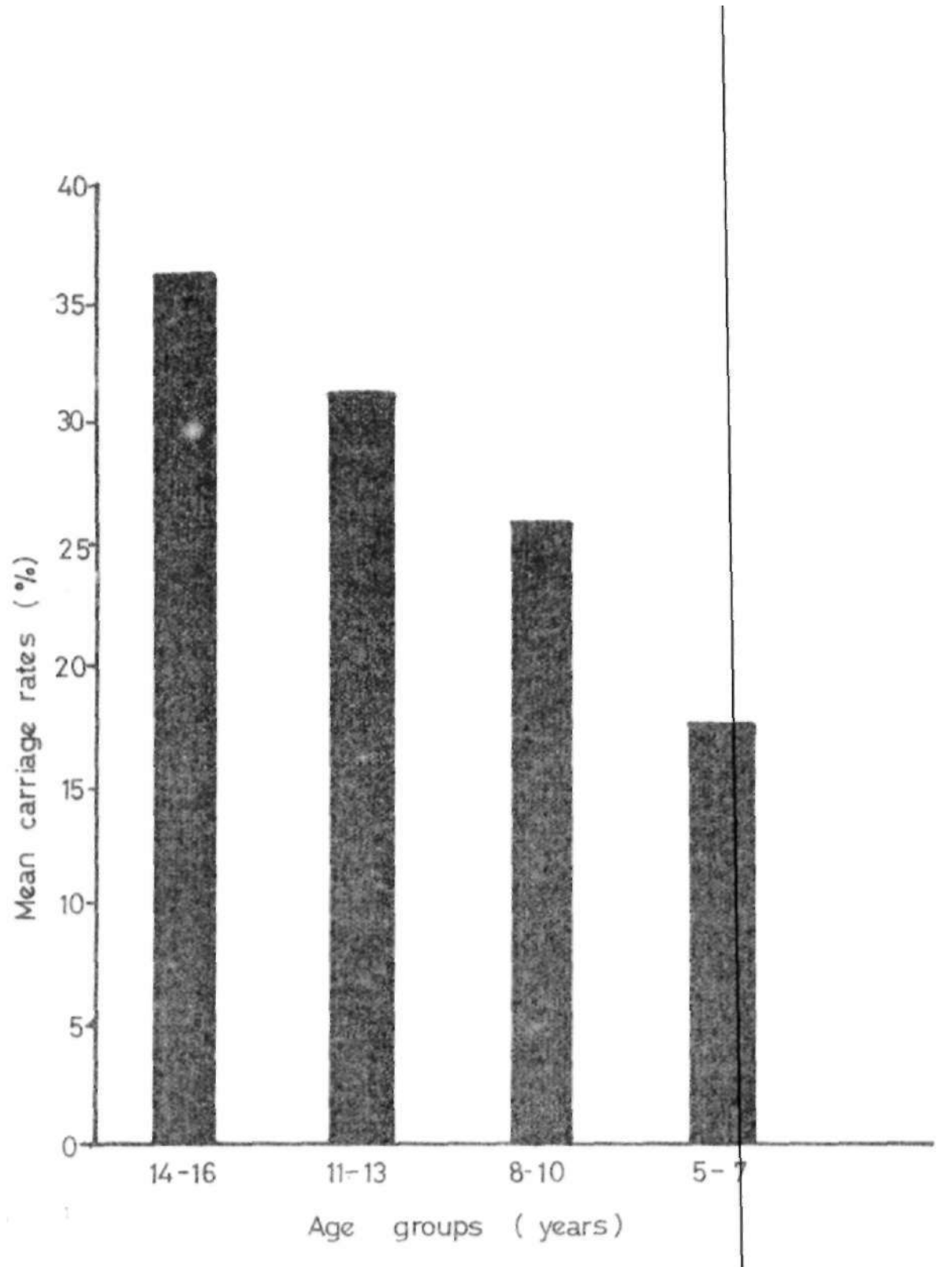


Fig. 1

AGE DISTRIBUTION OF CARRIERS IN ZARIA.

Age-group (Years)	No. of Children sampled	No. of Carriers detected	Mean Carriage rates (%)	Standard deviation
5 - 7	58	4	6.91	0.34
8 - 10	53	7	13.19	0.23
11 - 13	55	8	14.56	0.38
14 - 16	48	8	16.67	0.00

Table 4: Age distribution of carriers in Shika

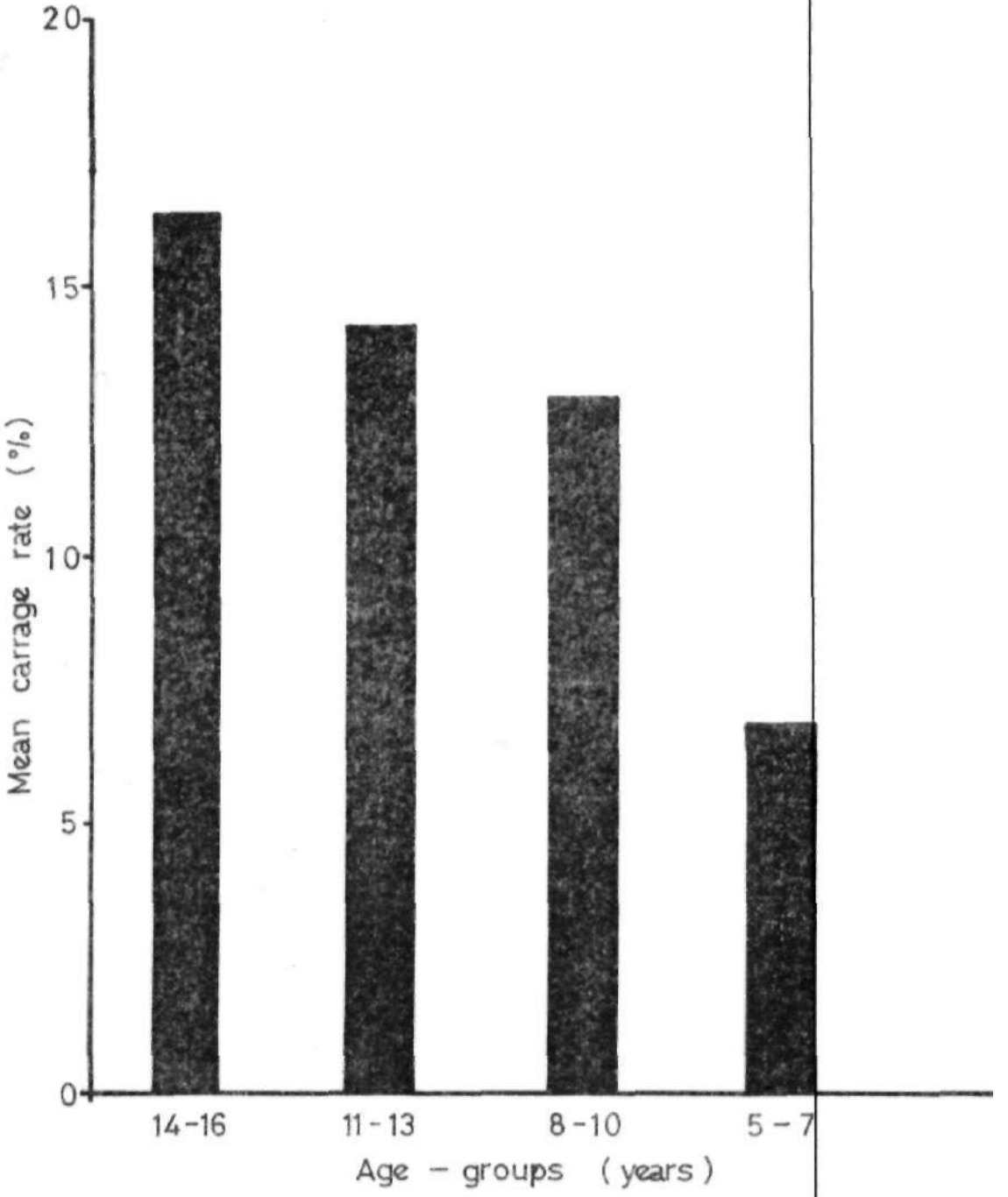


Fig. 2

AGE DISTRIBUTION OF CARRIERS IN SHIKA .

Age-group (Years)	No. of Children sampled	No. of Carriers detected	Mean Carriage rates (%)	Standard deviation
5 - 7	128	7	4.33	4.04
8 - 10	175	15	8.62	5.26
11 - 13	15	2	13.33	-
14 - 16	14	2	14.28	-

Table 5: Age distribution of carriers in Kano

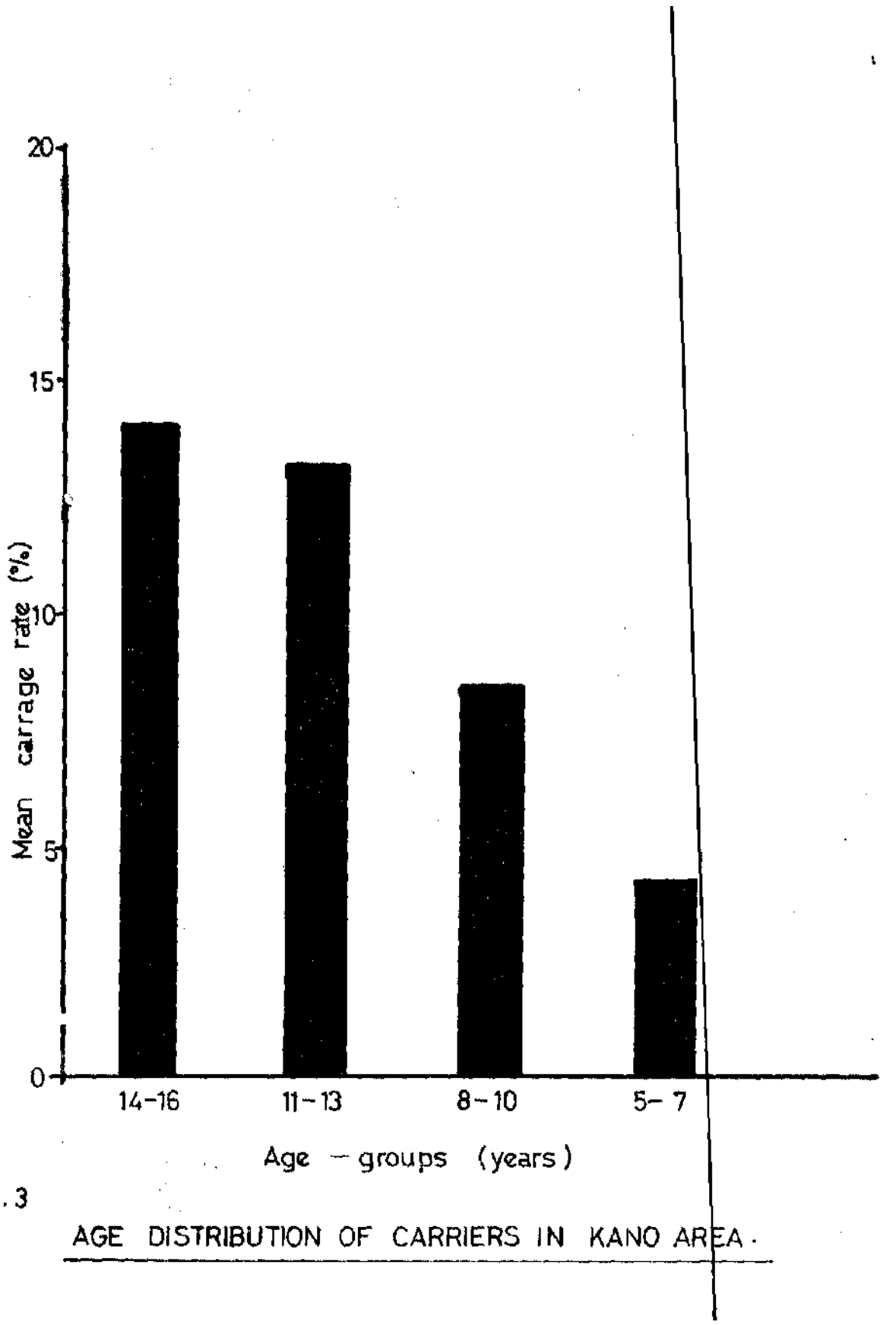
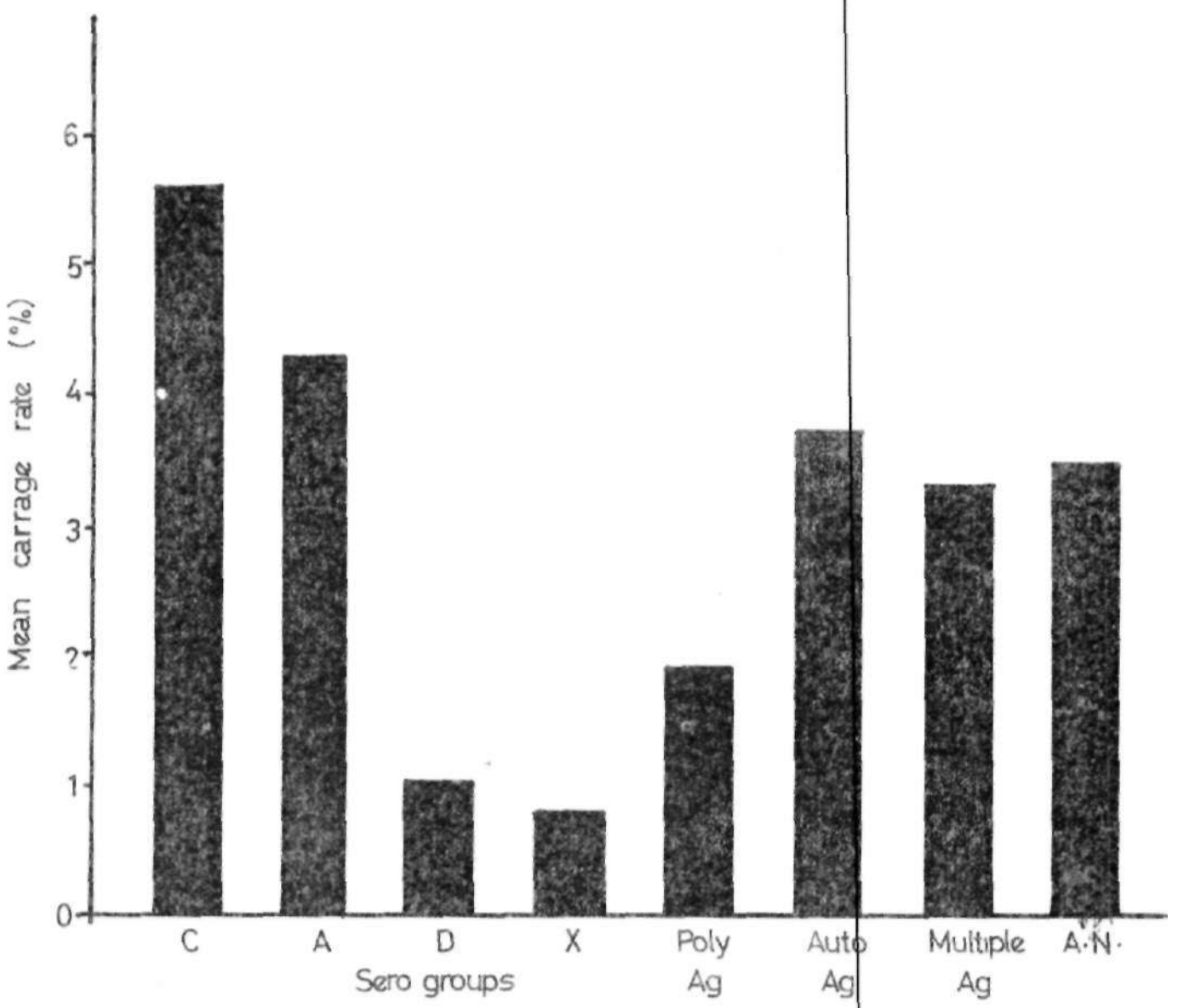


Fig. 3

AGE DISTRIBUTION OF CARRIERS IN KANO AREA.

Serogroup	No of Carriers detected in Zaria	Mean Carriage rate (%)	Standard deviation	No of Carriers detected in Shika	Mean Carriage rate (%)	Standard deviation	No of Carriers in Kano State	Mean Carriage rate (%)	Standard deviation
C	29	5.73	2.71	8	3.86	1.83	10	3.02	1.49
A	22	4.37	1.07	0	0	0	0	0	0
D	5	1.042	2.33	0	0	0	0	0	0
X	4	0.81	0.86	0	0	0	0	0	0
Poly Ag.	10	1.98	2.10	3	1.46	0.85	6	1.50	0.53
Auto Ag.	19	3.80	2.18	6	2.74	0.96	4	1.20	1.46
ultiple Ag.	17	3.396	0.93	3	1.28	1.81	2	0.57	0.50
Non - gglutinable	18	3.55	2.68	7	3.26	0.23	4	1.59	1.95

Table 6. Prevalence of serogroups in areas surveyed



Fig·4

PREVALENCE OF SEROGROUPS IN ZARIA.

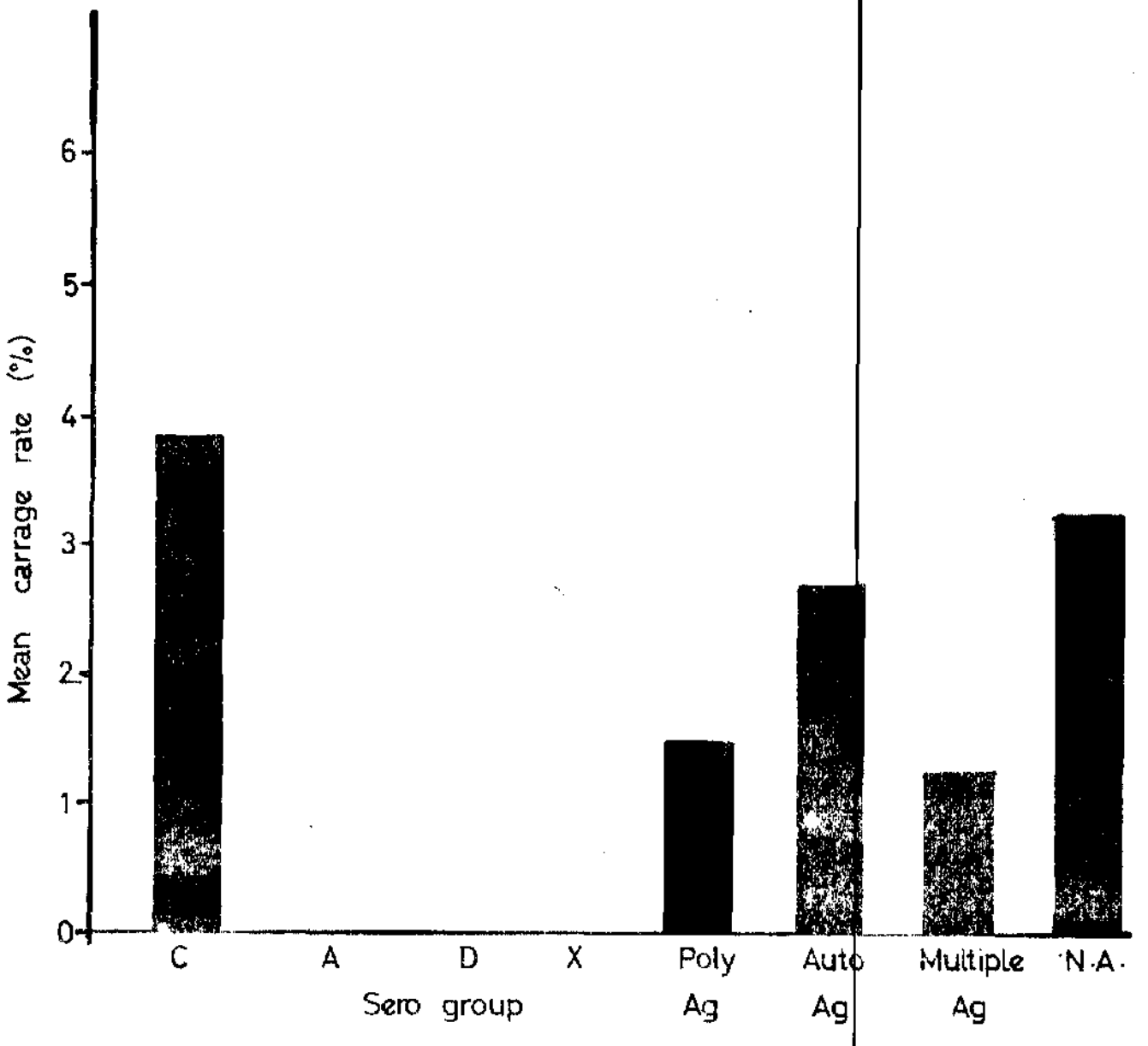


Fig. 5

PREVALENCE OF SEROGROUP IN SHIKA.

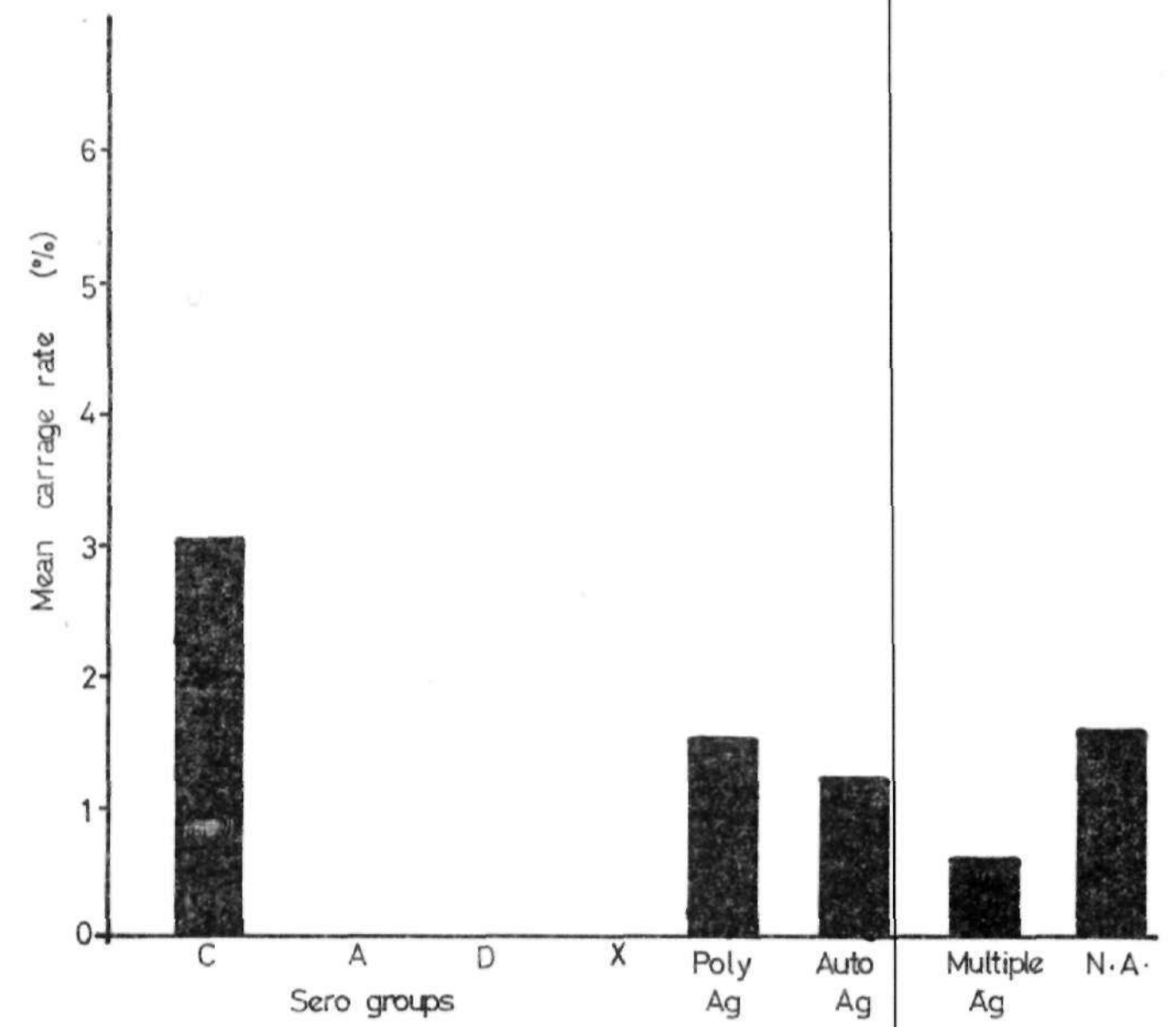


Fig. 6

PREVALENCE OF SEROGROUPS IN KANO AREA

Age-group	Mean carriage rate of serogroups (%)															
Years	C	SD	A	SD	D	SD	X	SD	Poly Ag	SD	Auto Ag	SD	Multi-ple Ag	SD	Non Aggl.	SD
5 - 7	3.42	2.45	3.20	0.84	0.47	1.04	1.092	1.52	2.29	3.82	3.16	2.21	2.57	2.63	1.68	1.56
8 - 10	4.84	2.36	4.96	1.95	0.53	1.18	1.04	1.42	2.004	2.23	4.911	2.95	4.35	1.91	4.22	4.88
11- 13	10.12	8.76	5.71	5.31	6.66	14.91	0	0	2.65	3.71	3.28	3.12	2.24	3.16	3.69	3.64
14- 16	19.45	4.81	0	0	0	0	0	0	0	0	0	0	5.56	9.62	9.72	8.67

Table 7. Age - group distribution of carriage of each serogroup in Zaria

Age-group (Years)	Mean Carriage Rate of Serogroups (%)															
	C	SD	A	SD	D	SD	X	SD	Poly Ag	SD	Auto Ag	SD	Multiple Ag	SD	Non Aggl.	SD
5 - 7	5.24	2.69	0	0	0	0	0	0	0	0	1.67	2.36	0	0	0	0
8 - 10	1.67	2.36	0	0	0	0	0	0	3.85	0.71	0	0	0	0	7.69	1.44
11 - 13	1.79	2.52	0	0	0	0	0	0	1.79	2.53	5.49	2.72	3.71	5.24	1.79	2.53
14 - 16	7.22	5.5	0	0	0	0	0	0	0	0	4.45	1.58	1.67	2.35	3.34	4.72

Table 8. Age - group distribution of carriage of each serogroup in Shika

Age-group (Years)	Mean carriage rates of serogroup (%)															
	C	SD	A	SD	D	SD	X	SD	Poly-Ag	SD	Auto-Ag	SD	Multiple Ag	SD	Non-Agg.	SD
5 - 7	2.00	2.00	0	0	0	0	0	0	0.67	1.15	0	0	1.33	2.31	0.33	0.58
8 - 10	3.06	1.61	0	0	0	0	0	0	1.67	0.39	2.57	2.26	0.70	0.97	0.70	0.97
11 - 13	6.67	-	0	0	0	0	0	0	0	0	0	0	0	0	6.67	-
14 - 16	7.14	-	0	0	0	0	0	0	0	0	0	0	0	0	7.14	-

Table 9. Age - group distribution of carriage of each serogroup in Kano.

Age-group at immunization	No of Sera Tested	Mean log_2 Post-Immunization titre (+ or - Standard deviation)	Percentage with log_2 titre of 4 or more	Per-cent-age with log_2 titre of 6 or more
3 - 6 Years	47	5.70 (± 1.09)	96	66
7 - 13 Years	22	7.86 (± 0.94)	100	96
Control groups Age 3 - 6 Years	53	4.09 (± 0.89)	73.58	0.00

Table 10. Persistence of Antibodies in Children aged 3 - 13 Years at Immunization - 3 years after immunization

Log 2 titre of antibody in serum	Frequency
2	4
3	10
4	16
5	23
Mean = 4.09	

Distribution of bactericidal antibody titre in non-immunized children aged 3- 6 years old

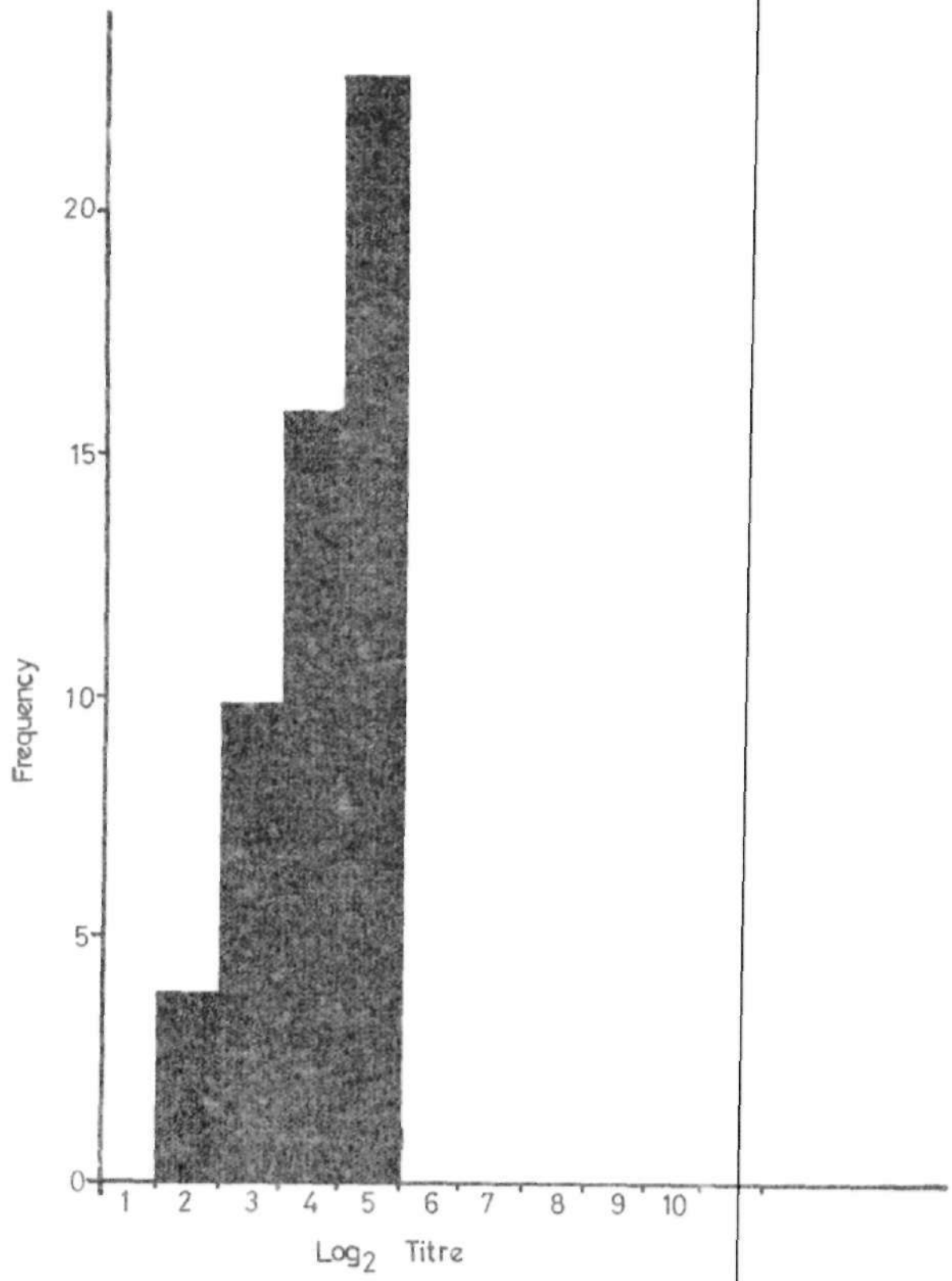


Fig. 7 DISTRIBUTION OF BACTERICIDAL ANTIBODY TITRES OF SERA FROM NON-IMMUNIZED SCHOOL CHILDREN AGED 3 – 6 YEARS.

Log 2 titre of antibody in serum	Frequency
3	2
4	7
5	7
6	19
7	11
8	1
Mean = 5.70	

Distribution of bactericidal antibody titre in children aged 3 - 6 years old immunized three years ago

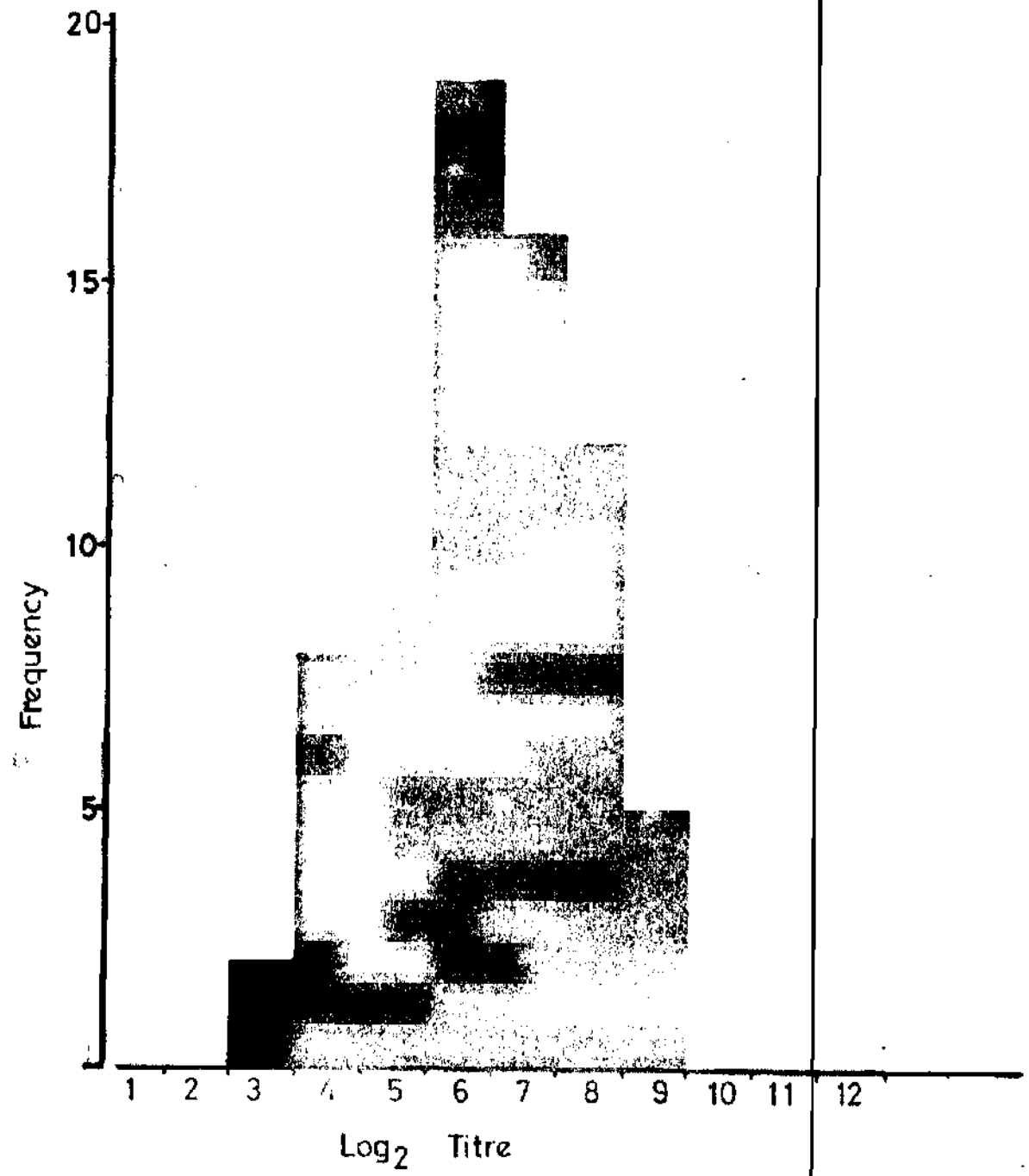


Fig. 8 DISTRIBUTION OF BACTERICIDAL ANTIBODY TITRE OF SERA FROM PUPILS AGED 3 – 6 YEARS IMMUNIZED 3 YEARS AGO.

Log_2 titre of antibody in serum	Frequency
5	1
7	5
8	11
9	5
Mean = 7.86	

Distribution of bactericidal antibody titre in Children aged 7 - 13 years old immunized three years ago.

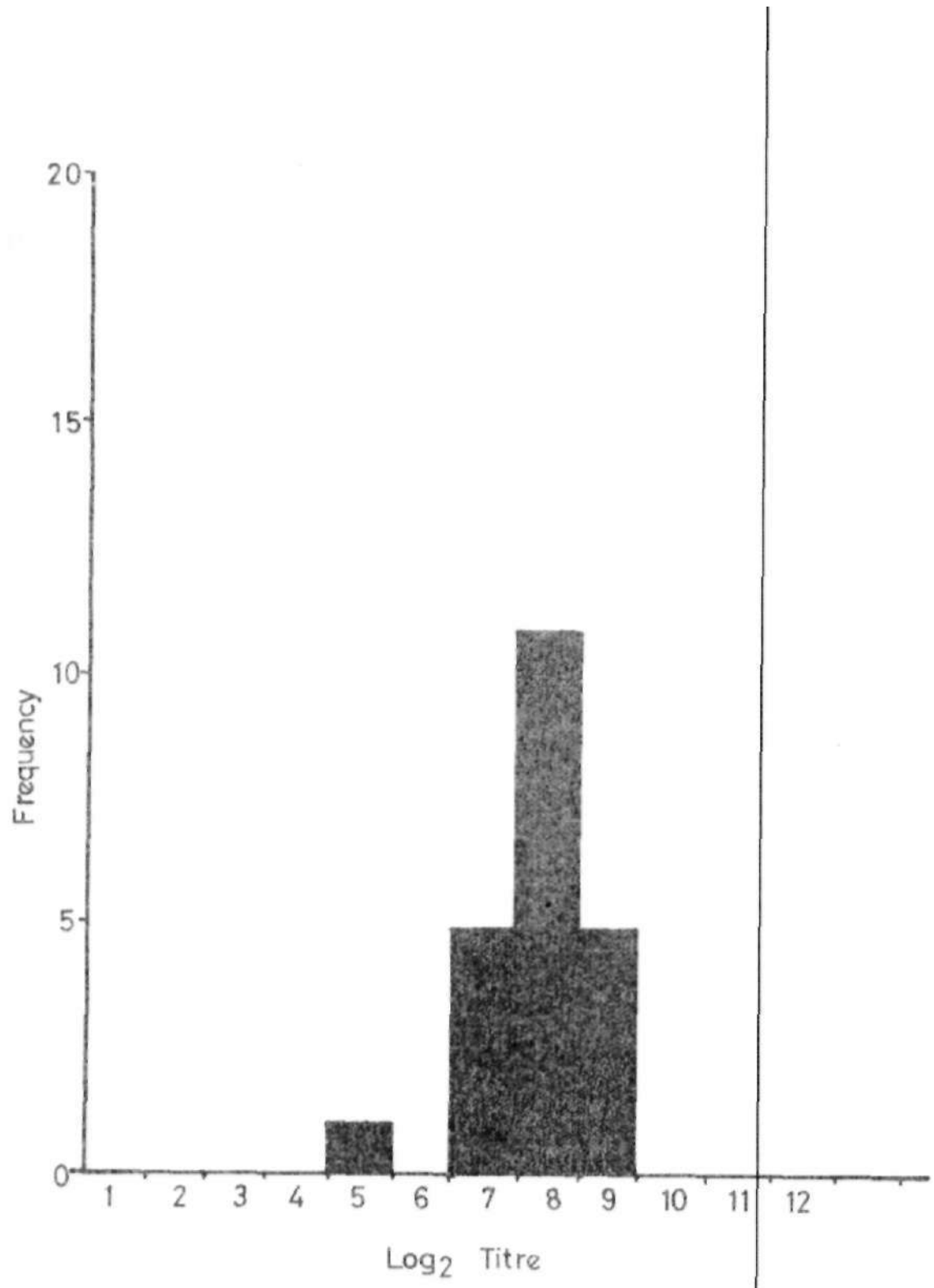


Fig.9 DISTRIBUTION OF BACTERICIDAL ANTI-BODY TITRE FROM SERA FROM PUPILS AGED 7 – 13 YEARS OLD IMMUNIZED 3 YEARS AGO.

RESULTS

The overall carriage rate of N. meningitidis in different locations in parts of Northern Nigeria is indicated in Table 1. In Zaria, carriage rate averaged 24.68% with a standard deviation of 1.33. In Shika, the average was 12.60% with a standard deviation of 0.31, while that of Kano averaged 7.88% with a standard deviation of 4.62. Carriage rate in Zaria is significantly higher than that in Shika (X^2= 13.17 P > 0.01) 16.71) and Kano (X^2 = 38.55 P >0.01) but there is no significant difference between carriage rate in Shika and Kano. Table 16 shows the carriage rate of N. meningitidis in immunized and non-immunized children in parts of Kano State. A X^2- test (X^2 = 3.86 P>0.05) shows that that carriage rate in non-immunized children is higher than in immunized children.

Table 2 shows the sex distribution of carriage rates in the parts of Northern Nigeria surveyed. A t-test shows no significant difference, between sexes at all age-groups, in carriage rates of N. meningitidis.

Tables 3 to 5 and figures 1 to 3 show the age distribution of carriage rates in the locations surveyed. Meningococcal carriage rates increased progressively with age in Zaria (17.67% SD = 5.0, 26.39 with SD = 6.80, 31.70% with SD = 7.34 and 36.67% with SD = 10.79 at 5 - 7, 8 - 10, 11 - 13 and 14 - 16 years of age respectively), Shika (6.91% with SD = 0.34, 13.19% with SD = 0.23, 14.56% with SD = 0.38 and 16.67 with SD = 0, at 5 - 7, 8 - 10, 11 - 13 and 14 - 16 years of age respectively) and Kano (4.33% with SD = 4.04, 8.62% with SD = 5.26, 13.33% and 14.28% at 5 - 7, 8 - 10, 11 - 13 and 14 - 16 years of age respectively. Carriage in children 11 years and older was significantly more common than in five

to ten year old children in all three locations surveyed (X^2 = 4.29, 4.9 and = 3.89 $P > 0.05$) in Zaria, Shika and Kano respectively).

Table 6 shows the prevalence of each serogroup of N. meningitidis in the three locations surveyed.
In Zaria, serogroups C and A ranked higher than serogroups D (X^2 = 16.10 and 9.74 $P > 0.01$ respectively) and X (X^2 = 18.05 and 11.41 $P > 0.01$ respectively). But there is no significant difference in carriage between A and C. There is also no significant difference between these two serogroups and all the categories of non-groupable meningococcai encountered in this survey (i.e. poly-agglutinable, auto agglutinable, multiple-agglutinable and non-agglutinable strains). In Kano, and Shika C was the only serogroup encountered. Serogroups A, D, X, and W135 were not isolated. There is no significant difference between serogroup C and all categories of non-groupable meningococci encountered in this survey. Non-groupable meningococci strains accounted for 51.2% in Zaria, 69.2% in Kano and 70.3% in Shika, of the strains isolated.

Tables 7 - 9 show the age-group distribution of carriage of each serogroup in the respective areas surveyed.
No significant difference was observed in the carriage between serogroups at all age - groups except that C and A tend to be higher in all age groups.

The mean $\log_2$ group A meningococcal bactericidal antibody titre found in the sera of healthy Nigeria children aged 3 - 6 years, 3 years after vaccination with 50 rg of group A and 50 rg of group C meningococcal polysaccharide vaccines was 5.70 (SD = 1.09) while that of age-matched controls (not vaccinated) was 4.09 (SD = 0.89). Vaccinated children aged 7 - 13 years old had a mean $\log_2$ bactericidal titre of 7.86 (SD = 0.94). These are all indicated in table 10 and figures 7 - 9 show the distribution of the bactericidal antibody titres in the sera sampled. A t-test shows a significant difference between the mean $\log_2$ bactericidal antibody titres recorded for the age groups indicated. The mean $\log_2$ group A meningococcal antibody titre of vaccinated children aged 3 - 9 was still significantly higher than that of age-matched controls 3 years after vaccination (t_{98} (<0.05) = 8.01 $P > 0.01$). Of the 53 control children sampled five were carriers but none carried group A. Sixty-nine vaccinated pupils were also sampled and five of them were also carriers. None, however, carried group A, but two were carriers of group C. All five carriers here have low-titre antibody (1.32 or less).

DISCUSSION

The average carriage rate of N. meningitidis in school children whose ages range from 5-16 years in Zaria was found to be 24.68% (SD = 1.33). In Shika it was 12.60% (SD = 0.31) while that in Kano area was found to be 7.88% (SD = 4.62). Previous reports on carriage rates both in Nigeria and elsewhere have been rather conflicting. However, the values obtained in all the locations surveyed fall within the ranges previously reported by some workers. Fraser and co-workers (1973), reported a carriage rate of 2% in the United States in civilian population while Altmann et al (1973) reported 60% in closed populations such as military establishments and schools. Gold et al, (1978), reported a carriage rate of 5.4% in children aged 14-17 years old in Danbury, Conn. U.S.A., Njoku-Obi & Agbo (1976) recorded an overall carriage rate of 10% in parts of Eastern Nigeria.
The higher carriage rates in children recorded in this survey is expected, as factors associated with poverty (like over crowding, filth, malnutrition etc) which increases the risk of contracting the infection, whether symptomatic or asymptomatic, (Fraser et al, 1973; Herman and Keynan, 1980) are more with us, here in Africa than in the developed parts of the world where some of the studies reported here were undertaken.
Our values, however, fall within the range previously reported by some workers in Nigeria. for example,

Sanborn et al,(1972), in Northern Nigeria, reported a carriage rate of 34% during the middle of the dry season and 7% at the end of the dry season.

Moreover, differences in epidemiological factors (such as endemicity of the disease in this part of Africa) probably contributed to the higher carriage rates recorded in this survey. The mean carriage rate of group A obtained in this age range was 4,37% (SD = 1.07) in Zaria. This value is fairly close to the value reported by Hassan King et al (1979) who recorded a value of 3% in Zaria, Northern Nigeria. However, this latter study included data from adults as well as children. This probably contributed to the slight difference.

Overall carriage rate is higher in Zaria than in the other two locations. This is probably due to the fact that most of the pupils sampled in Kano area have been previously immunized against groups A and C meningococci. Carriage rate in Kano area ranked lowest in all three locations, however a t-test shows no significant difference in carriage rates between Shika and Kano. Gotschlich and co-workers (1969) asserted that systemic immunization with group C polysaccharide gives rise to increased local defenses in the nasopharynx against meningococci as evidenced by the fact that a significantly lower percentage of vaccinated recruits became carriers of group C meningococci when compared to the control group. The same should also apply to immunization with group A polysaccharide vaccines, as the level of antibody

induced by group A immunization have been shown to be higher and persists longer than that induced by group C vaccine (Lepow et al, 1977, Greenwood et al, 1980).

There is no history of a previous immunization of the children sampled in Zaria and Shika. Sleeping proximity (Herman and Keynan, 1980) and the poorer living conditions of those pupils sampled in Zaria compared to those sampled in Shika and Kano areas, probably also contributed to the higher carriage rate observed in Zaria as the former group of pupils are boarders who live and sleep in overcrowded rooms and earn their living from begging for alms compared to the others who are cared for by their parents.

Carriage rate of serogroup C ranked highest in all locations. This was closely followed by serogroup A in Zaria, though A was not isolated in Shika and parts of Kano State. This is probably due to the fact that most of the epidemics that have been recorded in this area are due to these two serogroups. An epidemic due to serogroup C meningococci was recorded in Northern Nigeria in 1975 by Whittle et al and one of the most recent recorded epidemic witnessed in this area was due to group A organisms (Greenwood et al,1979). One of the ways of acquiring the carriage status, as earlier explained is through exposure to meningococci whether virulent or avirulent, though the factors that govern susceptibility to symptomatic infection is unknown.

There was no significant difference in meningococcal carriage rates between sexes in all age-

groups. Reports on influence of sex on carriage rate are also conflicting. However, this report is in agreement with that of Gold et al (1978), but Greenfield et al, 1971, reported that adult males were the most frequent carriers while Hassan-King et al,(1979), reported that older women were more often carriers than men. No literature to date to our knowledge has, however, shown any link between carriage status and the sex chromosomes or hormones.

Furthermore, our observation suggests a significant increase, with age group in carriage rate of meningococci in Zaria, Shika and Kano. These results are in agreement with those of other workers viz Gold et al, (1978), Hassan-King et al, (1979), Njoku-Obi et al, (1976). The factors responsible for the age-related variations in carriage rates of N. meningitidis are still unknown.

Increased opportunity for exposure to other carriers might help explain the increased prevalence of N. meningitidis with age. Gold et al (1978).

Previous reports of other workers have explained the occurence of the various categories of non-groupable meningococci isolates similar to those obtained in this survey. They accounted for 51.2% of those in Zaria 69% in Kano and 70.3% of those obtained in Shika. These are also fairly near values reported by other workers in this regard. Sippel and Girgis (1978) recorded about 51.3% of non-groupable meningococci from carriers. Gold et al (1978) also recorded about 59.6% of non-groupable meningococci from carrier surveys in children aged 3 months to 17 years old.

Goldschneider et al, (1969), regarded the non-groupable strains as avirulent since none has ever been isolated from patients. Sippel and Girgis, (1978), explained that the polyagglutinable and multiple agglutinable strains had more than one group-specific antigen as shown by counter immuno-electrophoresis of culture filterates. They also suggested that the non-typable strains i.e. those that were not agglutinated by any of the antisera were rough strains (i.e. lacking capsules) of one or more of the capsulated strains.

The two major problems encountered in slide agglutination, i.e. non-specific cross-reactivity and spontaneously agglutinable strains (Craven et al, 1978) were also encountered in this survey. The latter of these two problems was found to be reduced by growing the organisms overnight in tryptic soy broth and then using the broth cultures for the agglutination reactions.

The problems encountered with the sugar fermentation tests include decreased ability to degrade glucose and/or maltose by meningococci, contamination with other bacteria which degrade glucose and poor growth, or even death of the organisms under examination. As a result, all the isolates were, irrespective of the sugar fermentation reactions, serogrouped to circumvent the experimental error that would have resulted from wrong judgement based on these reactions.

The duration of post-immunization immunity is not known with certainty though available data for group A vaccine suggest a period of 1 - 3 years protection, while protection from Group C vaccine last for about a year, (Lepow et al, 1977; Galazka, 1982). Greenwood et al (1980) found low levels of antibody to group A and C meningococcal polysaccharides 2 years after immunization using the hemagglutination technique but found very high levels in those same sera, using the radioimmunoassay method in African villagers.

In the present study, the average bactericidal antibody level in immunized children was about two times higher than the bactericidal antibody level in the non- immunized control group (see Table No. 10). Consistently 100% of immunized children in the age group 7-13 years had such high levels of bactericidal antibodies, while 96% of immunized children between the ages of 3-6 years had comparably similar high levels of bactericidal antibodies in contrast with similar non-

immunized age range (3-6 Years), 57% of whom had levels of bactericidal antibodies about 2 folds lower than the mean in immunized groups. In the control group, about 26% of the number investigated had no traceable amount of bactericidal antibodies. This findings compares favourably with the results of Greenwood et al, (1980) in their radioimmunoassay studies.

The proportion of persons in any population with antibody levels above a certain "Protective" level may be a better reflection of immunity from infection than the mean antibody level (Kaythy et al, 1980). An antibody concentration of 2rg/ml, (Lepow et al, 1977) or a bactericidal $\log_2$ titre of 4 (Goldschneider et al, 1969) which is the mean antibody level in adult population, has been associated with protection against meningococcal disease (Goldschneider et al I, 1969). Ambrosch et al (1977), however recorded a mean $\log_2$ bactericidal titre of 6.2 in non-immunized adults. The difference in the findings of these two groups of workers is two doubling dilutions but the reasons for the difference is not apparent since both groups of workers used the same complement-dependent bactericidal antibody assay method. If we take the lower $\log_2$ bactericidal antibody titre of 4 obtained by Goldschneider et al (1969) as our standard, about 96% of immunized children aged 3-6 years had a protective anti-A meningococcal antibody titre three

years after vaccination while all the children aged 7 - 13 years still had protective levels of antimeningococcal antibody. The values are fairly close to earlier values reported by Kaythy et al (1980) and Lepow et al, (1977). If we use the mean antibody titre of 1:64 ($\log_2$ of value = 6.2) in adults obtained by Ambrosch et al, (1978), as our standard, none of the non-immunized children aged 3 - 6 years had this level of bactericidal antibody while 66% of the immunized children in this same age group still had this level of bactericidal antibody three years after immunization. About 95% of children aged 7 - 13 years still had this protective titre of antibody.

The results, here, however show a higher percentage in non-immunized children compared to the findings of the two groups of workers referred to above. This probably results from the fact that most of the children in Africa are more exposed to meningococci and other bacteria that cross-react with certain groups of meningococci than children in developed parts of the world.

It is important to mention here that whereas, neither hemagglutination technique nor radioimmunoassay technique could be assumed to measure bactericidal antibodies in the system, our technique which measured specifically bactericidal antibody activity of the sera is assumed to be a more reliable index of the state of meningococcal immunity amongst the children investigated. The results indicate that the persistence of antibodies to meningococci depend on age as the mean bactericidal antibody to group A meningococci was higher in children aged 7 - 13 years old at vaccination than in those aged 3 - 6 years old (t 67 (0.05) = 7.83).

Our findings reveal that most carriers have low antibody titre (i.e. 1.32 or less), and none of them carried group A meningococci. This observation is compatible with the findings of Goldschneider et al, (1969), who found that vaccination reduces the carriage rate of Neisseria meningitidis. It further implied that mass immunization campaign with divalent groups A anc C vaccine appear to eradicate group A carriage in school children, while reducing considerably the rate of serogroup C carriage in similar age range.

Goldschneider et al,(II 1969) and Reller et al, (1973) had also, earlier reported that, in addition to forming bactericidal antibodies to their own strains, carriers of Neisseria meningitidis also produce cross-reacting antibodies to heterologous strains of Pathogenic meningococci. These workers asserted that the development of bactericidal antibodies to serogroups of Neisseria meningitidis other than that of the homologous carrier strain indicates that other antigens, in addition to group specific polysaccharides antigens, are involved in immune sensitisation to meningococci during the carrier State. The results of our investigation agrees with the findings of Goldschneider et al, (II, 1969), and further reveal the complete absence of Neisseria meningitidis groups A, D and X in some areas where vaccination were carried out with divalent meningococcal groups A and C polysaccharide vaccine.

CONCLUSIONS

The results from this survey indicate a fairly high carriage rate of Neisseria meningitidis in the school - age children surveyed, particularly those in Islamic Schools, who sleep in overcrowded rooms and live under poor sanitary conditions. Efforts should be made to vaccinate these latter group of children.

Mass vaccination campaigns against meningococcal meningitis has been in progress in parts of Northern Nigeria since 1977 and the results so far have been encouraging. There has not been reports of any epidemic in the last three years. However, there are still some issues that need to be resolved before rational plans can be made for mass immunization programmes particularly in the African meningitis belt. A more detailed information on the immune response of people living in this part of the world is required. One way of evaluating the clinical efficacy of meningococcal vaccines is to monitor at varying periods the post-vaccination antibody levels in vaccinated individuals. The results of this survey indicate that group A antibody could persist for three years and even longer in children aged 3 - 10 years old at vaccination. In addition group A carriage seem to have disappeared in the vaccinated children, this suggests perhaps some relationship between immunization and carrier rate.

At present, there is also the problem of who, exactly, should be given meningococcal vaccines and whether booster does are necessary for certain age - groups or not. There is also the problem of the changing pattern of the serogroups responsible for epidemics. A polysaccharide vaccine containing the polysaccharide antigens of all the disease-causing serogroups, may therefore be necessary for complete eradication of meningococcal disease.

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APPENDIX 1



THE AFRICAN MENINGITIS BELT. (From I Mohammed, 1982)

288696

APPENDIX 2:

REACTIONS OF MENINGOCOCCAL ISOLATES

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
8	-	-	-	-	+	+	+	+	+	+	Nqg	-	D	D
35	-	-	-	-	+	+	+	+	+	+	+	+	C	C
36	-	-	-	-	+	+	+	+	+	+	-	-	D	D
37	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
39	-	-	-	-	+	+	+	+	+	+	-	-	X	X
42	-	-	-	-	+	+	+	+	+	+	-	-	A	A
47	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
52	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
58	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
66	-	-	-	-	+	+	+	+	+	+	-	-	D	D
67	-	-	-	+	+	+	+		+		-		D	
69	-	-	-	-	+	+	+	+	+	+	+	+	$C^{++}A^{++}C^{++}A^{+++}$	
71	-	-	-	-	+	+	+	+	+	+	+	+	A	A
73	-	-	-	-	+	+	+	+	+	+	-	-	X	X
78	-	-	-	-	+	+	+	+	+	+	-	-	A	A
80	-	-	-	-	+	+	+	+	+	+	+	+	$C^{+}A^{+}$	$C^{+}A^{+}$
81	-	-	-	-	+	+	+	+	+	+	-	-	D	D
86	-	-	-	-	+	+	+	+	+	+	-	-	A	A
88	-	-	-	-	+	+	+	+	+	+	-	+	$C^{+}A^{+}B^{+}$	$C^{+}A^{+}B^{+}$
89	-	-	-	-	+	+	+	+	-	-	-	-	NA	NA
65	-	-	-	-	+	+	-	-	-	-	-	-	$C^{+}B^{+}$	$C^{+}B^{+}$
96	-	-	-	-	+	+	+	+	+	+	-	-	$B^{+}A^{+}$	$B^{+}A^{+}$

TABLE 1: Reactions of Isolates from Islamic School Tudunwada, Zaria.

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
13	-	-	-	-	+	+	+	+	+	+	-	-	A	A
22	-	-	-	-	+	+	-	-	s+	s+	-	-	NA	NA
23	-	-	-	-	+	+	+	+	+	+	s+	s+	C	C
30	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
32	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
35	-	-	-	-	+	+	+	+	+	+	-	-	C^+A^+	C^+A^+
36	-	-	-	-	+	+	+	+	+	+	-	-	C	C
39	-	-	-	-	+	+	-	-	+	+	-	-	NA	NA
41	-	-	-	-	+	+	s+	s+	s+	s+	-	-	NA	NA
42	-	-	-	-	+	+	+	+	+	+	-	-	A	A
44	-	-	-	-	+	+	s+	s+	+	+	-	-	NA	NA
45	-	-	-	-	+	+	-	-	+	+	-	-	A	A
51	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
52	-	-	-	-	+	+	+	+	+	+	-	-	A	A
53	-	-	-	-	+	+	+	+	+	+	-	-	C	C
58	-	-	-	-	+	+	+	+	+	+	-	-	C	C
60	-	-	-	-	+	+	+	+	+	+	-	-	X	X
61	-	-	-	-	+	+	s+	s+	+	-	-	-	A^+C^+	A^+C^+
63	-	-	-	-	+	+	+	+	+	+	-	-	$A^+B^+C^+$	$A^+B^+C^+$
66	-	-	-	-	+	+	s+	s+	-	-	-	-	C	C
77	-	-	-	-	+	+	+	+	+	+	-	-	C	C
84	-	-	-	-	+	+	-	+	+	+	-	-	C	C
93	-	-	-	-	+	+	+	+	+	+	+	+	$A^{++}B^{++}$	A^+B^{++}
94	-	-	-	-	+	+	+	+	+	+	-	-	C	C
96	-	-	-	-	+	+	+	+	+	+	+	+	NA	NA

TABLE 2: Reactions of Isolates From Islamic School 1. Gyellesu, Zaria.

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
1	-	-	-	-	+	+	+	+	+	+	-	-	A^+C^+	A^+C^+
4	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
6	-	-	-	-	+	+	s+	s+	s+	s+	-	-	Ag	Ag
14	-	-	-	-	+	+	-	+	+	+	-	-	PAg	PAg
20	-	-	-	-	+	+	s+	s+	No growth		No growth		A	A
21	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
25	-	-	-	-	+	+	+	+	-	-	-	-	X	X
29	-	-	-	-	+	+	+	+	-	-	-	-	NA	NA
30	-	-	-	-	+	+	+	+	+	+	-	-	C	C
39	-	-	-	-	+	+	+	+	+	+	-	-	C	C
40	-	-	-	-	+	+	+	+	-	-	-	-	A	A
43	-	-	-	-	+	+	+	+	+	+	-	-	A	A
45	-	-	-	-	+	+	-	-	-	-	-	-	A	A
50	-	-	-	-	+	+	s+	s+	s+	s+	-	-	A	A
57	-	-	-	-	+	+	+	+	-	-	-	-	NA	NA
61	-	-	-	-	+	+	No growth		No growth		No growth		A	A
63	-	-	-	-	+	+	-	-	-	-	-	--	NA	NA
65	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
66	-	-	-	-	+	+	-	-	-	-	-	-	A	A
77	-	-	-	-	+	+	+	+	-	-	-	-	A^+C^+	A^+C^+
80	-	-	-	-	+	+	+	+	+	+	-	-	C	C
83	-	-	-	-	+	+	+	+	+	+	-	-	C	C
86	-	-	-	-	+	+	+	+	-	-	-	-	AAg	AAg
90	-	-	-	-	+	+	No growth		No growth		No growth		AAg	AAg
93	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
95	-	-	-	-	+	+	+	+	+	+	-	-	C	C
98	-	-	-	-	+	+	+	-	+	+	-	-	A^+C^+	A^+C^+

TABLE 3: Reactions of Isolates From Islamic School 2, Gyellesu, Zaria.

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
3	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
4	-	-	-	-	+	+	+	+	+	+	-	-	C	C
6	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
7	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
10	-	-	-	-	+	+	s+	s+	+	+	-	-	A	A
15	-	-	-	-	+	+	+	+	+	+	-	-	C	C
16	-	-	-	-	+	+	-	-	-	-	-	-	C	C
29	-	-	-	-	+	+	+	+	+	+	-	-	C	C
35	-	-	-	-	+	+	+	+	+	+	-	-	C	C
37	-	-	-	-	+	+	+	+	+	+	-	-	C	C
38	-	-	-	-	+	+	+	+	+	+	-	-	A	A
42	-	-	-	-	+	+	-	-	+	+	-	-	PAg	PAg
50	-	-	-	-	+	+	+	+	-	-	-	-	AAg	AAg
54	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
55	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
59	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
63	-	-	-	-	+	+	No growth		No growth		No growth		$A^{+}C^{++}$	$A^{+}C^{+}$
66	-	-	-	-	+	+	+	+	+	-	-	-	NA	NA
67	-	-	-	-	+	+	+	+	+	+	-	-	A	A
70	-	-	-	-	+	+	+	+	+	+	-	-	AAg	AAg
71	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
77	-	-	-	-	+	+	+	+	-	-	-	-	C	C
80	-	-	-	-	+	+	+	+	+	-	-	-	C	C
86	-	-	-	-	+	+	No growth		No growth		No growth		A	A
94	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
95	-	-	-	-	+	+	No growth		+	+	-	-	$C^{++}A^{++}$	$C^{++}A^{+}$

TABLE 4: Reactions of Isolates From Islamic School Kongo, Zaria

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
4	-	--	-	-	+	+	+	+	+	+	-	-	[illegible]	[illegible]
7	-	-	-	-	+	+	+	+	+	+	+	+	NA	NA
9	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
10	-	-	-	-	+	+	+	+	+	+	+	-	C	C
18	-	-	-	-	+	+	+	+	+	+	-	-	C	C
25	-	-	-	-	+	+	+	+	+	+	-	-	A	A
26	-	-	-	-	+	+	+	+	+	+	-	-	C	C
28	-	-	-	-	+	+	-	-	+	+	-	-	C	C
29	-	-	-	-	+	+	+	+	+	+	-	-	C	C
31	-	-	-	-	+	+	+	+	+	+	-	-	C	C
35	-	-	-	-	+	+	-	+	+	+	-	-	[illegible]	[illegible]
46	-	-	-	-	+	+	-	-	-	-	-	-	[illegible]	[illegible]
53	--	-	-	-	+	+	+	+	+	+	-	-	C	C
57	-	-	-	-	+	+	-	-	+	+	-	-	[illegible]	[illegible]
59	-	-	-	-	+	+	+	+	+	+	-	-	A	A
72	-	-	-	-	+	+	+	+	+	+	-	-	[illegible]	[illegible]
74	-	-	-	-	+	+	+	+	+	+	-	-	A	A
79	-	-	-	-	+	+	+	-	+	+	-	-	[illegible]	[illegible]
80	-	-	-	-	+	+	+	+	+	+	-	-	[illegible]	[illegible]
88	-	-	-	-	+	+	s+	s+	+	+	-	-	[illegible]	[illegible]
91	-	-	-	-	+	+	+	+	+	+	-	-	[illegible]	[illegible]
92	-	-	-	-	+	+	-	-	+	+	-	-	A^+B^+	A^+B^+
99	-	-	-	-	+	+	+	+	+	+	-	-	$A^+B^+C^+$	$A^+B^+C^+$
100	-	-	-	-	+	+	+	+	+	+	-	-	$A^*B^+C^+$	$A^+B^+C^+$

TABLE 5: Reactions of Isolates From Islamic School, Kongo, Zaria

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
9	-	-	-	-	+	+	+	+	+	+	-	-	C	C
14	-	-	-	-	+	+	-	+	-	+	-	-	C	C
17	-	-	-	-	+	+	+	+	+	+	-	-	*Ung*	*Ung*
18	-	-	-	-	+	+	No growth		No growth		-	-	NA	NA
21	-	-	-	-	+	+	+	+	+	+	-	-	B^+C^+	B^+C^+
22	-	-	-	-	+	+	+	+	+	+	-	-	C	C
25	-	-	-	-	+	+	+	+	+	+	-	-	A^+B^+	A^+B^+
55	-	-	-	-	+	+	+	+	+	+	-	-	*Ung*	*Ung*
58	-	-	-	-	+	+	-	-	+	+	-	-	NA	NA
81	-	-	-	-	+	+	+	-	+	+	-	-	NA	NA
100	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
106	-	-	-	-	+	+	+	+	+	+	-	-	P*Ung*	P*Ung*
107	-	-	-	-	+	+	+	+	+	+	-	-	*Ung*	*Ung*
111	-	-	-	-	+	+	+	+	No growth		No growth		*Ung*	*Ung*
114	-	-	-	-	+	+	+	+	+	+	+	+	B^+C^+	B^+C^+

TABLE 6: Reactions of Isolates From Islamic Schools 182

Shika

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
5	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
12	-	-	-	-	+	+	+	+	No growth		No growth		NA	NA
22	-	-	-	-	+	+	+	+	+	+	-	-	C	C
23a	-	-	-	-	+	+	+	+	+	+	-	-	Aag	Aag
23c	-	-	-	-	+	+	No growth		No growth		-	-	PAg	PAg
25	-	-	-	-	+	+	+	+	+	+	-	-	C	C
43	-	-	-	-	+	+	-	-	+	+	-	-	NA	NA
51	-	-	-	-	+	+	+	+	+	+	-	-	Aag	Aag
61	-	-	-	-	+	+	+	+	+	+	-	-	C	C
62	-	-	-	-	+	+	+	+	+	+	-	-	C	C
85	-	-	-	-	-	+	+	+	+	+	+	+	PAg	PAg
88	-	-	-	-	+	+	+	+	+	+	-	-	C	C

TABLE 7: Reactions of Isolates From Islamic Schools 2 & 3 Shika

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
4	-	-	-	-	+	+	-	-	+	+	-	-	PAg	PAg
14	-	-	-	-	+	+	+	+	+	+	-	-	PAg	PAg
26	-	-	-	-	+	+	No growth		+	+	-	-	PAg	PAg
37	-	-	-	-	+	+	+	+	+	+	-	-	C	C
53	-	-	-	-	+	+	+	+	+	-	-	-	C	C

TABLE 8

Reactions of Isolates From a Primary School in Dambatta, Kano State

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
4c	-	-	-	-	+	+	+	+	+	+	-	-	C	C
5c	-	-	-	-	+	+	+	+	+	+	-	-	C	C
5b	-	-	-	-	+	+	+	+	+	+	-	-	Pnc	Pnc
10a	-	-	-	-	+	+	+	+	+	+	-	-	C	C
13b	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
18c	-	-	-	-	+	+	+	+	No growth		-	-	Ang	Ang
20b	-	-	-	-	+	+	+	+	+	+	-	-	B^+C^+	B^+C^+
21a	-	-	-	-	+	+	+	-	+	+	-	-	NA	NA
27b	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA
29b	-	-	-	-	+	+	+	+	+	+	-	-	Ang	Ang
30a	-	-	-	-	+	+	+	+	+	+	-	-	C	C
30c	-	-	-	-	+	+	+	+	+	+	-	-	C	C
32b	-	-	-	-	+	+	+	+	+	+	+	+	Ang	Ang
34a	-	-	-	-	+	+	+	+	+	+	-	-	NA	NA

TABLE 9: Reactions of Isolates From Primary Schools in Barbura Gabas and in Kano State.

SERIAL NUMBER	GRAM REACTION		ONPG REACTION		OXIDASE REACTION		DEXTROSE FERMENTATION		MALTOSE FERMENTATION		SUCROSE FERMENTATION		SEROGROUP	
	a	b	a	b	a	b	a	b	a	b	a	b	a	b
5b	-	-	-	-	+	+	+	+	+	+	-	-	C^1	C
6a	-	-	-	-	+	+	-	+	+	+	-	-	C_2	C
8b	-	-	-	-	+	+	+	+	+	+	No	growth	C	C
16a	-	-	-	-	$+^1$	+	+	+	+	+	-	-	C^+D^+	C^+D^+
20b	-	-	-	-	+	+	+	+	+	+	-	-	Pnag	Pnag
31a	-	-	-	-	+	+	+	+	+	+	-	-	Pnag	Pnag
49	-	-	-	-	+	+	+	+	+	+	-	-	nag	nag

TABLE 10: Reactions of Isolates From Primary Schools in Kano State

APPENDIX 3

FORMULA FOR DULBECCO'S PHOSPHATE BUFFERED SATINE

SOLUTION A

Nacl - 8g.

Kcl - 0.2g

KH_2P_{o4} - 1.15g

Distilled water - 1,000ml.

1% aqueous phenol red - 1 or 2 drops or until solution is pale pink. Distribute in 100ml amounts and autoclave at 15ibs. presure for 20 minutes. Store at $4^{o}C$.

SOLUTION B

$Cacl_2$ - 0.2g.

$Mgcl_2.6H_2O$ - o.2g.

Distilled water - 100ml.

Dispense in 2.5ml amounts in bijoux and sterilise for 20 minutes at 15ibs. presure. Store at $4^{o}c$.

Add 0.5ml of solution B to 100ml of solution A, to make a complete solution.

1% Aqueous Phenol Red

Phenol red - 10g.

N/10 NaoH - 325ml.

Distilled water - 1000ml.

Distributed in 100ml amounts and autoclave at 15lbs.

Pressure for 15 minutes.

APPENDIX 4

FORMULA FOR STANDARD DEVIATION AND STUDENT TO TEST

1. Standard deviation $S = \sqrt{\frac{fd^2}{N-1}}$

where d = deviation from the mean.

f = frequency.

N = total number of observations.

2. $t = (m1 - m2)/S \sqrt{\frac{1}{n1} + \frac{1}{n2}}$

where m = sample mean.

n = no of observations.

s = poded deviation of the two variables.

Pooled deviation s is given by the formala.

$$s^2 = \frac{(n_1-1)\ s_1^2 + (n_2-1)\ S_2^2}{n1+n2 - 2}$$

where S_1 & s_2 represent the standard deviation of the two sets of variables.

Appendix 5 Formula for X^2 test and the statistical calculations for the analysis of results

Observed Nos	With Characteristic	Without Characteristic	Total
Sample 1	a	b	(a + b)
Sample 2	C	d	(c + d)
	a + c	b + d	N

$$\text{Test as } X^2 = \frac{N\,(ad - bc)^2}{(a+b)(a+c)(b+d)(c+d)}$$

Where there are entries of 5 or less on the 2 x 2 contingency table, then,

$$X^2 = \frac{N(Iad - bcI - \tfrac{1}{2}N)^2}{(a+b)(c+d)(a+c)(b+d)}$$

LOCATIONS

Zaria - Kano

Carriers	Non Carriers	Total
124	378	502
26	306	332
150	684	834

$$X^2 = \frac{834\,(37944 - 9828)^2}{502 \times 150 \times 684 \times 332} \qquad \frac{790509\,45 \times 10 \times 834}{65928488 \times 10^4}{17099726 \times 10^3}$$

$= 38.55$ Significant

Shika - Kano

Carriers	Non Carriers	Total
27	187	214
26	306	332
53	493	546

$$X^2 = \frac{546\,(4862 - 8262)^2}{214 \times 53 \times 493 \times 332} = \frac{63117600 \times 10^2}{18564131 \times 10^2}$$

$= 3.40$ Slightly Significant

Zaria - Shika

Carriers	Non Carriers	Total
124	378	502
27	187	214
151	565	716

$$X^2 = \frac{716\ (23188 - 10206)^2}{502 \times 151 \times 565 \times 214} =$$

$$= \frac{1206914 \times 10^4}{91652198 \times 10^2} = 13.17$$

Significant

Age Distribution of Carriers

a) Zaria

Age group (Years)	Carriers	Non-carriers	Total
5 - 10	88	302	390
11 - 16	36	76	112
	124	378	502

$$X^2 = \frac{502\ (6688 - 10872)^2}{390 \times 124 \times 378 \times 112}$$

$$= 4.39$$

Significant at 95% level

b) Shika

Age group (Years)	Carriers	Non-carriers	Total
5 - 10	11	100	111
11 - 16	16	87	103
	27	187	214

$$X^2 = \frac{214\ (765 - 1926)^2}{111 \times 27 \times 187 \times 103}$$

$$= 4.9$$

Significant at 95% level

c) Kano

Age group (Years)	Carriers	Non-carriers	Total
5 - 10	22	281	303
11 - 16	4	25	29
	26	306	332

$$X^2 = \frac{332\ (864 - 2064)^2}{303 \times 26 \times 306 \times 29}$$

$$= 3.89$$

Significant at 95% level

Prevalence of Serogroups

Zaria

C and D

Carriers	Non carriers	Total
29	473	502
5	497	502
34	970	1,004

$$X^2 = \frac{1004\ (2365 - 14413) - 502)^2}{502 \times 34 \times 970 \times 502} = \frac{13331011 \times 10 \times 1004}{83110919 \times 10^2}$$

$$= \frac{13384335 \times 10^4}{83110919 \times 10^2} = 16.10 \quad \text{Significant}$$

C and X

Carriers	Non carriers	Total
29	473	502
4	498	502
33	971	1004

$$X^2 = \frac{1004\ (1892 - 14442 - 502)^2}{502 \times 33 \times 971 \times 502} = \frac{14573491 \times 10^4}{80749641 \times 10^2}$$

$$= 18.05 \quad \text{Significant.}$$

A and D

Carriers	Non carriers	Total
22	480	502
5	497	502
27	977	1,004

$$X^2 = \frac{1004\ (10934 - 2400 - 502)^2}{502 \times 27 \times 977 \times 502} = \frac{64771076 \times 10^3}{66476135 \times 10^2}$$

= 9.74 Significant

A and X

Carriers	Non carriers	Total
22	480	502
4	498	502
26	978	1004

$$X^2 = \frac{1004\ (1920 - 10956 - 502)^2}{502 \times 26 \times 978 \times 502} = \frac{73120472 \times 10^3}{64079577 \times 10^2}$$

11.41 Significant.

Bactericidal antibody titres (t - test)

(a) Non-immunized and immunized at age 3 - 6 years

	Non-immunized	immunized
Mean	4.09	5.70
SD	0.89	1.09
n	52	46

$$t = \frac{1.61}{Sp\sqrt{0.022 + 0.019}}$$

$$Sp = \sqrt{\frac{52\,(0.89^2) + 46\,(1.09^2)}{98}}$$

$$= 0.99$$

$$t = \frac{1.61}{0.99 \times 0.203} = 8.01$$

tabulated t at $98^{\circ}F$ at 99% level of probability is 3.37 so the result is significant.

Difference in means of antibody titres between age groups in vaccinated children

	Age group 3 - 6 years	Age group 7 - 13 years
Mean	5. 7	7. 86
SD	1.09	0.94
n	47	22

$$t = \frac{2.16}{Sp\sqrt{0.022 + 0.048}}$$

$$Sp = \sqrt{\frac{46\ (1.09^2) + 21\ (0.94^2)}{67}} = 1.046$$

$$t = \frac{2.16}{1.046 \times 0.264}$$

$$= 7.83$$

tabulated t at 67 degrees of freedom and at 99% probability level = 3.46 so the difference is significant.