STUDIES ON THE EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE IN NIGERIA

BY

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A Dissertation submitted to the Postgraduate School, Ahmadu Bello University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Veterinary Microbiology.

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DECLARATION

I sincerely declare that this work was carried out by me in the Virology Division of the National Veterinary Research Institute, Vom, and The Animal Virus Research Institute, Pirbright, U.K. No part of this thesis has ever been submitted to any Institution for award of diploma or degree.

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CERTIFICATION

We the undersigned examiners hereby certify that this thesis by Adedipe Abegunde entitled "Studies on the Epidemiology of foot-and-mouth disease in Nigeria" meets the regulations governing the award of the degree of Doctor of Philosophy in Veterinary Microbiology of Ahmadu Bello University - Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this thesis to my wife and children who have had to cope with extensive absences by me from home during the course of this work

and

To my big brother Dayo Abegunde who guided and supported me through the early years of my formal education.

ABSTRACT

Foot-and-mouth disease (FMD) is endemic in Nigeria. Lossess resulting from outbreaks of the disease could not be estimated and no meaningful control measure could be embarked upon because information required for the formulation of FMD control policy is not adequate. This has been due to poor disease reporting and the nature of animal husbandry practices among the fulani pastoralis who control majority of Nigerian cattle.

This study examined historical documents and data relating to FMD in Nigeria since 1925 and conducted interviews with State and Federal Government Organs of disease control as a means of establishing what is so far known about the disease. This investigation established an apparent 0.012% incidence$_f$ 0.05% prevalence and *0.3%* mortality rates and that Type A, SAT_1 and SAT_2 viruses are still active in the field.

Serological survey was conducted using virus infection antigen (VIA) and 146S whole virion antigen in indirect ELISA to detect antibodies to FMD and thus establish the true incidence and prevalence of the disease in the field. Overall incidence of the disease among sampled population was *9.8%* while prevalence was *55%*. Incidence and prevalence varied widely from State to State.

Recent isolates of the virus from Nigeria (Type SAT_1: Nig. 4/81, Nig. 5/81, Nig. 10/81, Nig. 11/81 and Nig. 13/81) (Type SAT_2 (Nig. 6/81, Nig. 8/81 and Nig. 2/82), were compared serologically with one other and with standard vaccine strains for each type, to establish their relatedness and the ability of the vaccine strains to protect against infection caused by recent Nigerian strains.

All Type SAT_1 isolates of 1981 are closely related and could be regarded as belonging to the same subtype. The 1981 and 1982 Type SAT_2 isolates studied were also closely related.

Strain Nig. 2/82, the most recent type SAT-isolate was more closely related to Nig. 6/81 than Nig. 8/81. Cross neutralisation test in vitro showed that the standard vaccine strains could provide a measure of protection against infection caused by Nigerian strains with which they were compared.

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CHAPTER 1

GENERAL INTRODUCTION

Foot-and-mouth disease (FMD) also known as Fievré Aphteus (French), Maul und Klauenseuche (German), Fiebre Aftosa (Spanish) and Afta Epizootica (Italian) is a very important disease. It is a highly acute and contagious disease affecting all cloven footed animals (Callis, 1979).

Countries that are endemic or report the disease sporadically spend lots of resources to rid their livestock of the disease (Bachrach, 1981). Its importance stems from losses that could result from mortality, loss of milk production, loss of condition, depressed growth, abortion, subsequent infertility and cost of control or eradication (Callis, 1979). Any country that wants to partake actively and economically in international trade takes FMD seriously (Sellers, 1981).

The disease is characterised by the formation of vesicles and erosions of tongue, nose, lips and other mucous membranes of the mouth and on the skin between the hooves and around the coronary band above the hoof. Vesicles may be found on the teats of nursing sows and are frequently found on the snout and may sometimes extend into the nares in swine. In sheep,

goats and deer, foot lesions are characteristic; those in the mouth may be small and unnoticeable. In cattle, vesicles are most often seen on the feet and tongue. There is usually a drooling of ropy saliva and a rupturing of tongue vesicles leaving raw haemorrhagic eroded surfaces with ragged fragments of necrotic epithelium attached. Soreness of the mouth and tongue severely limits or prevents eating and foot lesions cause lameness. When secondary infections do not occur, the lesions tend to heal very rapidly (Bachrach, 1978).

The importance with which FMD is regarded by the Veterinary Authority of a country depends largely on the economics of livestock production to individual livestock owner and the political influence of livestock producers. It is no suprise therefore, that countries that have economically important livestock sectors yielding substantial foreign exchange from trade in beef, dairy products and other livestock products, take the control of FMD seriously.

FMD is known to be widespread in Nigeria (Anon, 1975). The distribution of the disease is not completely known (Owolodun, 1971, Asagba, 1982), because the system of disease reporting is not very efficient (Anon, 1975). This lack of sufficient information on the disease and its impact has made the formulation of control policies difficult.

The effect of FMD in Nigerian livestock has been described as mild with low mortality resulting from it (Owolodun, 1971; Smith, 1971). The low mortality has tended to make Fulani herdowners think of FMD as a transient nuisance. Losses to them are strictly based on mortalities resulting from infectious diseases. A disease like FMD that produces little or no mortality is therefore not reported and not regarded as a threat unlike Rinderpest or CBPP which is associated with devastating mortality.

It is evident from the studies of Owolodun, (1971) and Durojaiye, (1981) that what is reported is only a small fraction of total incidence of the disease and that economic losses resulting from loss of condition, retarded growth, abortion and infertility and loss of milk production could run into millions of naira (Smith, 1971; Nawathe and Goni, 1976).

The low ratio of animal protein to plant protein in total intake in the diets of Nigerians has long been recognised (Olayide et al., 1972; Oyenuga, 1974 and Ademosun, 1976). The adverse effect of this low protein intake on nutrition, growth and human productivity level has also been reported (Collins et al., 1961, Platt, 1954 and Oyenuga, 1967). Demand for beef and other livestock products has been surging upward in proportion to the growth in population and

increased affluence and economic wellbeing of the Nigerians. Nuru (1981) reported that because of the inadequacy of the traditional (extensive) system of animal production, demand for animal products outstrips supply in Nigeria. As a result Nigeria accounted for about 20 and 21% of the total Argentine beef export to African in 1976 and 1977 respectively. The huge foreign exchange involved in the importation of beef alone was ₦2.7 million in 1978.

In an attempt to increase the supply of beef and animal products from Nigerian livestock, it has been proposed (Nigerian National Development Programme, 1980-85) that production methods have to become more intensive and local breeds has to be improved with exotic high producing breeds. Supporting this proposal Nuru (1981) has proposed establishment of intensive cattle production systems. Along with improvement in livestock breeds, production methods and productivity of the animals, improved disease control measures are called for. Emphasis will continue to shift from Rinderpest and CBPP to FMD and it would grow in economic importance once these "Killer" diseases are firmly under control. The exotic breeds imported at very high costs for improvements of dairy and beef cattle would be in serious danger from FMD.

For any disease control measure to be meaningful and successful, it is important to understand; the

disease agent, its environment, its mode of transmission and its survival in nature. This is why this study on the epidemiology of foot-and-mouth disease in Northern Nigerian was undertaken.

Three major antigens are produced as a result of the replication of FMD virus in tissue culture or animals. Whole virions (146S), subunits (12S) and virus infection associated antigen (VIA) (Newman, 1979). Antibodies are generated against all the three antigens after infection; those against the structural capsid protein (146S and 12S) are type specific whereas those against VIA antigen are not. Antibodies produced against VIA antigen do not react with purified virus or subunits of it. The VIA antigen has been shown to be the virus polymerase VP 56 on polyacrylamide gel electrophoresis (Newman, 1979).

VIA antibodies produced in infected animals peaks at about 9 - 14 days post infection and then decays at the expected half life of immunoglobulins. Thus it is possible for one to detect antibodies against VIA for 90 -120 days following infection (Lobo, 1976). In this study therefore, the VIA antigen is chosen to detect the antibodies to FMD virus to determine the incidence of FMD infection.

In the same manner the 146S antigen was chosen to determine the overall prevalence of FMD infection.

Objectives

1. To investigate outbreaks of the disease, occuring during the study period and isolate the virus causing current infection in the field.
2. To determine the distribution of foot-and-mouth disease in Nigeria by determining its incidence and prevalence among Nigerian cattle.
3. To determine antigenic relationship of recent Nigerian isolates of the virus and to compare them with standard vaccine strains.

CHAPTER 2

REVIEW OF LITERATURE

History

Foot-and-Mouth disease (FMD) was first described in an outbreak in Italy in 1514 (Brooksby, 1982). Foot and Mouth disease occurs in most livestock producing areas of the world at the present time with the exception of U.S.A., Canada, Panama, Australia and New Zealand. The last outbreak of FMD in Australia was in 1872 and in North America in 1954 and it has never occured in Central America, North of the Panama - Colombia border (Callis, 1979). Ireland, Norway, Greenland, Iceland, Japan and most of the smaller Islands of Oceania and the Carribean are free of FMD. England is free of the disease for years at a time. The largest and most costly epizootic in England occured late in 1967 and early 1968. This outbreak involved more than 2,300 premises and resulted in the destruction of 430,000 animals before the disease was brought under control (Brooksby, 1967).

THE AMERICAS

The United States has experienced 9 outbreaks of FMD, the first in 1870 and the latest in 1929. The disease was eradicated in each instance by slaughter of the infected and exposed animals (Callis, 1979).

Type O, FMD virus escaped from a laboratory building at the Plum Island Animal Disease Centre in September 1978 and infected cattle in a normal cattle holding area. There was no evidence that the virus escaped to the mainland (Callis, 1979). FMD occurred in Canada in 1952 but it was eradicated within weeks. The disease occured in Mexico in 1946, and the country was not declared free of the disease until 1954, after nearly one million animals were slaughtered and approximately 60 million vaccinated (Callis, 1979).

The Type A_{24} and O, FMD virus are responsible for enzootic disease in Colombia, Venezuela, Ecuador and Peru. In Brazil, 6,061 outbreaks caused by types), A and C in 1978 were reported. The country still remains enzootic with 1,033 reported outbreaks in 1983 one third of the number of outbreaks reported in 1982 (3,014). Bolivia reported five outbreaks of type O virus and four outbreaks of Type A virus in 1978. In the last three years, outbreaks have remained at a low level. In 1983, 23 outbreaks were registered in which FMD type O, A_{24} and C_3 were diagnosed. No epizootic situation was reported in the country in 1983 (Anon, 1983).

Paraguay experienced 8 outbreaks of type O and two outbreaks of type C virus in 1978. Type C virus has not been isolated since 1979 (Anon, 1980). In 1983, 10 cases of type A and O were diagnosed. The incidence of the disease in Chile has dropped in recent years. In 1978 there were only 2 outbreaks in cattle imported for slaughter. Chile was however declared free of FMD in January 1981. The Agriculture and Livestock Raising Services (SAG) has been implementing a rigorous external disease preventive programme with special emphasis on FMD. There were 28 outbreaks in Uruquay in 1978, caused by types A, O and C. In 1983, 20 outbreaks of FMD were recorded in the country just like 1981. The disease is widespread in the oriental region of the country (Anon, 1983). The three types are still prevalent.

In Colombia in 1983, 1,120 outbreaks were recorded, a figure notably higher than the annual record for 1981 (785) and 1982 (597). More outbreaks were recorded in 1979 (1693) and in 1980 (1765). Type O and A virus were diagnosed. During 1983 and 1984 Canada, United States of America, Mexico, Panama, Guyana, Chile and the countries of the West Indies reported no FMD outbreaks. (Anon, 1979, 1981, 1984).

EUROPE

Although FMD continues to occur in Europe, the disease is at a low incidence in many European countries. It last occured in Finland in 1960, in Norway in 1952, Sweden in 1966 and Ireland in 1941. The disease has never occurred in Iceland and has been reported in the United Kingdom in 1982 (Anon, 1982a).

Foot-and-mouth disease caused by type O virus occurred in France in March 1979 and caused the government to destroy 514 pigs, 1,619 cattle, 17 sheep and 4 goats. There was an outbreak of FMD in Switzerland in 1978, the German Democratic Republic and the European part of the Soviet Union caused by type C virus. Albania has been free of the disease since 1960. Cyprus since 1964 and Malta since 1975. The incidence of FMD in Europe has been reduced by the strict annual application of well produced and tested trivalent vaccines (Anon, 1983c).

Spain, Turkey, Portugal and the European part of the USSR still remain endemic areas. Turkey reported a total of 856 outbreaks in 1980 in cattle and sheep, 836 outbreaks in 1981 also in cattle and sheep, 604 outbreaks in 1982 and 406 in 1983 in cattle, sheep and goats. Types O and A were implicated in these outbreaks. Portugal reported the outbreaks of type C virus after nearly ten years of freedom

from FMD. Type C outbreaks were reported in May 1980 as a result of illegal importation of piglets from Spain. Type C virus was reported in cattle again in 1981 with a total of 303 outbreaks. In 1983, subtype A5 were reported to have caused 164 outbreaks in cattle, sheep, goats and pigs (Anon, 1981).

In Spain, type C was reported in pigs in 1980 and type A has been reported in the years 1980 through 1983 in sheep, cattle, goats and pigs. A mass vaccination campaign with trivalent vaccine A, O and C was implemented (Anon, 1983c).

The USSR has consistently reported type O outbreaks in the past three years in Georgia, Armenia, Azerbaijan and the South Eastern provinces. In Denmark, one outbreak of type O was reported in cattle in January 1983 in the Island of Fumen. Stamping out and strict sanitary measures were carried out and the country was declared free of FMD in February 1983. In the Netherlands, Subtype O_1 was reported to have caused outbreaks of the disease in cattle in December 1983 but it was quickly stamped out and the country was declared free from FMD in March 1984 (Anon, 1984b).

ASIA

The Asian continent has remained basically an enzootic region with the exception of Japan, Korea and the Island of Taiwan which have remained free

of FMD (Anon, 1980).

Israel which had no report of FMD for a short period up to 1977 reported new outbreaks in 1978 and 1979 and type O and A were recorded in 1980. Israel however, did not report the disease between 1981 and 1983 (Anon, 1983). Type O was reported in 1985 and 1986.

In Kuwait, 454 cases were reported in 1977, 1,710 cases in 1978 and the disease outbreaks were reported in 1979 and 1980 (Anon, 1980). Type C virus was reported for the first time in Kuwait in 1982 (Anon, 1983). The disease was reported in Bahrain in 1978 and 1982 (Anon, 1983). In Iran it was enzootic, with a total of 154 outbreaks for 1978, 1979 and 1980. Types A and O were isolated (Anon, 1981).

In Saudi Arabia, 967 outbreaks were reported in 1977 and the outbreaks continued into 1980. Types A and O were prevalent. In 1983, type O_1 caused outbreaks in Saudi Arabia. In Oman, Yemen and United Arab Emirates, types A and O viruses caused outbreaks in 1977 and 1978 and continued to cause outbreaks in 1980s. Iran experienced 58 outbreaks in 1977 and 37 in 1978 caused by type O. SAT Types 2 and 3 have not been reported in Iran at all while SAT 1 virus has not been reported in Iran since 1964 (Anon, 1979). Syria reported type O outbreaks in 1977, 1978 and 1979 and types O_1 and A_{22} in 1980, 1981 and 1983 (Anon, 1983).

In India, FMD is enzootic, with an average of 6,000 outbreaks per year. Although cattle and buffaloes are the most commonly affected animals, the disease also affects sheep, goats, pigs, captive animals and wild animals. Type O, A, C and Asia 1 are prevalent in most parts of the country but type O outbreaks predominate (Anon, 1979, 1982, 1984).

Malaysia, Laos and Indonesia reported FMD due to type O in 1977 and 1978 and the disease remains enzootic in these countries. The 1980-81 outbreak in Malaysia was brought under control by vaccination, restriction of movements and other measures. FMD eradication programme in Indonesia launched after 1974 with assistance from Australia reduced the incidence of the disease from about 11,000 cases in 1974 to only one reported case in 1979 (Anon, 1979). From 1980-1982, there were no outbreaks reported. However in 1983, one outbreak was reported on the Island of Java (Anon, 1983).

In Hong Kong, FMD was first recorded in 1954/55. The disease has been reported in cattle, pigs, buffaloes and deer. Type O, A and Asia 1 were reported in the fifties and sixties. Since 1970, typeO virus has remained predominant. All the outbreaks occuring in 1983 were due to type O (Anon, 1984).

In Bangladesh, the disease is enzootic and occurs throughout the year. FMD virus type O, A, C and Asia I have been identified. The last outbreak caused by type C ocurred in 1975 but types A, O and Asia 1 continue to be isolated. The disease occurs mostly in cattle especially those involved in mass movement.

In Pakistan FMD is widespread and occurs throughout the year in areas of high livestock density particularly in the irrigated area of Punjab. Type O virus is predominant (Anon, 1984). The disease is enzootic in Sri Lanka with a seasonal incidence largely during the monsoon between November and February. In recent years, only type O has been recorded in cattle, pigs, buffaloes and goats (Anon, 1980, 1981, 1983).

AFRICA

Foot-and-mouth disease was first described in Southern Africa in 1795 by Le Vaillant (Brooksby, 1982). Galloway in 1948 discovered that there were three different types of foot and mouth disease virus active in Southern Africa and not similar to the existing European types. These types were subsequently called Southern Africa Teritories SAT_1, SAT_2 and SAT_3. SAT Foot and mouth disease virus types are still the major cause of FMD outbreaks in Southern Africa even though types O, A and C have occurred elsewhere on the

continent (Mannathoko, 1982).

Morroco in North Africa which was free of FMD since 1965 reported 1,611 outbreaks caused by type A in 1977. (Bolekali, 1982). This epizootic continued to 1979 and may have been responsible for the outbreaks in 1982, 1983 and 1984.

Algeria reported 23 outbreaks of FMD in 1977 due to type A virus. Type A virus caused FMD in Libya and Egypt in 1979 and type O virus in Egypt in 1977, 1978 and 1979. Libya still continues to report outbreaks of type O FMD in 1981, 1982 and 1983 (Anon, 1983).

In Western and Central Africa, Gambia reported type SAT_2 outbreaks in 1979 and 1980. Sierra Leone and Liberia have not reported FMD. Senegal reported outbreaks of type SAT_2 in 1980, 1983 and 1984 (Anon, 1984). Ghana reported outbreaks of SAT_1 and SAT_2 in 1974 and 1975. Type SAT_1 was identified in Niger in 1976, 1978 and 1979. In Cameroons, Type A virus caused outbreaks in 1976, 1978 and 1979. Upper Volta experienced outbreaks in 1976 and 1978 due to type SAT_1.

In East Africa, types A, O and SAT_2 caused outbreak in Sudan in 1977 and 1978 and type O in 1981, 1982 and 1983. Ethiopia reported FMD in 1978 due to types O, A and C. Type O was also reported in 1982 (Anon, 1982). In Kenya, types O, A, C, SAT_1 and SAT_2

caused outbreaks in 1976, 1977 and 1978. No virus has been isolated from outbreaks in Kenya in recent years (Ngichable and Chema, 1982). FMD is enzootic in Somalia; types O and A are prevalent.

In Uganda, FMD types A, O, SAT_1 and SAT_2 have been reported in 1978 but in recent years only type O virus has been reported. Zambia had no reports for five years, the disease reappeared in 1975. It was caused by type SAT_2 thought to have been introduced by wild buffaloes. Type SAT_3 caused disease in Malawi in 1976, SAT_1 in 1981, SAT_2 in 1981 and 1983 while type O was reported in 1981 (Anon, 1983, 1984).

Tanzania has never been free of the disease since it was introduced into the country from Southern Africa. Types A, O, SAT_1 and SAT_2 viruses have been responsible for outbreaks in 1975, 1976, 1977 and 1980 (Anon, 1980). Type SAT_3 was reported in 1955 reappeared in 1975 (Anon, 1975). Mozambique reported types SAT_1 and SAT_2 in 1979 and 1980 and SAT_2 in 1981 and 1982 (Mannathoko, 1982). Botswana did not report FMD between 1968 and 1977. Types SAT_1 and SAT_2 caused outbreaks in 1977 and 1978 (Mannathoko, 1982) and the country has not reported FMD again.

No outbreak has been reported in Swaziland since 1970 and in Angola since 1974. In the Republic of South Africa, however, FMD types SAT_1 and SAT_2

continued to be reported. Zimbabwe has reported outbreaks due to types SAT_2 and SAT_3 in 1981, 1982 and 1983 (Anon, 1983).

NIGERIA

The earliest recorded history of FMD in Nigeria dated back to 1924 when the then colonial government was setting up Nigeria's embryonic veterinary service (Anon, 1925). Before that period, FMD probably existed in Nigeria but was undocumented. In the 1924 report of the veterinary department of Nigeria, mention was made of the lack of information on the disease in the country. Since no reported outbreak was brought to the notice of the colonial veterinary department, it was concluded that if FMD occurs in Nigeria at all, it must have been of such mild nature to escape clinical detection.

FMD was detected and diagnosed clinically among trade cattle in Borno Province in 1929 (Anon, 1929). In the following year the disease spread widely throughout Nigeria. Adult stock suffered mildly, mortality among suckling calves in some infected herd was high (Anon, 1930). The outbreak was reported to have died out towards the end of the year. Clinical detection of FMD continued in trade cattle along several trade routes in the Northern part of Nigeria in the thirties and in the forties but no control

measures were introduced (Anon, 1943). The colonial authorities paid little attention to the disease since it did not have the same economic importance it has in British livestock. In 1943, there was an increase in the number of outbreaks reported from most provinces in the North. No records were kept about the number of outbreaks occuring or the number of animals involved in each outbreak (Anon, 1943). Several outbreaks of FMD occured at immunisation centres and high mortality among young stocks reacting to rinderpest immunisation were attributed to FMD (Anon, 1943). It was noted that certain herds that had passed through an attack of FMD in December 1942, did not contact the disease when exposed to new infection again in September 1943. The disease was reported among pigs at one government farm. No mortality was reported among adult pigs but some piglets lost one or more hooves (Anon, 1943).

Sporadic outbreaks occured in 1946, 1947 and 1948 among trade cattle. Outbreaks in trade cattle was mostly in young stocks which became infected during the dry season. Ten scattered outbreaks were reported in Bamenda area (now part of Cameroon) in trade cattle from Adamawa Province and the Mambilla Plateau (Anon, 1948).

The outbreaks of 1955 and 1956 were important because of their severity and the epizootic nature

of the disease during that year. Unlike previous sporadic incidences, the disease was reported to have spread rapidly throughout the country. By 1957 there was a remarkable reduction in the incidence of the disease. It was reported in seven minor outbreaks in two provinces.

Four samples were dispatched to the World Reference Laboratory - Pirbright, for virus isolation and typing. Type O FMD virus was isolated from three of the four samples. During 1958/59 only a single outbreak of the disease was reported. It was brought to the highland of the Mambilla Plateau from the Cameroon by trade cattle. Trade cattle originating from Mambilla in turn carried the disease to the Benue Province (Anon, 1959).

The nineteensixties witnessed an improvement in the compilation of information on the disease. The veterinary department began routine despatch of samples to Pirbright for virus isolation and typing. In 1960, the disease appeared in epizootic proportion in the Eastern half of the country but it was reported to be of a mild nature. The disease was recognised mainly among trade cattle and it spread along cattle trade routes. Aphthisation was used to spread the disease quickly among incontact animals. In that same year, two severe outbreaks were reported in Benue Province and one severe outbreak in Jos. In the former, out

of 774 cattle exposed to the disease, 128 died. In the Jos outbreak,characterised by very unusual severe symptoms,some animals died. No specific figures on mortality and morbidity were recorded. Type A FMD virus was isolated from one such severe outbreak in Gombi in Adamawa Province. In 1961 the disease was seen in almost all the provinces in the North. It was not reported in most cases by herdsmen. Type A virus was again isolated from an outbreak in Jalingo (Anon, 1961).

During 1962/63 outbreaks were reported in nearly all the Northern provinces; thirty eight outbreaks were documented, 7495 animals were involved and only 41 deaths were recorded (Anon, 1964). Type A FMD virus was again isolated during the year. During the same period, Type SAT_2 virus was appeared in Nigeria probably introduced by movement of animals from Chad to Nigeria. Type SAT_2 FMD virus was isolated from a severe outbreaks in Sardauna Province. Libeau's (1960) review of the foot-and-mouth disease situation in Africa in which he reported only 10 sporadic cases of FMD in Northern Nigeria and no outbreaks of the disease in Eastern and Western Nigeria characterises the scanty nature of information on FMD in Nigeria at that time.

As a result of improvement in the collection and despatch of field samples to the World Reference Laboratory, Pirbright, more virus isolations were made from several outbreaks in many of the Northern Provinces. During 1963/64, 45 outbreaks were reported officially involving 8,149 animals and 53 mortalities. Type A FMD virus was isolated from an outbreak in Adamawa, Type O virus from an outbreak in Bauchi Province, Type SAT_1 from outbreaks in Bauchi, Borno and Zaria Provinces. Type SAT_2 was isolated in Zaria Province (Anon, 1965).

During this period SAT_1 was isolated in Nigeria. The virus spread across the continent of Africa through Sudan in the course of 1963 and it entered Nigeria through Maiduguri with trade cattle from Chad in December 1963. From there it spread to Bauchi and Zaria Provinces in 1964. Reviews of FMD outbreaks in Nigeria (Owolodun 1971, Anon, 1975; Nawathe and Goni 1976 and Asagba 1982) have documented what little information is available on FMD in Nigeria. These reviews of FMD virus distribution tend to indicate that before the early 1970s FMD outbreaks had been limited to mostly the Northern states of Nigeria and among trade cattle moving from south either on hoof or in trucks or train. From 1972/73 however, outbreaks

of FMD were reported at Agege and the University of Ibadan Farm where dairy herds, pigs and sheep were severely affected (Anon, 1974). In 1975 outbreaks of FMD were reported at Obudu Cattle Ranch and other parts of the Cross River State. Thousands of cattle were involved but mortality was low, (Anon, 1976). Between 1975 and 1976, cattle from presumably foot-and-mouth disease free Mambilla Plateau came down with FMD after attending a livestock show in the Cameroons (Osma, 1976).

The clinical picture of FMD is described by Tomori et al., (1974) and Smith (1971) appear consistent with the clinical picture described for cattle in other areas of the world. Smith (1971) described an outbreak at Vom involving both Friesians, white Fulani and Wadara breeds. The Friesians showed all the classical clinical signs and symptoms. Morbidity rate was higher (72.8%) among Friesians than the White Fulani 33.3% and Wadara 65%. The disease was more severe among Friesians and milk yield dropped drastically from a daily average of 23.4 lbs to 13.4 lbs per animal per day in about 12 days. The drop in milk yield was highest in Friesians. Smith (1971).
Control practices have been based on quarantine and restriction of movement of trade cattle. Vaccination, limited in scope, has been carried out in some exotic

dairy herds e.g. (Kano State 1975, 1976; University of Ibadan Farm 1965, 1968 and 1972; Agege Farm 1970, 1971 Anon, 1971, 1977). Vaccines against FMD had been imported on an adhoc and emergency basis from England. Some of these imported FMD vaccines were not formulated strictly based on the prevalent type variant predominant in the field at the particular time (Anon, 1975), and consequently performed suboptimally in most cases under Nigerian conditions (Mowat et al., 1975). Vaccines strains used for vaccine production for East Africa were used in formulating most of the vaccines imported earlier into the country. Studies conducted at Wellcome Laboratory, Pirbright had shown that Nigerian SAT_1 and SAT_2 virus types are serologically different from virus strains used for producing vaccines for East Africa and Southern Africa (Forman, 1974). Nawathe and Majiyagbe (1981) found that two commercial polyvalent vaccines formulated from non Nigerian Type A, SAT_1 and SAT_2 viruses did not give satisfactory results in Nigerian cattle. Limited studies had been carried out on the suitability of Nigerian field strains for the formulation of vaccines for use in Nigeria. Mowat et al., (1975) and Preston et al., (1982) have shown that suitable vaccine could be made from Nigerian strains. Nicholls et al., (1983) reported satisfactory protection of Nigerian cattle using inactivated

vaccines formulated with Nigerian strains.

FMD Serotypes in Nigeria.

No consistent and systematic classification or subtyping of FMD virus strains isolated in Nigeria has been carried out. This in part is due to the little interest shown in particular FMD virus strains that do not constitute a threat to Europe where the typing and subtyping reference laboratory is located, and in part to the absence of a clear control policy on FMD in Nigeria.

Vaccines have been produced for use in Nigeria on a number of occasions (Mowat, et al., 1975, Preston, et al., 1982 and Nichols, et al., 1983). In each instance, a group of Nigerian FMD virus isolates were examined and serologically compared with existing vaccine strains in use elsewhere in South and East Africa. Forman (1974) examined a group of 1974 field isolates of type A, SAT_1 and SAT_2 and found that they were not related to existing vaccine strains in use in Africa. Type A NIG. 5/72, SAT_1 NIG. 9/72 and SAT_2 NIG. 10/72 were chosen on account of their ability to produce high yields of antigen in 24 hours in BHK-21 cultures (Mowat et al., 1975). Preston et al., (1982) compared Nigerian and other West Africa subregional isolates of 1974 - 1976 in an attempt to select vaccine candidates for use in Nigeria. As a result of that

study Type A NIG. 10/76, SAT_1 NIG. 8/76 and SAT_2 NIG. 1/74 were incorporated into vaccine. Type A NIG. 10/76 tissue culture harvest did not show complement fixing activity against antiserum raised against type A NIG. 5/72. Arrowsmith (1984) (personal communication) has shown in serological comparison with other reference type A strains that A NIG. 10/76 may be a new subtype.

Nichols et al., (1983) selected strains SAT_1 NIG. 4/81 and SAT_2 NIG. 6/81 for the formulation of vaccines for current use in Nigeria based on a comparison of strains recently isolated in the field from 1980 to 1981. The vaccine provoked sufficient antibody in Nigerian cattle to effect protection provided revaccination is carried out every six months. This vaccine has not however been used widely for the control of FMD in Nigeria and outbreaks continue to occur.

Aetiology

FMD virus is classified under the genus Aphthovirus within the family Picornaviridae (Cooper et al., 1978), This classification is based on various characteristics of the virus. It has naked capsids, is ether-resistant and acid labile, has an icosahedral symmetry, is 20-25 nm in diameter and contains a single stranded linear RNA genome (Bachrach et al., 1964) with a molecular weight (Mw) of $2.6x10^6$ (Wild and Brown, 1970). The RNA is infective and acts as messenger. RNA (Polatnick and Arlinghaus, 1967; Lazarus and Itin,

1974). Mature virions contain a major polypeptides resulting from the post-translational cleavage of a single precursor (Vande Woude and Ascione, 1974; Black, 1975). Viral synthesis and maturation occur in the cytoplasm , independent of cellular DNA function.

Infective particles of FMD virus have a sedimentation coefficient of 146S in sucrose gradients (Strohmaier, 1971) and a bouyant density of 1.43g/ml in CsCl gradients (Trautman and Breese, 1962). The total molecular weight is estimated to be 8.3×10^6 (Talbot and Brown, 1972; Vande Woude et al., 1972). The 146S infective particle dissociates into subunits and viral RNA when the pH is lowered to 6 (Bachrach et al., 1957). Mussgay, (1959) or by heating at 56°C (Brooksby, 1953; Brown and Crick, 1958; Brown and Cartwright, 1961). The subunit has a sedimentation coefficient of 12S in sucrose gradients and a mol. weight of 2.65×10^5 (Talbot and Brown, 1972).

Capsids devoid of RNA (empty particles) occur naturally in some FMD virus cultures (Graves et al., 1968). These have a sedimentation coefficient of 75S in sucrose gradients (Graves et al., 1968; Rowland et al., 1975) and a density of 1.31g/ml in CsCl gradients (Graves et al.,1968).

Structure and Chemistry of the Virus

Although Loeffler and Frosch (1897) speculated on the particular nature of the virus, as opposed to the contagium virumfluidum concept, the next 30 years saw little advance in thought on the nature of the particle. It was seen to be readily filterable and well below the resolution limits of the conventional light microscope. Attempts were made, by Barnard and Elford (1937) to use the greater resolution of the UV microscope, which had demonstrated the elementary body' of the pox viruses, but particles of FMDV were not seen. Elford (1937) had made another approach through the preparation of a series of colloidon membranes, of decreasing pore sizes, the Gradocol membrances and applied them to the determination of particle size of a range of viruses. By this method, Galloway and Elford (1937) estimated the size of FMDV as in the range of 8-12 n_m. That this was about half the actual size was due to the application of a falsely high factor for the relation between particle size and the pore diameter of the limiting membrane through which the virus passed. All viruses studied by Elford (1937b)in this lower range were subject to the same error, perhaps most notably the dysentary bacteriophage (Y6R) which, having the same particle size as FMDV, was used as a control in many

experiments. With larger viruses the size estimations were closer to those which have been obtained by direct observation. Thus the virus of vesicular stomatitis was estimated to be 70-80 nm in diameter, a little greater than the diameter of the bullet shaped particle. Elford himself speculated that some virus particles were not spherical and even in 1941 discussed the possibility of a filametous form of FMDV (Elford et al., 1941).

Another attempt at the determination of particle size in the 1930s followed Sverdberg's development of the ultracentrifuge for estimating the size of proteins. In so far as animal viruses were concerned, the concentrations available gave no hope of detecting a sedimenting band by optical methods, and animal virologists were envious of their colleagues in plant virology and their observation on the sedimentation of such viruses as tobacco mosaic virus. For the animal viruses, the measurement of sedimentation rate depended on virus titration in samples of suspension that had been centrifuged. Collection of these samples without disturbance was difficult enough but an even more serious problem was the titration of virus. In the case of FMDV the standard deviation of the end point using 5 guinea pigs per dilution in 10-fold series of dilutions would be of the order of log 0.5. It says

much for the technique of the workers concerned that the size value calculated from the studies was 20 nm, which was approximately correct (Elford and Galloway, 1937; Schlessinger and Galloway, 1937). Traub and Mohlmann (1946) had made a new approach to the use of complement fixation test in FMD and it was shown that it could be made more reproducible than it had been in the hand of Cuica 12 years earlier. Traub and Pyl (1943) applied the test to samples of centrifuged antigen and found that 50-70% of the complement fixing activity of a virus suspension remained in the supernatant even after prolonged centrifugation at speeds which would deposit 99% of infective virus. So called soluble antigen had been demonstrated in vaccinia (Craigie and Wishart, 1932) and in influenza (Craigie and Wishart, 1936), but with FMDV, the particles of which were already so small, the term seemed to be a misnomer, and most worker tended to think of smaller complement fixing material as viral subunits. Bachrach (1950) in Denmark and Bradish (1952) in England followed up Traub and Pyl's (1943) observations. The estimation of infectivity was still difficult and even the estimation of complement fixation, although much more precise, was hampered by the need for larger volumes of material than could be readily obtained from the ultracentrifuge. Nevertheless by 1953 it had been established (Bachrach, 1952),

(Bradish, 1952) that virus suspensions contained not only the virus particles sedimenting at approximately 140S and responsible for the infectivity and for about 50% of complement fixing activity of freshly harvested virus, but also a smaller particle associated with the remaining complement fixing activity. This had a sedimentation constant of about 12S and an antigenic type specificity that was less than that of the large particle (Bradish, 1952).

It was found that the larger particle could be disintegrated, with loss of infectivity, by aging, by mild heat treatment or by exposure to adverse pH (6.5 or lower). The main product identified in the disintergrated virus had a sedimentation constant of 12S and appeared to be identical with the small particle found in fresh virus preparations.

The complement fixing activity of the virus particle increased by 30-50% on disintegration, and it was postulated might consist of a structure of 12S subunits which when separated, exposed new antigenic sites of less specificity than in the intact virus particle. The small particle was also deficient in another important respect for it failed to produce an immune response.

Work on FMDV proceeded in parallel with that on other picorna viruses, and the identification of the

structural proteins and the study of the viral genome were the common goals of biochemist working on polio virus, encephalomyocarditis virus and other viruses of the family. A first step was the concentration and purification of the virus, and suitable methods were developed by Brown and Cartwright (1960, 1963). Brown and Crick (1959) demonstrated the importance of preserving the 140S particle intact during the inactivation of its infectivity if the product were to immunise animals effectively. They also confirmed the earlier data on the relation between 140S and 12S antigens and the lowered antigenic specificity of the 12S component.

Other components were detected in virus preparations. Planterose and Ryan (1965) found a 65S particle which fixed complement with antisera against either 140S or 12S antigens.

Cowan and Graves (1966) produced evidence for yet another antigen, VIA (virus infection associated), which they detected in concentrated virus grown in BHK cells and in concentrated extracts of infected cells disrupted before the appearance of 140S or 12S components. This antigen was specific for the virus of FMD but not specific for serotypes. Cowan and Graves suggested that the VIA antigen might be an enzyme involved in virus replication. This has

subsequently been established and the antigen is now recognised as an RNA polymerase (Newman et al., 1979).

The demonstration of separate antigens of varying specificity stimulated attempts to separate the antibody species and to prepare antisera. Brown and Graves (1959) had provided an explanation of the lack of serotype specificity in sera collected in the early convalescense when they showed that 1gM, which predominated in sera 7-10 days after infection, was of much lower specificity than the later developing 1gG. Brown and Smale (1970) combined the serological approach with a study of morphology in the electron-microscope, by photographing virus with attached antibody of both 1gM and 1gG species. 1gM was shown to combine with sites at the vertices of the particles and believed to be the location of the immunogenic antigen of the virus. 1gG also attaches to this site and to two others, on the margin and surface of the icosahedral structure. These pictures, while failing to resolve the detected surface of the particle, do imply the distribution of surface antigens in a pattern consistent with icosahedral symmetry (Brown et al., 1970).

Investigation of the viral RNA began when Gierer and Schramm (1956) succeeded in obtaining infective RNA from polio virus by phenol extraction. A similar

technique was employed on FMDV. and infective RNA was demonstrated in 1958, almost simultaneously by workers in England (Brown, 1958) and Germany. Subsequently the viral genone was established as single strand of RNA of molecular weight about 2.8×10^6. The complex structure referred to as the replicative intermediate, involved in the reproduction of viral RNA has not been completely understood.

The use of polyacrylamide gel electrophoresis opened a new chapter in the study of the viral proteins. Wild et al., (1969) used this technique to demonstrate in FMDV the four major polypeptides, (virus protein) VP_1 - VP_4, recognised throughout the picornavirus groups. La porte (1969) presented similar evidence and went on to identify the terminal amino acid residues in each. VP_1 was found to be cleaved by trypsin and identified as the immunogen. Laporte et al., (1973) and Bachrach et al., (1975) found that the isolated and purified VP_1 would produce an immune response in pigs, though a much greater dosage was needed than for the same antigen in the intact but inactivated virus particle. Talbot et al., (1973) compared the patterns in acrylamide gel electrophoresis and showed that VP_4 was common to all seven serotypes but that the pattern given by VP_1, VP_2 and VP_3 could be used

to differentiate the serotypes and even strains within a given serotype. The sequencing of the polypeptides continued and differences between serotypes were established - (Bachrach et al. 1973; Strohmaier et al., 1978).

These studies have led to major development in the last 10 years, the biochemical map which links the base sequence of the RNA and the amino acid sequence of the polypeptides. The gene order has been established, as it was for Poliovirus (1971) and EMC virus (1972) (Brooksby, 1982) and the primary proteins which are the precursors of VP_1 - VP_4 have been identified. Recently, its mapping information has been used in recombinant DNA experiments (Brooksby, 1982), and Kupper et al., (1981) have produced evidence of the production of immunising antigen VP_1 in Escherichia coli containing a plasmid carrying the appropriate codon.

Virus stability

Various authors have investigated the viability of FMD virus strains in tissue culture under controlled conditions of pH and temperature, and in the presence of chemical inactivating agents (Bachrach et al., 1957; Brown et al., 1963; Bachrach et al., 1964). The virus was found to be stable at neutral pH but could readily be disrupted at acid pH (Bachrach et al.,

1957). However, virus integrity could be maintained at pH 5.25 at a NaCl tonic strength of 2.8 and a pH 9.5 when the tonic strength of NaCl was 10^{-4} (Bachrach, 1968).

Virus suspensions maintained at a neutral pH may remain infective for 18 weeks at 4°C, 11 days at 20°C, 21 hours at 37°C, 7 hours at 43°C, 1 hour at 49°C, 20 seconds at 55°C and 3 seconds at 61°C (Hyslop, 1970).

The effect of freezing and thawing or freeze-drying on stability of the virus varies with the preparation (Bachrach, 1968). Virus from mouse muscle was reported to withstand several cycles of freeze-thawing when diluted in glycerine, phosphate or tris buffer or in saccharose (Storz, 1958). Virus in tissue culture fluid containing serum was found to withstand repeated freeze-thawing cycles without loss of infectivity. When freeze dried and stored at 4°C it lost $10^{0.8}$ mouse lethal dose 50/ml (LD_{50}/ml) per year and was rapidly inactivated on storage at 23°C (Fellows, 1965; Ramyar and Traub, 1967).

Highly purified virus in 0.05M phosphate at pH 7.5 was unstable on freezing unless protected with 1% sodium ethylene-diamine tetra-acetate (EDTA) (Trautman and Breese, 1962). Lucam et al., (1964)

investigated the action of 21 common disinfectants on the virus and Sellers (1968) described comparative tests on the inactivating properties of several chemical compounds. N - acetylethyleneimine (AEI) is commonly used as virus inactivant in vaccine production (Brown et al., 1963).

Virus growth in tissue culture

FMD virus has been grown in embryonic guinea pig, bovine, porcine and ovine tissues (Maitland and Maitland, 1931). Frenkel (1951) used fragments of bovine tongue epithelium to propagate FMDV for large scale vaccine production. Brooksby and Wardle (1954) used bovine tongue epithelium for the titration of virus infectivity.

Sellers (1955) demonstrated that FMD virus produced cytopathogenic effects in monolayers cultures of calf and swine kidney cells and used this for the titration of infectivity by plague formation.

Ubertini et al., (1960) used primary calf kidney cultures for the industrial production of FMD vaccine and Mowat and Chapman (1962) introduced the use of baby hamster kidney 21, clone 13, cell line (BHK-21) in FMD virus research.

The variability of different cell cultures in their sensitivity to FMD virus is of interest. Sellers et al., (1959) showed that some strains of FMD virus were less pathogenic in calf kidney cultures than in cultures of pig kidney. Cottral et al., (1965) compared the sensitivity of calf kidney cultures, cattle, unweaned mice and guinea pigs to 16 strains of FMD virus. They found that guinea pigs were insensitive to all strains and that calf kidney cultures were more sensitive than cattle or mice. Snowdon (1968) has shown that calf thyroid cell cultures are more sensitive to FMD virus than are pig kidney cultures, BHK-21 cells, cattle or unweaned mice.

When FMD virus randomly encounters a susceptible cell, the particle attaches to a receptor site (Cavanagh, 1976). Adsorption of the virus to cells was shown to be temperature -calcium ion dependent. The half time of penetration at 37°C is 30 seconds (Thorne and Cartwright, 1961; Thorne, 1962; Brown et al., 1962).

Treatment of FMD virus with trypsin greatly decreased the ability of the particles to attach to host cells, resulting in a 100 fold loss of infectivity (Wild and Brown, 1967; Wild, et al., 1969; Cavanagh, 1976). Cavanagh (1976) has shown that only 20% of the

virus attached to cells could be recovered but these had a lower sedimentation coefficient and bouyant density than that of the native virus, and he suggested that this might be due to the association of the virus with cell membrane components.

Virus penetration is followed by a latent period of 2½ hours (Cartwright and Thorne, 1959), at the end of which, infected cells start producing virus. Within 3 hours the cells begin to retract from one another and cell sheets become fenestrated (Pay, 1957). Four to five hours after infection, many cells produce large bulbous crytoplasmic protrusions which gradually coalesce. Finally, large spherical cells are produced which resemble cells showing hydropic degeneration found in the early stages of a developing vesicle in the epithelium of infected animals (Pay, 1957). Pay also reported that certain cultures of adult bovine tongue epithelium continue to produce virus at a constant low level for more than 30 days.

Antibodies to FMD virus

Antibodies to the 146S virus particle and the 75S and 12S subunits are produced in animals after a disease or vaccination. Antibodies to virus infection antigens (VIA) are usually found in convalescent animals (Cowan and Graves, 1966). However Dawe and Pinto (1978) and Pinto and Garland (1979) detected

antibodies to VIA antigen in repeatedly vaccinated cattle, but not in cattle vaccinated once against FMD.

Early antibodies of the 1gM class are detectable in cattle infected with FMD virus within 3 to 5 days post infection, reaching a peak at 7-14 days and gradually declining within a month (Brown et al., 1964; Cowan, 1966). This antibody has a sedimentation coefficient of 19S (Brown, 1960) and is sensitive to cleavage with 2 - mercaptoethanol (2 - ME) (Brown et al., 1964). It neutralises and precipitates the virus but does not fix complement to any appreciable extent (Brown and Graves, 1959; Cowan, 1966). At 10-14 days post infection, a second class of antibody is detected (Brown and Graves, 1959) which reaches a peak at about 28 days and can persist for some years after infection (Cunliffe, 1964; Cowan, 1966; Graves et al., 1972). The late antibody belongs to the 1gG class of immunoglobulins, having a sedimentation coefficient of 7S (Brown, 1960; Cowan, 1966) and being resistant to 2 - ME (Brown et al., 1964). This 7S antibody shows neutralising, precipitating (Brown and Crick, 1958) and complement fixing activity (Cowan, 1966).

Cattle response to vaccination with inactivated FMD virus vaccines closely resembles that described

as following infection (Mckercher and Giordano, 1967; Chappuis et al., 1971; Garland, 1974).

New born calves from vaccinated dams are essentially devoid of antibody but acquire protective antibody within a few hours after ingesting colostrum. The transferred colostral antibody appears to protect young calves until they are 2-4 months of age (Graves, 1963; Sruban, 1966).

Measurement of antibodies

Antibody to FMD virus has been detected and measured by serum neutralisation test (SNT) (Brooksby, 1949; Skinner, 1953; Brooksby and Wardle, 1954; Van Bekkum, 1959; Capstick et al., 1959; Golding et al., 1976), complement fixation (Brooksby, 1952), agar gel immunodiffus ion (Brown and Crick, 1958; Cowan and Graves, 1966; McVicar and Sutmoler, 1970), indirect haemagglutination (Warrington and Kawakami, 1971; Warrington and Kawakami, 1972) and radioimmunoassay (Crowther, 1977), and by indirect enzyme-linked immunosorbent assay (ELISA). (Elzein and Crowther, 1978).

The 146S, 12S, 75S and VIA antigens react in complement fixation, agar gel diffusion and radio-immunoassay tests but only antibodies to 146S and 75S antigens appear to neutralise virus infectivity

specifically. Antibodies against intact 146S are mainly type specific, whereas antibodies to the subunits (12S) and VIA show a considerable degree of cross reaction between types in precipitin and complement fixation tests (Brown and Crick, 1958).

Serotypes

Vallee and Carree (1922) were the first to recognise the difference in serotype between strains of an animal virus. They observed reinfection of animals in the field and conducted cross immunity experiments with the then prevalent French Strain and a 'new' strain throught to have been isolated in Germany. The two serotypes from these experiments were labelled O (from the department of Oise) and A (from Allemagne). The finding was confirmed by Waldmann and Trautwein (1926) at the Insel Reims, with strains which they labelled A and B and a third which they described in 1926 as C. This led to arguments and discussions which were later resolved at the Office International des Epizootics in Paris and the three types were labelled Vallee O, Vallee A and Waldmann C. As the situation stabilised, the names were dropped and the three serological types have since been known as O, A and C till today.

The arrival of FMD samples from Southern Africa led to the discovery of the hitherto unknown sero types.

several strains from the then Rhodesia and Benchuanaland had been examined from 1931 onward at Pirbright, and cross immunity experiments had given equivocal results. The development of complement fixation test for FMDV in 1948 led to re-examination of some of the old isolates from Southern Africa. In the 1948 tests, two new serotypes were found among field specimens, SAT_1 (Southern African Territories) from Benchuanaland and SAT_2 from Rhodesia. SAT_3 serotype was identified in a Southern Rhodesian sample in 1937. The final discovery in the serotypes came in 1954 (Brooksby and Rogers, 1957) when type ASIA 1 was detected in a sample from Pakistan.

Seven serotypes and 65 subtypes of the virus exist. The degree of difference between them is such that animals immune to one type, remains susceptible to other six types. Because of antigenic drift, each type contains a number of subtypes distinguishable on the basis of cross immunity and serological tests (Brooksby, 1982).

Typing and Subtyping

Virus samples were typed by cross-immunity tests in guinea pigs or cattle until Traub and Mohlmann (1943) applied the complement fixation (CF) test to the detection of virus from bovine materials.

The method used in the World Reference Laboratory (WRL) at the Animal Virus Research Institute (AVRI), Pirbright, for typing FMD virus samples were described by Brooksby and Rogers (1957) and by Buckley et al.» (1975).

Pereira (1977) proposed that subtyping of FMD virus for epidemiological purposes must take into account $^{M}r_{,}^{f}$ rather than "R" values; so, for a virus to be considered a new subtype, he suggested that "r" value relationships with all existing subtypes must be lower than 0.25 in both directions. He also suggested that new subtype designation should be limited to strains of epidemiological importance. As for vaccine selection, Pereira (1977) suggested that a list of reference vaccine strains should be selected from the smallest possible number of viruses covering current epidemiological situations and that field strains should be related to reference vaccine strains. A knowledge of the subtype situation is important especially for the selection of correct vaccine and also in revealing the existence of newly evolving strains which may cause outbreaks of disease in animals already immunised.

Other serological techniques for typing FMD virus have been described, such as passive haemagglutination-

inhibition (Warrington and Kawakami, 1972) and flourescent antibody tests (Sugimure and Eissner, 1976).

Methods of Subtyping

The methods used for differentiation of FMD subtypes are based on reciprocal antigenic relationships as demonstrated by the use of in vitro serological techniques or by crossprotection tests in vivo. The antigenic relationships are based on difference between homologous and heterologous reactions expressed as ratios. For each pair of viruses two ratios (r_1 and r_2) of heterologous and homologous serum titres are obtained and a reciprocal relationship is calculated as percentage: $R = r_1 \times r_2 \times 100$ (Ubertini et al., 1960; Archetti et al., 1950).

On the basis of such relationships obtained by CF test, Brooksby (1968) proposed that strains related to each other by 70% or more should be considered as belonging to the same subtype, by 32% to 70% as different subtypes, by 10% - 32% as widely different subtypes.

Anderson et al., (1974), Arrowsmith (1975) and Forman (1975) have proposed lower 'R' values (25-40%) for subtype differentiation and have suggested the formation of subtype groupings related to selected reference strains of existing subtypes in such a way

that given isolates may be classified into more than one subtype group.

Transmission and Persistence

FMD virus is spread primarily from infected animals (Bachrach, 1968). Virus is present in all organs, tissues, secretions and excretions of infected animals.

Virus has beem detected in ambient air surrounding cattle before and for two weeks after clinical onset of disease (Hyslop, 1965). During the 1967-68 epizootic of FMD in England, high titres of virus were found in milk collected from cows in the prodromal stages of the disease. Although the spread of this particular epizootic has been attributed to winds, the transportation and marketing of virus ladden milk might have been a contributory factor in the spread of the disease (Burrows, 1968; Hedger and Dawson, 1970). Virus in dried blood, milk, carcases, hay, farm equipment, shoes and clothings can remain viable for long periods of time and serve as sources of infection (Cottral, 1969). The disease has also been disseminated by wind (Sellers and Parker, 1969). Aerogenous dissemination may result in the spread of the virus over distances as great as 60km (Hugh-Jones and Wright, 1970). The velocity of the prevailing wind and its direction and the of rainfall at the

time affect the spread. There are peaks of spread at dawn and dusk (Hugh-Jones and Wright, 1970). It seems probable that the portal of infection with aerogenous spread is the respiratory tract (Norris and Harper, 1970). Infected animals shed maximum quantity of virus when the vesicles in the mouth and on the feet rupture.Pigs are known to excrete the largest quantity of virus followed by cattle and sheep (Donaldson et al., 1971). There are variations in the quantity of virus shed in case of infection by different strains of virus (Donaldson, et al, 1971). Infected animals are seldom infective to other animals for more than four days after the rupture of vesicles, except in so far as the virus may persist in the skin or the hair, however, some animals may remain as carriers for very long periods and the intermittent shedding of small quantities of virus may lead to the establishment of new outbreaks. The virus has been reported to persist in the mammary gland for 3 to 7 weeks (Burrows et al., 1971). The transmission of FMD is exacerbated by the more than 30 animal species that are natural hosts of FMD. Not all these species are however equally important in maintaining the natural chain of transmission of FMD (Martinic, 1977). Many species of wild animals have been reported as having been infected with FMD (Macaulay, 1963) and FMD

virus has been isolated from some species in the natural state (Meeser, 1962) while serological surveys (Condy, Herniman, and Hedger, 1969) have suggested that other wild ungulates are susceptible to FMD. Studies carried out in Botswana and Zimbabwe (Hedger, 1970) have proved that the African buffalo is the carrier of the virus in that area.

Man may be a vehicle for transmission of FMD virus (Sellers, 1970). The virus has been recovered from the nasal mucosa of persons working with infected cattle for up to 24 hours after contact. Nose blowing does not eliminate the virus and cotton face mask does not prevent the infection. All tissues including bones remain infective for long periods especially if quickly frozen but to a less extent in meat frozen slowly. The survival of the virus is closely associated with pH of the medium. The development of acidity in rigor mortis inactivates the virus but quick freezing suspends acid formation and the virus is likely to survive (Bachrach, 1977). However, on thawing, the suspended acid formation recommences and the virus may be destroyed. Prolonged survival is more likely in viscera, bone marrow and in blood vessels and lymph nodes where acid formation is not so great.

to infect susceptible cattle by direct exposure with carrier animals have been unsucessful to date. Factors which are incompletely understood such as immunological deficiencies, physiological imbalances and physical stresses in recipient or carrier animals seem to trigger off the infection.

Control

Many factors influence the procedures used to control FMD in an infected country. Measures short of eradication are complicated by a number of intractable characteristics of the disease. These characteristics and other considerations such as climate, geographical isolation, wildlife and livestock populations, international trading status, technological and economic development, past experiences in control programmes and political consequences are used in formulating control programmes for FMD (Bachrach, 1968).

In poor countries where the disease is enzootic, cattle owners practice little more than aphthisation, which is the practice of synchronizing an outbreak by rubbing virus from infected cattle onto the tongue of all cattle in the herd (Bachrach, 1978). Indirectly, recent evidence suggests that this method may be a way of preventing the formation of new subtypes. For example, the passage of FMDV in immune or partly

immune cattle has been reported to induce the formation of distinctly new virus subtypes (Hyslop and Flagg, 1965).

Systematic vaccination with inactivated vaccines is used to control FMD in many countries of Europe, South America, Asia and Africa, and the Middle East (Brooksby, 1982). The short lived immunity in FMD complicates these vaccination programmes. After infection or vaccination, the immunity of cattle to virus of the same type generally lasts not more than 1 year and rarely more than 3 years (Frenkel, 1964).

Nevertheless, in enzootic countries, systematic vaccination of cattle once or twice a year with inactivated vaccines of proven potency have been shown to bring the disease under control and to interupt the natural chain of transmission (Brooksby, 1982). Such programmes in Holland and other European countries in the past 25 years have been responsible for a remarkable drop in the incidence of FMD. However, some Western European countries still experience sporadic outbreaks, especially of type C FMD in swine (Sellers, 1982). The systematic vaccination has not been practical because vaccines adjuvanted with aluminium hydroxide gel protect swine for only 2 months. Extensive swine outbreaks can be controlled either by

a single dose of monovalent Frenkel or calf kidney cell - derived vaccine containing four to ten times the antigenic mass required for cattle or by two doses of regular strength Frenkel vaccine spaced 2 weeks apart (Ubertini et al., 1964; Van Bekkum et al., 1967). Because of antigenic variability of subtypes of FMDV, vaccines often have to be made from the same virus subtype (especially for subtype A) that is present in the field.

The ability to adapt FMD to unweaned mice, day old chicks, chick embryos, rabbits and other small animals and tissue cultures led to the development of experimental live attenuated FMDV vaccines. The testing of such vaccines formerly received considerable attention, but because of real and potential dangers, live attenuated FMDV vaccines are now only used in Venezuela (Olascoaga, 1982). Their use is prohibited in Western Europe (Sellers, 1982). Most, if not all, attenuated FMDV vaccines are too virulent for swine and for cattle under stress. Also, the danger exists that some of the many natural hosts of FMDV may induce live attenuated viruses to revert to virulence.

Stamping out or slaughter method has been practiced in several countries (Bachrach, 1982). Farmers, livestock men and veterinarians often receive

informations to keep them aware of the need for early recognition and reporting of suspected outbreaks of FMD , and a capability for its rapid diagnosis if maintained at all times. In the event of an outbreak , government supervised quarantine measures are imposed as quickly as possible within and surrounding the affected areas. Infected animals and all exposed susceptible livestock are killed and buried or burned on the premises. When all available signs indicate that the outbreak has been stamped out, the premises are cleaned and disinfected. Contaminated areas are sprayed with alkali or other approved disinfectant; materials that may by be damaged by such treatment are soaked or fumigated with formaldehyde and those of little value are burned or buried. Thirty days later, a few susceptible animals are placed on the premises (farm) by the government for 30 days. If no signs of the disease develop in the animals, the farmer gradually acquires new stocks. All restrictions are removed if FMD does not occur during the next 90 days (Anon, 1974), (Anon, 1981).

Outbreaks in the USA and the one in Canada in 1952 have been eradicated by stamping out (Anon, 1953). England also relies on stamping out method, even though the disease has a history of being reintroduced at

varying intervals. In the 1967-1968 stamping our campaign, about 500,000 infected and exposed animals were slaughtered and destroyed many of them by burning (Great Britt, 1968).

Most countries of South America and Western Europe in which the disease is enzootic or which are exposed repeatedly from without, usually try to control outbreaks principally by quarantine and vaccination (Sellers, 1982). A method that combines the slaughter of infected animals with ring vaccination around the focus of infection was practiced in Denmark and in the extensive outbreak in Mexico in 1946-1954 (USDA, 1955). Stamping out alone was used during the first years of the Mexico campaign, but it was unsuccessful and impacted heavily on the social and economic structure of the country. Consequently ring vaccination has to be incorporated into the eradication programme.

Differentiation of FMD virus Strains

Traub and Mohlmmann (1946) showed that immunogenically different strains could be differentiated by complement fixation test. Groups of cattle vaccinated with one strain withstood homologous challenge but were only partially protected against challenge with a heterologous strain. Similar qualitative results, were obtained by Henderson et al., (1948), Martin et al., (1966), Hyslop, Davie and Carter (1963).

The complement fixation test first described by Cuica, (1929) has been used from 1947 onward for comparison of strains of foot-and-mouth disease virus. It has been found that the reproducibility of results obtained in the complement fixation test and its convenience are of advantage when a large number of strains of virus of one type have to be examined. Traub and Mohalmann (1946) used the CFT to demonstrate differences in the titre of immune sera reacting with homologous and heterologous antigens. Brooksby, et al., (1948) used the amount of complement fixed as a measure of such reactions. Graves, (1960) combined the titration of complement with the titration of antisera in order to compare homologous and heterologous antigen - antibody combination. Bradish, et al., (1960) and Bradish and Brooksby (1960) made detailed examination of the reactions of foot-and-mouth disease antigens and antisera in CFT and established working principles for the comparison of virus strains.

They demonstrated that the amount of complement fixed by two or more strains' specific antisera in an excess of one antigen allowed a more sensitive detection of differences between strains of virus than when two antigens and one antiserum were used.

The CFT as currently used is based on the basic procedure developed and described by Brooksby (1952)

and modified by Buckley, et al., (1975) based on a microtitre technique described by Takatsky (1955) and subsequently modified by Casey (1965). The CF test has been the method most widely used to differentiate FMD virus strains (Brooksby, 1968; Forman, 1975 and Pereira, 1977).

However the CF test is reported to suffer major setbacks in that it involves non immunogenic, non specific as well as immunogenic antigens (Cowan, 1973). Its specificity is reported to be influenced by the method of antiserum preparation in guinea pigs (Cowan, 1973). Type and subtype differentiations are based on complete or partial lack of crossprotection respectively between given FMD viruses. Antigenic relationship are based on differences between homologous and heterologous reactions expressed as ratios. Brooksby (1968) proposed that strains related to each other by 70% or more should be considered as belonging to the same subtype, by 32 to 70% as different subtypes, by 10 to 32% as widely different subtypes and by less than 10% as different types. The criteria has provided a useful baseline for serological differentiation of FMD types and subtypes. It has been felt however by many workers that strict adherence to the above criteria would lead to an excessive number of subtypes and would obscure important epidemiological relationship (Pereira, 1976).

Other immunoassays have been examined to determined their suitability in the differentiation of strains of FMD virus. Such new approaches include the use of ELISA (Crowther, 1978) for the subtyping of FMD virus and for the titration of FMD antigen (Ouldridge et al., 1982). Crowther (1978) also explored the use of radioimmunoassay for the subtyping and differentiation of FMD virus strains. Of the several methods proposed, the virus neutralisation test has been advocated and accepted as the preferable reference in vitro test (Anon, 1981) because of its correlation with animal protection tests (Bekkum, 1959; Pay et al., 1977). The international Association of Biological Standardisations permanent committee on FMD has endorsed the suggestion that the virus neutralisation test should be regarded as a reference in vitro test against which other tests could be standardised (IABS, 1982).

Serological surveys in epidemiology

In the 1920s and earlier an apparently insoluble problem that confronted epidemiologists was a means of measuring degree of susceptibility and resistance to disease in human and animal hosts. The usual way for the inquisitive physician to find out whether a given individual was resistant or susceptible to measles or chickenpox for example was by asking the patient or his parents whether he had experienced those common contagious diseases. To find out whether an individual had been vaccinated against smallpox, a similar approach could be used, with a search for a vaccination scar. Measuring immunity in the population as a whole was a different matter.

By the 1920s however, advances had been made in testing immunity to some infections, and two tests, the Schick and to a lesser extent, the tuberculin skin tests were available. Such skin test survey measured tissue reactivity and indicated the presence of some degree of immunity based on previous infection with the causative agents.

When serological tests were introduced, naturally they were first used for diagnostic purposes on individual cases. Williams (1920) did routine Wasserman test on all patients attending his prenatal clinic with

the sole aim of mapping out the distribution of syphylis in segments of urban population of Baltimore. The year 1930 saw the development of the neutralisation test for the detection of antibodies to Poliovirus. The test was used to determine immunity patterns of a given population (Aycock and Kramer, 1930).

In the field of arboviruses, the earliest serological survey was conducted by Soper and his associates in 1933 in Brazil on the prevalence of antibodies to yellow fever virus. Serological surveys for influenza antibodies also date back to the mid 1930s. Swine infuenza virus was first isolated in 1931 and human influenza virus in 1933. A neutralisation test in ferrets and later in mice permitted differentiation of two strains and demonstration of antibodies in human and animal sera. In 1935 a survey for swine and human influenza antibodies in different age groups in individuals from Great Britain, United States and Alaska was made by Andrewes et al., (1935). Similar surveys were conducted in the USA at about the same time by Francis and Magill (1936), Shope (1936), and Brown (1936).

By the middle of the twentieth century the World Health Organisation was quick to adopt surveys as part of its programme in epidemiology. Not only in the field of poliomyelitis where it has made its biggest impact

but in a number of others, particularly viral diseases. This approach has proved to be a far more accurate indicator of susceptibles and immunes, and of how widespread a given infection might be in a given population, than is indicated by reports of clinically diagnosed cases. With the availability of a variety of serological techniques, the immune status of a man or an animal can be measured with resonable accuracy in respect of many infectious diseases.

Advances in serology and serodiagnostics epidemiology has been adapted for various animal viral diseases. Serological surveys have been found useful in understanding how widespread a given disease is in a given animal population where essential data of mortality and morbidity rates are lacking. In the field of veterinary research, serological surveys has been used to determine the immune status of Nigeria's National herd after the major anti-rinderpest vaccination campaign, (Taylor and Ojeh, 1981), distribution of African horse sickness in Nigerian horses (Parker et al., 1977), to show evidence of Pest des Petits Ruminant (PPR) infection in small ruminants (Durojaiye, 1982), (Zwart and Rowe, 1966). In Foot and Mouth Disease research, serological survey has been employed in a variety of

test systems to demonstrate the role of wild life in the spread of the virus. Antibodies to FMD virus in wild life have been reported by a number of authors, Macauley (1964), Hedger et al., (1972) and Grosso (1977).

Lobo et al., (1977) using the double agar gel diffusion test established a 12% prevalence rate of virus carrier cattle in Northern Colombia. Serological surveys are of immense value in Nigeria in the study of epidemiology of many infectious diseases since the disease reporting system is inadequate.

Various substances related to epidemiology of a given disease can be measured in the serum. The number and variety of such substances and the number of tests used to detect them have increased steadily and continue to increase. Among the many methods that can be used to detect antibodies to foot and mouth disease virus in cattle are complement fixation test (CFT), serum neutralisation test (SNT), Agar gel diffusion test (AGDT), Enzyme linked immunosorbent assay (ELISA) etc.

Enzyme - labelled immunosorbent assay (ELISA) is a serological technique which depends on the assumption that an antibody or antigen is linked to an enzyme while retaining both immunological and enzymatic activity in the resultant conjugate.

Since the 1940s there has been a phenomenal increase in the number and variety of immunodiagnostic tests performed on various disease agents. One of the reason for this has been the development and perfection of methods which used labelled antigens or antibodies, resulting in tests with very high levels of sensitivity and specificity, Flourescent labels have been attached to antibodies, and these conjugates are very useful for the rapid identification of organisms responsible for infectious diseases as well as for measurement of antibody levels especially in infectious and autoimmune diseases (Nair, 1976). Isotopes were found to be suitable, especially as labels on antigens and radio-immunoassay becomes a method of choice for sensitive assays of both small and large molecular weight substances (Sonksen, 1974). These assays were of special value for steroids (Cameral et al. 1975).

However, both immunoflourescence and RIA have their limitations. Immunoflourescence is tedious, time consuming and not easily automated so it can only be used for small batches of tests. In contrast, RIA is particularly suitable for large scale operations but the short shelf life of reagents, the rather sophisticated and expensive equipment coupled with strict regulatory controls on the use of isotopes have tended to exclude RIA from any small laboratories.

These considerations led workers to search for alternative labels for antibodies and antigens. Many candidates were proposed for this role, for example, bacteriophages (Haimovitch, et al, 1970), metals (Cais, et al., 1977) and free radicals (Leute, et al, 1972). Enzymes have been found to fulfil that role better. The enzymes can be linked to antibodies or antigens such that the complexes have immunological and enzymatic activity. Degradation by enzyme of a chromogenic or flourogenic substance yields an amplification factor, which enables accurate and sensitive detection of the presence of enzyme.

The precursors of ELISA are the homogenous enzyme immunoassay (EIA) (Rubinstein, et al., 1972), enzyme labelled assay (EIA) (Sanders and Wilders, 1974), competitive enzyme linked immunoassay (CELLIA) (Yorde, et al., 1976) and enzyme linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1971).

Enzyme labelled antibodies have been used in histopathology to detect virus antigens in tissue sections since 1966 (Nakane and Pierce, 1966; Avrameas and Uriel, 1966) but their use in quantification was introduced by Engvall and Perlmann in 1971, when they used an assay for determination of rabbit IgG using plastic tubes as solid phase in a way in which the reactivity of the immunological components was retained.

This formed the basis of the ELISA technique.

ELISA has been used widely in parasitology to detect both antigens and antibodies. The test was developed in malaria research (Voller et al., 1974), Schizostomal infections in man (Voller, et al, 1976b), babesiosis (Purnell, et al, 1976), amebiasis (Bos, et al., 1975) and in detecting trichinella spiralis in man (Engvall and Ljungstrom, 1975).

It has also been suscesfully applied to the study of bacteria and rickettsial diseases. Carlson, et al., (1972, 1975, 1976) introduced its use in bacteriology and were able to assay antibodies to salmonella antigens, for assay of antibodies to Pasteurella haemolytica in sheep sera (Burells et al., 1979), for antibody assay in vibrio cholera (Holgren and Svennerhold, 1973) for detection of antibodies to mycobacteria tuberculosis (Nassau, et al., 1976).

The introduction of ELISA into medical virology by Voller and Bidwell (1976) has opened a new chapter in the field of virus research. Most of the application of ELISA in animal virology have been in the clinical testing of human viruses and has enabled the testing of many dirty samples such as faeces in which antigens may be present in minute quantities.

The application of ELISA to influenza research has demonstrated that it is more sensitive than the CFT (Lambrei and Kasluri, 1979) and superior to haemagglutination inhibition test in the measurement of antibodies to influenza (Delia et al., 1977). The screening of animal sera for hog cholera antibodies by ELISA was reported to be sensitive and rapid (Sanders and Clinard, 1976, Sanders, 1977). Detection of antibodies to respiratory syncythia virus by ELISA was reported by Richardson et al.,(1978) to be 100 times more sensitive than the CFT. Souvres et al., (1979) used ELISA with success for the quantification of poliomyelitis virus D antigen.

Its use in detecting foot-and-mouth disease virus antigen has been described (Yolken et al., 1977; Ellenc and De Leeuw, 1977, Crowther and Abu El zein 1979a). Double sandwhich ELISA for FMDV has been used for typing from epithelial samples and with appropriate antisera and dilutions, cross reactions due to subunit and non structural proteins (which present problems in CFT) could be avoided (Crowther and Abu Elzein, 1979b). ELISA has been adapted for the serology of bovine rhinotracheitis (Payment et al., 1979; Harring, et al., 1980).

Soluble antigen prepared from African swine fever (ASF) infected cells was used to develop an ELISA which could detect 5ng amounts of ASF - antibody (Hamdy, et al., 1979) and produced good differentiation of normal and convalescent pig sera (Wardley et al., 1979). Description of assays for other veterinary viruses have included those where herd screening for evidence of infection may be particularly required, rather than individual diagnosis. Aujesky's disease and Enzootic bovine leucosis can now be tackled using ELISA methodology (Braire et al., 1979; Ressang et al., 1978). Anderson and Rowe (1982) also applied ELISA for large scale epidemiological surveys of field sera to detect antibodies to rinderpest virus.

Cowan and Graves (1966) first described virus infection associated (VIA) antigen in foot-and-mouth disease and showed that although this antigen is virus specific, it is not type specific. It has now been shown that VIA is identified with the RNA - dependent RNA polymerase (Polatnick, et al., 1967), (Newman, et al., 1979) and that it contains a major virus coded protein of molecular weight 56,000 (Sangar et al., 1977, Lowe and Brown, 1981). Much of the available information on antibody responses to VIA antigen has come from double immunodiffusion

(DID) studies in sera from domestic animals (Mcvicar and Suttmoller, 1970), (Fernandez et al., 1975, Rosenbery, et al., 1977). In these studies the test for antibody to VIA antigen provided a useful and accurate method for differentiating animals immunised by inactivated vaccine from those that have experienced infection. Lobo et al., (1977) using the double agar gel difussion test to detect antibodies to virus infection associated antigen established a 12% prevalence rate of virus carrier cattle with VIA antibody in Northern Colombia.

CHAPTER 3

INVESTIGATION OF OUTBREAK OF FMD IN NIGERIA BETWEEN 1981 - 1984

Introduction:

Foot-and-Mouth disease is enzootic in Nigeria (Nawathe and Majiyagbe, 1981). Outbreaks occur ed sporadically in the Northern part of Nigeria before 1960 (Owolodun, 1971) and in epizootic proportion since then. Four virus types O, A, SAT_1 and SAT_2 have so far been isolated from outbreaks in the field (Asagba, 1982).

Outbreaks occur every year around the country but not all outbreaks are reported (Durojaiye, 1981). No systematic attempts to control the disease have yet been undertaken probably because of the existence of more deadly diseases like rinderpest and CBPP (Smith, 1971). Occassionally requests for the supply of vaccines for the control of the disease have been made and vaccines have been imported to meet such demands. These vaccines have not been strictly formulated to meet the requirements of Nigeria.

In an attempt to increase and improve the production and supply of beef, it has been suggested by Nuru (1981) that improvement in production method will have to go on hand in hand with improved disease control measures. It is expected that rinderpest and CBPP would be

controlled if current mass vaccination programmes are carried out successfully and that other less important diseases would assume prominence.

It is the objective of this study to carry out thorough investigation of outbreaks of foot-and-mouth disease occuring during the study period in order to understand the sources of infection mode of transmission and the types of virus causing outbreaks and why nomadic herd owners do not report outbreaks of the disease. It is envisaged that a better understanding of the disease would enable the government to formulate a good control policy.

Background Information

Livestock Husbandry Methods

Livestock production in Northern Nigeria is still under the traditional transhumace methods. This age old cultural method of livestock production reflects long term adjustments to limitations imposed by unfavourable climatic and ecological factors and diseases.

At the begining of the dry season October/ November, the local herdsmen reduce the size of their stock and move them southwards in preparation for the long dry season. In their place, herdsmen from Niger, Chad and Cameroon migrate into Nigeria from their arid areas around the Sahara desert to take

the places vacated by Nigerian herdsmen who had gone southwards (Jiya, 1974; Khalil, 1974). These patterns of movement of nomad herdsmen has subjected lands in these areas to overgrazing all the year round.

The Nomads and Settled Fulani

The migratory Fulani's major occupation is rearing livestock. The settled Fulani on the other hand combines livestock keeping with some amount of arable land cultivation, planting such crops as millet, guinea corn, maize and other crops. The nomad sees his cattle as his wealth and therefore places more emphasis on quantity rather than quality. The nomad rarely parts with his animals except in cases of disease where he has to dispose of an animal to pre-empt its death. The settled Fulani on the other hand parts with some of his animals and places less emphasis on quantity. This is because the settled Fulani has more material needs to satisfy, housing, transportation (motorcycles, cars) and education of their children.

Both the settled Fulani and the migrant ones do occasionally hire herdsmen from outside their family circle. These hired hands take animals over long distances and are required to return and report to the owners regularly. These movements back and forth

constitute a major problem in disease control. Hiring of herdsmen is a far more common practice among settled fulani who usually divide their large herd into smaller herds in different locations as insurance against disease and shortage of grazing land.

Grazing time varies between the seasons during the wet season, grazing starts when the sun is already high in the sky and some of the dew on grasses has evaporated and may continue until dusk. During the raining season, animals of both migrant and settled Fulanis depend solely on grazing. In dry season, fodder is provided by the settled Fulani in addition to grazing on remnants of plants that have not dried up.

In terms of disease control, the settled Fulanis are more aware of the need to visit the veterinary services and therefore do more to control diseases than the migrant Fulani. The settled Fulanis do have more disease problems and hence patronise government veterinary services more frequently. The migrant nomads tend to move away from danger zones i.e. diseased areas and depend more on herbs for curing their animal diseases. They have the perpetual fear that the government might restrict their movement or destroy their animals during an outbreak of a contagious disease.

Government Ranches and Livestock Investigation and Breeding Centres (LIBC)

The government ranches are mainly cattle fattening centres. They operate as a commercial enterprise - purchasing stocks from different areas of the Northern States subjecting them to extensive feeding, raising them to certain market weights after which they are sent for slaughter.

The ranches have clearly demarcated and limited territory and hence feeding of animals is based on grazing on the limited grazing land and provision of supplimentary feeds. The origin of animals coming to these fattening centres vary a great deal. Most of them are purchased from cattle market in the extreme north. Animals of such markets are from both settled and migrant nomads.

Animal health services are readily available from the State governments. Outbreaks of contagious diseases are not very common except on occasions when nomad cattle stray into the ranches or an animal incubating disease is purchased into the fattening group.

The LIBCS were established by state governments as breeding centres for upgrading stocks or as dairy centres. Animals in these LIBC's are usually under the care of the state's veterinary and livestock

services and hence their performances vary according to the amount of funding which the ministries receive from the state government.

Private Ranches

The emergence of private ranches is a recent development in Nigerian livestock industry. The ranches are still very few. The private ranches visited are still not organised on specialisation basis. They practice a mixture of cattle fattening and breeding. The large ones have resident animals health officers who look after disease problems.

Animals in such ranches are not confined solely to their territory. In extreme dry season conditions, some of them are taken out to graze like migrant Fulani herds. Supplimentary feeds are provided in addition to grazing.

MATERIALS AND METHODS

The Area under study and its geography:

Nigerian's border states of Sokoto, Kaduna, Kano, Borno and Gongola were the main target states in this study. Other Northern states with a substantial cattle population (Benue, Bauchi, Plateau and Niger) were sampled. The border states are the major portal of entry of livestock and diseases from our neighbouring countries of Niger Republic, Chad and Cameroon.

The area under study lies between 7^{o} and 14^{o} latitudes north of the equator. A good proportion of this area, especially Sokoto, Kano and Borno States fall within areas of the world described as arid or at least semi-arid as the area comes within the Sahel region of West Africa (UNESCO, 1958). These are the four main states of Nigeria with the largest livestock population and production capacity. These areas are known for the vital trans-sahara trade link dating back as far as the 18th and 19th century when African Empires flourished (Ukwu, 1981). The five states are strategically important because they share borders with Chad, Niger and Cameroon and also serve as major livestock trade link with these countries.

Livestock raising is a major occupation of peasants in these areas and the main cattle rearers are the nomadic and semi nomadic Fulani and Shuwa Arab pastoralists. Drought greatly affects livestock production in these areas. Rain starts by late May - early June and stops late in September - early October. Areas still further north of latitude 12^{o} have rains starting by July and August and these may even be erratic (Khalil, 1974). Ambient temperatures and humidity vary according to seasons. The maximum temperatures can vary from below $30^{o}C$ by December/January to between $40^{o}C$ and 45^{o} by April/May. Relative humidity varies between 0-5% by December/January to about 40-45% by July/August.

Reporting of Outbreaks

Letters soliciting the help of veterinary investigation officers of the National Veterinary Research Institute (NVRI) and the Chief Veterinary Officers (CVO) in the study areas were sent out before the study started. The officers were requested to notify the investigator at the NVRI - Vom, by the quickest means, about outbreaks of FMD in their states.

Any information about outbreaks of FMD that were brought to the notice of the investigator were officially documented as required by the disease

reporting regulation and investigated.

Investigation of Outbreaks:

The herdsmen, in case of an outbreak in a migrant herd, or the veterinarian/animal health superintendents in case of an outbreak in a government or private ranch were questioned on the following:

(1) Movements of their animals, if any, before the outbreak of the disease.
(2) Any previous history of FMD in the herd before.
(3) Contact if any with any other herd diseased or healthy.
(4) History of the particular outbreak - The day signs were first noticed. How long had the disease signs lasted before the investigator arrived.
(5) Number of animals at risk.
(6) Number of animals showing signs (morbidity).
(7) Number of deaths if any
(8) Clinical signs
(9) Measures taken by veterinary authorities if any. (Details in Appendix 1).

Laboratory Samples:

Samples for culture taken from vesicular fluid and epithelial scrappings from ruptured vesicles were collected in buffered glycerine and kept in an ice chest until transfered to the laboratory. Blood samples were also collected for serology. Samples of tongue epithelium were stored at -20C before processing.

Virus Isolation:

Virus isolation was undertaken at the Animal Virus Research Institute Pirbright using primary calf thyroid cell culture. Tongue epithelium scrappings were ground and made into a 10% (w/v) suspension in Veronal buffer. The suspension was used to infect primary calf thyroid cells. Two or three blind passages were carried out on each specimen. The cultures were discarded if no cytopathic effect (CPE) was observed after three blind passages. In a case where CPE was observed, the cell culture fluid containing virus was tested in a complement fixation test (CFT) with known positive antisera against FMD whole virion. If the result was positive, the virus isolate was then typed using complement fixation test and serum neutralisation test (SNT).

RESULTS

Outbreaks reporting

Outbreaks of FMD were reported directly to the investigator from 12 locations in the following states - Benue, Plateau, Gongola, Sokoto, Borno, Niger, Kaduna, Kano and Bauchi, during the course of the study. Two outbreaks each were reported from Gongola, Borno and Sokoto states respectively. In addition, 6 outbreaks were reported from other places in the states under study but these particular outbreaks were investigated by the veterinary officials of the states. 8 other reported outbreaks that were not investigated were cases where information about the outbreak reached the investigator too late and in the judgement of the investigator, the outbreaks would have resolved and the migrant herdsmen would have moved away. The investigator learnt from herdsmen that many more outbreaks occur in the field but were not reported for fear of restrictions of movements. In addition, herdsmen do not bother to report the disease because no help had been rendered by the veterinary authorities when they had outbreaks in the past.

Investigation of Outbreaks:

The 12 outbreaks reported above were investigated. In seven cases in Borno, Sokoto, Kano, Gongola and Niger states respectively, the investigator arrived at the location of reported outbreaks only to find that the herd involved had moved away and samples had been collected by the state authorities.

In the remaining five outbreaks, the investigator was lucky to find the herd where they were restricted at the onset of the outbreak. The results of the investigations are set out in a tabular form in table 4.

Finding from investigation of outbreaks:

Outbreaks occurred among migrant herds along trade routes in 19 cases out of a total of 26 cases that were reported officially between 1981 and 1984. 4 outbreaks were reported among herds owned by settled Fulanis. 2 outbreaks were reported among herds in confined territory on ranches.

Contact with infected animals was the major mode of infection. In 23 cases out of 26, contact with an infected animal either during grazing or at watering points was cited as the source of the infection.

In two cases movement of the animals or contact with already infected migrating herd was responsible

for the outbreaks. Only in one case was an infected herd reported to have crossed an international border from a neighbouring country.

Mortality rate during the outbreaks investigated was low varying from 0 - 2.1%. Mortality was reported only in young calves whose ages were below eight months. Morbidity rate ranged from 5 - 58%.

Clinical findings:

Among indegenous cattle herd, the spread of the disease was usually gradual. 2 or 3 animals were affected at the onset and these spread the disease to the remaining animals in the herd. There was no significant difference in the rate of spread of the disease noticed among different breeds of indigenous Zebus. The rate of spread among exotic breeds e.g. Santa Getruda at Benue Carney ranch, was rapid.

The signs were typical in the exotic breeds - profuse salivation, smacking of teeth and lameness in some cases. In the indigenous breeds, the signs observed among young animals were typical of foot-and mouth-disease. In older animals, salivation though always present was not so profuse. Formation of vesicles was more pronounced on the gum and palate and less on the tongue. Some of the vesicles ruptured easily on examination revealing raw eroded surfaces.

Viruses Isolated:

Two virus isolates NIG. 13/81 and NIG. 2/82 were obtained from samples collected by the investigator. Six other virus isolates were obtained by the AVRI from samples collected and dispatched the World reference centre by the Veterinary authorities of the states viz NIG. 4/81, NIG.5/81, NIG. 6/81, NIG. 8/81, NIG. 10/81 and NIG. 11/81.

TABLE 4

INVESTIGATION OF OUTBREAKS

	TIME OF YEAR	FARM OR HERD LOCATION	SOURCE OF INFECTION	METHOD OF HUSBANDRY	ANIMALS AT RISK	MORB-IDITY	MORT-ALITY	MORT-ALITY RATE	VIRUS ISOLATED
1.	JAN. 1981	Fulani Herd Wase in Plateau State.	Contact with infected herd	Migrant Herd.	335	140	-	-	SAT_1
2.	JAN. 1981	Vom Plateau State.	Contact with infected herd	Settled Fulani Herd	391	107	2	1.8	SAT_1
3.	FEB. 1981	Potiskum Borno State.	Trade Cattle	Migrant Herd	329	113	-	-	SAT_2
4.	MARCH "	Gujba Borno State	Trade Cattle	Migrant Herd	79	26	-	-	SAT_2
5.	OCT. 1981	DUTSIN WAI District Kaduna State.	Contact with Herd	Migrant Herd	70	5	-	-	SAT_1
6.	JUNE 1981	Kano	Contact with Trade Cattle	Trade Cattle	56	15	-	-	SAT_1
7.	OCT. 1981	Jaji via Zaria	Contact with infected Herd	Migrant Herd	605	343	5	1.4%	SAT_1

TABLE 4

INVESTIGATION OF OUTBREAKS

	TIME OF YEAR	FARM OR HERD LOCATION	SOURCE OF INFECTION	METHOD OF HUSBANDRY	ANIMALS AT RISK	MORBIDITY	MORTALITY	MORTALITY RATE	VIRUS ISOLATED
8.	NOV. 1981	Mukani Farm Gongola State.	Contact with Trade Cattle	Ranching	407	156	1	0.75%	NVD
9.	APRIL 1982	Kangare Galambi District Bauchi State.	Contact with Trade cattle	Settled Fulani Herd	856	26	-	-	NVD
10.	JUNE 1982	Kolaku in Waja District Bauchi State.	Unknown	Fulani Migrant Herd	429	105	-	-	NVD
11.	JUNE 1982	University Farm Ibadan	Unknown	Ranchin	220	100	2	0.002%	NVD
12.	SEPT. 1982	Michika Gongola State	Infected Herd	Migrant Fulani Herd	654	106	-	-	NVD

TABLE 4

INVESTIGATION OF OUTBREAKS

	TIME OF YEAR	FARM OR HERD LOCATION	SOURCE OF INFECTION	METHOD OF HUSBANDRY	ANIMALS AT RISK	MORB-IDITY	MORT-ALITY	MORT-ALITY RATE	VIRUS ISOLATED
13.	NOV. 1982	DULAHI Sade	Contact with infected Herd	Migrant Fulani Herd	77	38	1	-	NVD
14.	SEPT. 1982	Benue-Carney Ranch	Carrier cattle	Ranching	642	42	-	-	SAT_2
15.	JAN. 1983	Alhaji Magaji Ruwa, Asara District Sokoto State	Migration of stock toand from Niger Republic	Migratory Herd	176	12	-	-	NVD
16.	AUG. 1983	Alh.Tangale Waja LGC, Bauchi State	Extract source of infection unknown but animals had contact with trade cattle.	Settle Fulani	105	73	-	-	NVD
17.	MAY 1984	Talata Mafara Sokoto State.	Unknown	Migrant Fulani Herd	125	46	1	2.1%	NVD

TABLE 4

INVESTIGATION OF OUTBREAKS

	TIME OF YEAR	FARM OR HERD LOCATION	SOURCE OF INFECTION	METHOD OF HUSBANDRY	ANIMALS AT RISK	MORB-IDITY	MORT-ALITY	MORT-ALITY RATE	VIRUS ISOLATED
18.	MAY 1984	Alh. Ciroma's Herd. Ganawu Plateau State	Contact with infected Herd.	Settled Fulani Herd	1678 made up of 3 Herds	408	2	0.49%	NVD
19.	SEPT. 1984	Sigire in Fufore LGD Gongola State.	Contact with Trade Cattle	Migrant Fulani Herd	107	50	-	-	NVD
20.	DEC. 1984	Langtang Plateau State.	Contact with infected Herd.	Migrant Fulani Herd	139	107	1	1.01%	NVD
21.	FEB. 1986	Miango Plateau State	Contact with Trade Cattle	Migrant Fulani Herd	506	243	2	-	NVD
22.	MARCH 1986	Durr in Dass LGA Bauchi State.	Source unknown	Settled Fulani Herd	91	20	-	-	NVD
23.	MARCH 1986	Bururu in Bauchi State.	Contact with infected Herd.	Migrant Fulani Herd	335	140	-	-	NVD

NVD = No virus detected

CHAPTER 4

STUDY OF OFFICIAL RECORDS ON FMD

Introduction

The keeping of records on animal diseases started in Nigeria after 1925 (Anon, 1926). Accurate records were kept on important notifiable diseases which were brought to the attention of the colonial authorities. Foot and mouth disease did not feature prominently in reports earlier than 1930 because it was presumed that the disease did not exist in Nigeria because it was not reported by nomadic herdsmen (Anon, 1930).

Records of outbreaks of Foot-and-mouth disease were kept from 1932 onward. No virus isolation was attempted before 1954 because of infrastructural difficulties of lack of appropriate laboratory to handle samples from such outbreaks.

This study was therefore to undertake retrospective examination of accumulated official data on FMD inorder to determine its apparent incidence and prevalence in the country.

MATERIALS AND METHODS

Documents examined:

(1) Records of diseases diagnosed at the National Veterinary Research Institute - Vom from 1925 - 1985.

(2) Annual reports of the National Veterinary Research Institute - Vom from 1925 - 1985.

(3) Annual reports of the Veterinary services of Northern Nigeria from 1948 - 1967.

(4) Annual reports of the Ministry of Agriculture and Animal Resources of all Northern States from 1968 - 1985.

(5) Statistics on Livestock trade and movements - Federal Livestock Department, Kaduna from 1972 - 1985.

(6) Summary of recorded Incidence of disease of cattle in Nigerian Abbatoir 1972 to 1985 (Federal Livestock Department).

(7) National Bulletin on Livestock Diseases in Nigeria (Epidemiological Department, Federal Livestock Department, Kaduna).

(8) Virus Isolations and Typing 1940 - 1985, The Animal Virus Research Institute - Pirbright, U.K.

Visits

Visits were made to the Ministries of Animal Health of the Northern States, The Federal Livestock Department - Kaduna, National Veterinary Research Institute - Vom and The Animal Virus Research Institute - Pirbright, U.K.

In each case, the incidence, prevalence, morbidity and mortality rates of FMD and animal movement records were examined.

The Chief Veterinary Officers of the Northern States were interviewed on their state's FMD outbreaks and control programmes in their state.

The Epidemiological Officer at the Federal Livestock Department was interviewed on the method of disease reporting from the states. Disease reporting forms (Returns of outbreaks) were examined to determine deficiencies in the disease reporting system.

Analysis of Accumulated Data

Data collected from sources described above were analysed to determine:

(1) Incidence (annual) and Prevalence rates of FMD.
(2) Morbidity and mortality
(3) Quarterly movement rates of animals on hoof, by rail or by truck.
(4) Quarterly movement of imported cattle

(5) Quarterly (3 monthly) distribution of outbreaks of FMD.

(6) Methods of spread of the disease

(7) Breeds and species of animal affected.

RESULTS

THE DISEASE

Outbreaks, Morbidity and Mortality

Table 2 shows the distribution of outbreaks annually since 1969 as officially reported throughout Nigeria during the period. The origins of the main epizootiological types (SAT_1, SAT_2 and A) of FMD virus that had infected animals in Nigeria were reported traceable to Chad, Cameroon and Niger Republics and Nigerian states that share a common border with these countries. No virus typing was undertaken earlier than 1950. Between 1950 - 1960 only Type O virus was isolated in Northern Nigeria.

Thus type A FMD virus apparently entered Nigeria in 1960 from Chad and Cameroon via Adamawa province of the present Gongola State - and from there it spread rapidly to other states in the Federation. It became the predominant type until 1968 when type SAT_1 first appeared in Nigeria presumably through Chad following the Westward spread of SAT_1 epizootic accross the continent of Africa. The entry of a new type of the virus was generally marked by increased number of outbreaks involving several thousands of cattle (Table 3). In 1964, a total of 68 outbreaks were reported officially, 21,223 animals were involved.

This marked a sharp rise in the number of outbreaks and number of animals involved when compared with figures recorded for the previous five years before 1964.

A similar trend of an upward surge in outbreaks and morbidity rate was noticeable in 1968. This was as a result of the entry of SAT_1 virus which caused outbreaks alongside type A which had before then become more or less enzootic. Between 1973 and 1974 there was another noticeable rise in the number of outbreaks and morbidity rate. In that year type SAT_2 first appeared in Nigeria and caused epizootics alongside type A, and SAT_1 which had now become entrenched in the country. The number of outbreaks decreased gradually until 1979 when there was another rise in the officially reported cases of FMD. This rise was apparently due to reintroduction of a new subtype of type SAT_1 from Niger Republic. A similar trend was noticeable in 1981 with the introduction of a new variant of type SAT_2 from Chad (Fig 12).

Table 2 shows the distribution of outbreaks of FMD on a three monthly-quarterly basis. Outbreaks occured throughout the year. No seasonality in the outbreak of the disease could be established.

But the last quarter of each year (October - December) which marks the beginning of the long dry season in Northern Nigeria and the begining of the movement of cattle southwards in anticipation of the dry season, has a higher frequency of distribution of outbreaks.

Disease Reporting (FMD)

The problem of reporting of outbreaks of foot and mouth disease is part and parcel of the animal disease reporting system in Nigeria. The regulations governing the reporting of classes A and B disease (FAO, Rome) are well spelt out in the various animal diseases act of Nigeria and Northern Nigeria in particular prior to Independence in 1960. A variety of problems complicate the reporting of epizootic diseases in Nigeria thus making official statistics on animal diseases inadequate and sometimes unreliable.

Infrastructural difficulties are experienced by field officers in trying to reach areas where communication is poor. Most traditional herdsmen move and migrate about over vast territories where only foot tracks are the only acess available. This inaccessibility makes it difficult in most cases for them to get veterinary assistance when there is an outbreak of disease. The attitude of a traditional herdsmen to animal disease depends largely on the

severity of the disease in question in his herd. If the disease spreads quickly and kills large numbers of animals, the herdman goes out of his way to seek veterinary assistance. In cases of mild diseases, the herdsman does not regard it as potential threat to his animals. FMD runs a mild course in zebus. Morbidity may be high or low depending on herd immunity but mortality is generally very low in adult animals, usually less than 0.7%. Thus Fulani herdsmen do not usually report outbreaks of FMD.

Samples for diagnostic purposes were collected from a small proportion of the reported cases, and only a few of these had viable virus. Between 1963 and 1973, 60 samples were submitted to the World Reference Laboratory, virus was isolated in only 17 cases. Many of the samples were probably poorly collected or inadequately preserved while in transit to the laboratory.

In 1979, a total of 22 outbreaks of FMD were reported throughout Nigeria but diagnostic samples were collected from only 7 cases out of which two different types (type A and SAT_1) of FMD virus were isolated. In like manner 25 cases were officially

reported in 1981 but samples were collected from only 8 cases. It is therefore clear that official statistics may not reflect the true epizootiological picture of FMD in Nigeria. The inadequacy of information on FMD coupled with its mild course in local cattle has created the impression that FMD is of no economic importance in Nigeria. Table 3 illustrates the number of outbreaks reported officially over a 20 year period (1964 - 1984).

Information available at the Epidemiological Unit (FLD - Kaduna) and Diagnostic Department (NVRI - Vom) show clearly that most of the disease reports sent in from the Veterinarians in the field do not give sufficient information on outbreaks.

Animal Movement

Fulani herdsmen move their cattle South towards the coast every year at the onset of the dry seasons and North again during the rains mainly in search of better pastures but also for purposes of trade. The contrast in climatic conditions between the North and South of the country has created an inbalance in animal population between the North and the South. As a result, livestock especially cattle, sheep and goats have become important articles of trade between the North and the South. Trade cattle are moved

Southwards all year round in trains, trucks and on hoof (Oyenuga, 1976, De Leauw, 1974). The volume of cattle moved south by truck is highest during September - October at the end of the rains. The volume of cattle moved on hoof is however highest in December and January. Cattle moved by truck and rail are mainly trade cattle destined for consumption at the high population centres of the South. On the other hand, the peak in movement on hoof registered in December/January are made up of breeding cattle moving south to escape the dry grazing lands and trade cattle that their owners could not afford to transport by rail or truck.

Table 1 presents data on the number of animals moved by various means of transportation and on hoof each year, as well as animals imported into Nigeria yearly from neighbouring countries. The animals moved on hoof by Nigerian herdsmen and those moved across Nigerian border from neighbouring countries constitute the greatest danger to the control of major epizootic diseases.

Spread of the Disease

Among the various usual means of spread of FMD known, contact and movement "trade route spread" of the disease were reported to be important in Nigeria. The records dating back to 1925 show that the disease

spread mostly along trade cattle routes.

Contact Spread

Contact of infected with susceptible animals is aided by the method of animal husbandry (transhumance/nomadism) and inadequate surveilance and control. Fulani herdsmen do not often report FMD outbreaks since this would result in movement restrictions and in turn in limitations of grazing land and watering points. Infected animals disseminate the virus into the environment, contaminating communal grazing land and watering points. This is exacerbated during the dry season when most herds migrate to areas around river basins resulting in high concentrations of livestock. The Gongola/Benue River Basin, the Sokoto/Rima Basin, Hadeija River Basin and the Niger River Basin become meeting point for a large population of Nigerian and migrant cattle herds from neighbouring Chad and Niger Republics. Millions of cattle share common grazing land and watering point during the long dry season making the spread of epizootic disease an easy affair.

Contact infection also takes place at cattle markets. Among the cattle markets visited, Sokoto state (Sokoto, Ilella and Jaredi), Borno

state (Maiduguri and Potiskum) and Kano State (Kano, Birnin Kudu) markets, report outbreaks of FMD frequently. Through these markets the Fulani herdsmen try to get rid of unproductive and diseased animals in their herds and even in some cases trade in breeding cattle. It is not uncommon therefore to observe outbreaks of FMD among these market stocks or for a purchased animal to develop signs and symptoms of FMD soon after it has been purchased or has mixed with infected stocks at the market.

The trade cattle that are moved mostly on hooves accross the country play the most significant role in the dissemination of FMD virus and in the spread of the disease to healthy herds. Outbreaks result from the purchase of occasional animal from trade route and mixing them with healthy village herds. It has also resulted from healthy animals grazing over or near pastures transversed by infected trade cattle. A 1968 outbreaks in Mangu - Plateau State involving 323 cattle started as a result of animals grazing over pastures on which infected trade cattle had grazed the previous week.

Animals handlers, their clothings and equipments also aid the spread of FMD virus from place to place. The role these objects play in the spread of FMD cannot be placed as highly as animal movement and contact since no control programme is presently in operation.

TABLE 1

TRADE CATTLE MOVEMENTS IN NIGERIA (1976-1981)

YEAR	LOCAL CATTLE MOVE BY TRUCK OR RAIL	IMPORTED TRADE CATTLE MOVE BY RAIL OR TRUCK	CATTLE MOVE ON HOOF
1976			
1st Quarter	101,552	46,979	77,544
2nd Quarter	117,629	47,089	70,979
3rd Quarter	123,625	28,179	54,306
4th Quarter	NA	NA	NA
1977			
1st Quarter	134,912	60,694	67,634
2nd Quarter	179,597	53,332	70,167
3rd Quarter	140,607	46,968	76,574
4th Quarter	171,380	73,844	86,098
1978			
1st Quarter	130,386	69,830	54,288
2nd Quarter	114,422	65,133	63,609
3rd Quarter	143,460	77,335	78,655
4th Quarter	188,537	104,622	99,931
1979			
1st Quarter	171,430	91,559	90,950
2nd Quarter	170,379	96,216	82,681
3rd Quarter	171,651	73,500	42,631
4th Quarter	177,018	85,650	60,866
1980			
1st Quarter	175,499	100,628	41,358
2nd Quarter	191,904	85,993	16,843
3rd Quarter	156,787	76,852	13,462
4th Quarter	142,598	92,333	14,947
1981			
1st Quarter	199,249	97,569	18,484
2nd Quarter	175,152	106,259	18,479
3rd Quarter	168,030	95,793	30,637
4th Quarter	183,672	114,952	37,774

SOURCES: Federal Livestock Department, Statistics on Livestock Trade, Kaduna (1977 - 1983).

NA = Information not available.

TABLE 2

SEASONAL DISTRIBUTION OF OUTBREAKS

YEAR	1ST QUARTER JANUARY - MARCH	2ND QUARTER APRIL - JUNE	3RD QUARTER JULY - SEPTEMBER	4TH QUARTER OCTOBER - DECEMBER
1969	-	1	2	3
1970	-	2	2	12
1971	-	4	3	2
1972	6	1	8	8
1973	2	6	8	26
1974	3	20	2	4
1975	3	2	4	6
1976	2	2	3	2
1977	3	2	4	6
1978	2	2	2	8
1979	11	7	8	5
1980	2	2	4	9
1981	8	2	8	2
1982	3	-	-	-

SOURCES: (1) Federal Livestock Department, Statistics on Livestock Diseases 1969 - 1982.

(2) National Veterinary Research Institute, Vom. FLD file.

TABLE 3

SUMMARY OF OUTBREAKS REPORTED 1964 - 1984

YEAR	NO OF OUTBREAKS	NO OF CATTLE INVOLVED	MORTALITY	% MORTALITY	TYPE OF VIRUS ISOLATED	AREAS AFFECTED
1964-65	70	19,128	59	0.65	A	Borno, Gongola, Kaduna, Kano, Plateau State.
1965-66	21	2,404	3	0.08	A	Kaduna, Kwara
1967-68	60	10,338	20	0.03	A, SAT_1	Sokoto, Borno, Kano, Gongola, Plateau, Kwara.
1968-69	18	3,169	3	0.19	SAT_1	Borno, Kaduna, Bauchi, Kano, Kwara, Plateau.
1969-70	2	751	0	0	SAT_1	Benue, Plateau
1970-71	5	1,813	3	0.16	A	Borno, Bauchi, Gongola, Sokoto, Kano and Kaduna.
1971-72	21	5,326	1	0.018	A	Sokoto, Kaduna, Kano States.
1972-73	40	8,967	6	0.06	A, SAT_1	Borno, Bauchi, Kano, Gongola, Kaduna, Plateau.
1973-74	60	20,000	50	0.25	A, SAT_2	Borno, Bauchi, Kwara, West, Lagos, Gongola, Sokoto, Kano.
1974-75	28	13,625	64	0.46	A, SAT_2	Sokoto, Borno, Bauchi, Gongola, Niger, Benue, Anambra.
1975-76	4	-	-	-	A, SAT_1	Kano, Kaduna, Borno, Plateau, Oyo, Gongola.

TABLE 3

SUMMARY OF OUTBREAKS REPORTED 1964 - 1984

YEAR	NO OF OUTBREAKS	NO OF CATTLE INVOLVED	MORTALITY	% MORTALITY	TYPE OF VIRUS ISOLATED	AREAS AFFECTED
1977	3	826	-	0	-	Oyo, Borno
1978	6	819	1	0.12	-	Kaduna, Lagos, Borno, Bauchi.
1979	22	3,966	7	0.17	A, SAT_1	Niger, Kano, Sokoto
1980	19	4,565	8	0.017	SAT_1	Borno, Bauchi
1981	25	7,865	68	0.13	SAT_1	Borno, Kano, Kaduna
1982	14	368	-	0	SAT_2	Bauchi, Anambra
1983	5	165	-	-	NVD	Kaduna, Sokoto and Ondo
1984	4	426	7	1.6	NVD	Kaduna, Sokoto and Gongola
1985	7	1,591	7	0.43	NVD	Plateau, Kano and Benue

SOURCES: FLD - Epidemiological Department, Kaduna

NVRI - Vom - Diagnostic Records

AVRI - Pirbright - Typing of FMDV

NVD - No virus detected

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CHAPTER 5

ANTIBODIES TO VIA AND 146S ANTIGENS IN NIGERIAN CATTLE

Introduction

Antibodies represent foot prints either faint or distinct, of an infection experienced in the remote or recent past. When animals are infected with foot and mouth disease virus, antibodies are generated against the three major antigens produced as a result of the replication of the virus in the animal.

Antibodies produced against the structural capsid protein (146S and 12S) are type specific while antibodies against VIA antigen are not type specific. VIA antibodies produced as a result of FMD virus infection peak at 9 - 14 days (Crowther, 1984) post infection and could be detected in such animals up to nine month post infection by Agar gel diffussion test (Lobo et al, 1976).

VIA antibodies have been detected in animals that have recovered from FMD infection or are carriers of the virus (Hedger and Pinto, 1977). Detection of VIA and 146S antibodies in animals can therefore provide answers to many vital questions to which answers could not be provided from information available from the disease reporting organ of government. This study is therefore aimed at

determining (i) the prevalence of FMD in Nigerian cattle generally and in cattle under different management conditions.

(ii) the presence of apparently healthy carriers of FMD among cattle and other ruminant populations.

(iii) the serotypes of FMD virus that have been active in Nigeria.

MATERIALS AND METHODS

Viruses

The following FMD viruses were used in the test:

01 PACHEKO } they were supplied by the World Reference
OBFS 1860 } Laboratory - Pirbright.

SAT_1 NIG.4/81 - Isolated in Borno State in 1981, underwent two passages in Bovine thyroid (BTY) cells and two passages in Baby hamster kidney (BHK).

SAT_2 NIG. 8/81 - Isolated from Ikyogen-Benue State in 1982 underwent one passage in BTY cells and two passages in BHK cells.

A NIG. 4/79 - Isolated in Niger State in 1979, underwent two passages in BTY cells and two passages in BHK.

Tissue Culture

Baby hamster kidney (BHK), strain 21, clone 13 cells (Macpherson and Stoker, 1962) were grown in Eagles medium with 10% tryptose phosphate broth, 10% bovine serum and antibiotics (100 units per ml penicillin and 100 units per ml polymixin). Cells were grown in Roux flask incubated at 37°C until confluent.

Conjugate

Rabbit anti-Bovine IgG conjugated with Horse radish peroxidase, Rabbit anti-Guinea pig IgG and Rabbit anti goat IgG all conjugated with horse radish peroxidase were obtained from Dakoppatts, Copenhagen. Working dilutions of 1:1000 of the conjugate was determined by means of a checker board titration. PBS with an addition of 0.005% Tween - 20 and 3% ovalbumin was used as diluent for the conjugates. Working solutions were prepared daily before the test and kept at + 4°C.

Media The following culture media were supplied by the tissue culture preparation unit of AVRI - Pirbright.

(a) Eagle's medium (Eagle, 1955)

(b) Eagles overlay medium: Eagles medium containing 0.65% Noble agar (W/V) final concentration.

(c) Versene-Trypsin solution obtained from the tissue culture unit at AVRI and consisting of 8.0gm NaCl, 1.0gm glucose, 0.1 Trypsin 0.1gm versene, 0.55gm $NaHCO_3$, distilled to make one litre, 0.1% phenol red and antibiotics (100 units per ml. penicillin and 100 units per ml polymixin, pH 7.6.

Microtitre Plates for ELISA

Rigid tissue culture polystyrene plates (Nunc Immuno 96F Plate 1, Nunc Intermed, Denmark) were used in all tests. Decision to use this particular plates was based on Mc. ullough and Parkison (1984) findings on biding capacity of the Nunc Immuno plates.

Enzyme Substrate

Orthophenyenedimine dihydrochloride (OPD) (Sigma Chemical CO) was prepared by dissolving 5.11gm citric acid, 9.15gm Na_2HPO_4 and 0.4gm OPD in one litre of water pH 6.6.

DE - 52 Cellulose Diethylaminoethyl cellulose - ion echanger for column chromatography was obtained from Whatman Ltd. Springfield Hill, Kent England.

SUCROSE was obtained from BDH Chemical Ltd., Poole - England. Bovine serum albumin (BSA) was obtained from Miles Laboratory, U.K.

Tween - 20

Polyoxyethylene sorbitan monolaureate was obtained from Sigma Ltd., U.K.

Acetylethyleneimine (AEI) was obtained from Welcome Research Laboratories, Beckenham, U.K.

Antigen and its fixation to the carrier

VIA antigen prepared as described below was fixed to microtitre ELISA plates in PBS/Tween buffer pH 7.4 at 37°C for 2 hours. The working dilution of VIA antigen was determined by checkerboard titration with guinea pig anti VIA IgG.

Washing Solution

The plates were washed with PBS with 0.05% Tween-20.

Antisera

O_1 PACHEKO Guinea pig anti 146S serum was kindly supplied by the WRL. Guinea pig anti VIA serum was raised by immunising guinea pigs.

Cattle Sera

Cattle sera were collected from non vaccinated Nigerian cattle based on random sampling. Three categories of sera samples were collected (Table 13).

(a) Sera from Fulani migrant herds and

(b) Sera from government and privately owned ranches.

(c) Sera from trade cattle meant for slaughter at abbatoirs or collected at trade cattle markets.

In the case of group (a), not less than 20 sera samples were collected randomly from 5 Fulani herds in each of the states surveyed. Thus a total of 100 sera from Fulani migrant herds. In case of group (b), 50 sera samples were collected from 3 government or privately owned cattle ranches. Thus 150 samples were collected from this group of animals. In the case of group (c) 500 samples were collected randomly from each state.

Blood was collected in 10ml syringes individually from each animal's jugular vein. Blood was allowed to clot in the syringe and retracted. The sera were collected in individual Bijou bottle, labelled, stored in ice chests until transported to the laboratory where they were stored at -20^{o}C. Each serum sample was diluted one hundred fold with PBS containing Tween-20 and bovine serum albumin for the VIA ELISA test.

Preparation of VIA antigen

Twenty Roux flasks of BHK-21 cells were infected at high multiplicity (10-100 pfu/cell) with FMD virus

01 Pacheko. After 6 hours, the cells were frozen at -20°C, thawed and harvested 24 hours later. Harvests were clarified by centrifugation at 2,000 x g for 20 minutes. Chloroform was then added at 10% volume of harvest supernatant and this was stirred for 2 hours at 4°C. The chloroform and the lipid containing layer was then removed after centrifugation at 2000 x g for 20 minutes and the remaining bottom layer was clarified by filtration through 'O' grade Whatman filter paper. The filtrate was then added to 20 CM^3 of DE 52 cellulose (Whatman) in 0.015M Nacl in disposable syringe, sealed at the bottom with an 'O' grade filter pad. Whole virus (146S) and subunits (12S) were eluted from the cellulose column with 0.015M Nacl in PBS using twice the volume of the supernatant. The remaining attached protein mainly VIA antigen was eluted with 1M Nacl PBS, pH 7.4. Fractions of the eluate were collected in bijou bottles and the protein content estimated spectrophotometrically at 280nm. The peak fraction containing VIA was concentrated and dialysed against PBS containing 0.015M Nacl. The antigen was further purified by centrifugation on a linear 5-25% sucrose density gradient (6 x 37ml in 42-1 Beckman Rotor).

Each VIA preparation was tested against guinea pig anti VIA and anti 146S IgG. Only VIA preparation showing no positive reaction with anti 146S IgG was used in the tests.

Preparation of 146S Antigen

Each FMD virus listed above was grown in monolayers of BHK-21 tissue culture cells, concentrated with polyethyleneglycol (PEG), sedimented and pelleted at 30,000 rmp for 2½ hours, resuspended in tris-buffer with 0.5M Nacl and purified by sucrose gradient centrifugation (Brown and Cartwright, 1963). The concentration and gradient profiles of each FMD virus was determined spectrophotometrically at 280nm using the method of Bachrach et al., (1964). Purified (146S) virus was divided into aliquots and stored away at -70°C in siliconised glass vials.

Titration of VIA on Microtitre Plates

The VIA antigen was diluted in a 2 fold series (8 rows accross using a multichannel pipette) in the microtitre plate. Plates were incubated at 37°C for 2 hours on an Orbital Shaker and later washed with PBS/Tween.

Serial two fold dilutions of a control serum for VIA antigen were made and added to the sensitised plates. Thus giving a chequeredboard titration of antibodies and antigen. After 2 hours incubation at 37°C the plates were washed, and 1:1000 dilution of HRP conjugated antispecies antiserum was added and the plates incubated a further 1 hour at 37°C. The plates were washed again and the enzyme substrate added. The reaction was stopped by adding 1.25M H_2SO_4 after 15 minutes when colour had developed. Fig. 2 shows a typical result for such a titration relating the colour developing in the wells sensitised with different concentrations of antigen for different serum titration curves. From this an optimum level (as indicated in Fig. 2 for the antigen to sensitise the plates was determined.

Further tests were carried out using the chosen dilution of VIA antigen to examine the reaction of serum showing no antibody against VIA antigen as compared to positive sera, in order to establish levels of non specific reactions of the various antiserum dilutions under standard conditions. Other tests to establish the optimum conjugate dilution was also carried out using an optimum concentration of VIA antigen, constant known positive and negative

antiserum, titrations were made using 3 different dilutions of the conjugate. In this way, the concentration of VIA antigen, the relationship of positive and negative serum titration curves and the dilution of conjugate necessary to give optimum colour in 15 minutes was established.

Indirect ELISA

Antibodies against VIA antigen were measured by an indirect micro ELISA test (Abu Elzein and Crowther, 1978) as modified by Crowther (1984). 50 microlitres of antigen diluted in PBS were adsorbed unto micro ELISA plates by incubation at 37°C for 2 hours. The plates were washed 5 times with PBS and flicked free from residual liquid. Aliquots (50ul) of antiserum dilutions (Bovine serum diluted 100 fold) in PBS-Tween were added to the test wells. Each serum sample was tested in duplicate. The plates were incubated for 2 hours at 37°C on an orbital shaker (Luckhams, England), then washed 5 times with PBS to remove unattached (unreacted) antibodies. Rabbit anti cow immunoglobulins conjugated to horse raddish peroxidase (Dako pattis,Denmark) diluted in PBS containing 1% bovine serum albumin (BSA) and 0.05% Tween 20 were added at 50 microlitre to each well.

The plates were again incubated for 30 minutes on orbital shaker, washed 5 times with PBS and orthophenylenediamine (OPD) substrate containing hydrogen peroxide was added at 50 microlitre per well. Colour developed within 30 minutes and the reaction was stopped with the addition of 50ul per well of sulphuric acid (1.25M).

Antigen, antiserum, conjugate and normal (FMD VIA negative) cattle sera were included in the test. Results were read spectrophotometrically at 492nm using Titertek multiscan (Flow Laboratories, Irvine, Scotland) ELISA reader. The mean of the optical density (OD) readings recorded for the normal (negative) bovine serum control wells were then substracted from each of the corresponding test wells.

Standardisation of ELISA

(a) Standardisation of Conjugate

To determine the optimum working dilution of the conjugate, (Manufacturers' recommendation 1/100 to 1/2000 dilution for ELISA tests) plates were sensitised with two fold dilutions of VIA in PBS and used in a checkerboard type titration with guinea pig anti VIA serum - 50ul per well. Control wells were similarly treated. Plates were incubated at 37°C for 2 hours and washed five times with PBS/T.

Bovine reconvalescent serum (known to be positive for VIA antibodies) were added at a dilution of 1:100 and 50 ul per well and incubated for 1 hour at 37°C, washed after incubating 3 times with PBS/ Tween. Four dilution strengths of the conjugate (1:500, 1:1000, 1:1500, 1:2000) were made in PBS/ Tween containing 1% BSA. Each dilution was added to four rows of wells at 50ul per well and incubated for 1 hour at 37°C. The plates were washed and substrate added at a predetermined concentration 50ul/well. The reaction was stopped with 1.25M H_2SO_4 after colour had developed at room temperature 20°C for 15 minutes. Colour intensity was read in an ELISA reader. The appropriate dilution of conjugate was used in all ELISA tests.

(b) Standardisation of Levels of Positivity and negative of Bovine Sera

In order to control the VIA ELISA, known positive and known negative Nigerian cattle serum were usually incorporated to each test plate. To establish the level of positivity, (acceptable positive OD reading) plates were sensitised with 2 fold dilution series of VIA - Plates were incubated at 37°C for two hours, washed five times and ten

different known positive bovine serum and known negative bovine serum on separate plates were added in 2 fold dilutions in checkered fashion and incubated at 37°C for 1 hour. The plates were washed three times after incubation with PBS/Tween. Rabbit antibovine IgG conjugate at predetermined concentration was added to each well at 50ul per well, incubated for 1 hour at 37°C and washed three times. Substrate was then added at predetermined concentration and colour allowed to develop for 15 minutes at room temperature and stopped with sulphiric acid. The plates were read and ODs recorded.

(c) Effect of Bovine Serum inactivation on the VIA ELISA reaction

To determine the effect of heat inactivation of Nigerian bovine sera on VIA ELISA reaction, a group of twenty Nigerian sera were diluted 1:100. Each serum dilution was divided into two groups (A and B). Group A sera were heat inactivated at 56°C for 30 minutes before they were used in VIA ELISA test. Group B sera were not inactivated and were used in parallel to group A sera. These plates were then used in indirect ELISA using VIA.

(d) Determination of Background reaction by Bovine Sera on Plates

To determine the level of background colour reaction of the rigid polystyrene plate used (Nunc Immuno-plates), a plate was divided into 3 groups of rows (3 horizontal rows per group). Group 1 and 2 rows were sensitised with 2 fold dilutions of VIA antigen. Group 3 rows were not sensitised with VIA. The plate was incubated at 37°C and washed 5 times with PBS/Tween. Known positive bovine serum (1:100 dilution) was added at 50ul per well to replicate wells per VIA dilution to group 1 wells. Known negative bovine serum (1:100) was added at 50ul per well to replicate wells per non VIA wells in group 2. Group three had no serum. The plates were incubated for 1 hour at 37°C and washed 3 times. Routine ELISA was then performed.

Preliminary Experiment in the Application of VIA Indirect ELISA

Preliminary studies were carried out to detect VIA antibodies from a collection of bovine post infection (natural and experimental) sera using a dilution method in which sera were diluted in a two fold series and added to VIA coated plates in a checkered board form. The plates were used in routine ELISA.

Detection of antibody against FMD Purified Virus (146S) in Cattle Sera.

Viruses (146S) purified by the method described above were used as antigen in indirect ELISA to detect levels of 146S antibodies to four FMD virus types (A, O, SAT_1 and SAT_2). The standard indirect ELISA procedure was used. Virus concentrations (4 ugm/ml) were diluted in carbonate buffer and added to microtitre plates 50ul per plate. The plates were incubated at 37°C for 2 hours and used in indirect ELISA.

Determination of Levels of 146S antibodies against four different types of FMD Virus

Plates were subdivided into four parts and each part coated with appropriate concentrations 4 ugm/ml of FMD type A, O, SAT_1 and SAT_2, 146S antigen as determined above. The 146S antigens to the four serotypes were prepared as described earlier. The plates were incubated at 37°C for 2 hours and unattached viruses washed off.

Bovine sera (diluted 1:100) were added to each well at the rate of 50ul per well and incubated at 37°C for 1 hour. ELISA was carried out to measure antibodies to 146S antigen.

RESULTS

Preparation of VIA Antigen

Fig (1) shows the profile of VIA antigen on sucrose density gradient. The additional one step purification by sucrose density gradient centrifugation led to the preparation of VIA antigen devoid of other subparticles of FMDV. Fig (2) illustrates the titration of VIA antigen preparation against guinea pig anti VIA serum, guinea pig anti 146S serum and bovine reconvalescent serum. The reaction of the VIA preparations against anti VIA serum was specific. None of the VIA preparations used reacted against anti 146S serum.

Titration of reconvalescent, Prevaccination and Post Vaccination Sera

Fig (3) illustrates the titration of the three sera against standard dilutions of VIA antigen in a chequeredboard titration. The VIA antigen discriminates between reconvalescent serum which is expected to contain anti VIA antibodies and prevaccination and post vaccination sera which is not expected to contain anti VIA antibodies.

Standardisation of ELISA

(a) Standardisation of Conjugate

Fig (4) illustrates the titration of various dilutions of the conjugate against standard working dilution of VIA antigen. A working dilution of 1:1000 was therefore chosen for the test since it is the dilution that produces a curve very close to the standard curve of VIA and anti VIA serum.

(b) Standardisation of levels of positivity and negativity of Nigerian bovine sera

The OD readings of the various known positive and known negative sera at VIA's standard working dilution of 1:50 were recorded and an average of three titration taken. The average values were used as control for all subsequent titrations into which known positive and known negative control sera were incorporated. After a given titration of field samples, the OD reading of the positive and negative control sera were compared with the standard average. Any value of control sera exceeding or below the standard positive value were regarded as abnormal and the test was repeated.

(c) Effect of heat inactivation of sera on VIA ELISA

Figure 5 illustrates the titration of a group of twenty selected field sera that had been heat inactivated. It could be seen that the OD reading of the heated sera do not vary more than 30% from the mean OD values of the non heat inactivated sera. Hence it was decided that all sera samples would be tested as heat inactivated.

(d) Determination of background reaction of bovine sera on Plates

Figure (6) illustrates the titration curve of VIA antigen against known positive bovine serum and non VIA coated wells against known positive bovine sera. The result shows that although some amount of background colour was produced by the bovine sera that sticks to the wells, the stickiness was not significant to overshadow the reaction of VIA antigen with anti VIA antibodies present in the bovine sera examined.

Titration of VIA on Microtitre Plates

A chequered board type titration of VIA antigen preparation against anti VIA serum and anti 146S serum raised in guinea pigs demonstrated the degree of purity of the VIA preparation (Fig. 2). The working dilution

of the VIA antigen and antiserum was also determined from the titration. The VIA was used at 1:50 dilution in PBS, while the 146S antiserum was used 1:2 dilutions.

Other preliminary titrations using the working dilution of the VIA helped in establishing the working dilution of other reagents (field serum samples, conjugate and substrate).

Serological Survey using Indirect ELISA

The result of the Indirect VIA ELISA serological survey is illustrated in tables (14a and 14b). About 9.8% of the 5,200 cattle sera examined had demonstrable antibodies to VIA antigen. About 37.96% of the 324 goat and sheep sera examined were positive. Tables (16 and 17) illustrates FMD VIA antibody profile distribution by farms. Among the abbatoir samples, Bauchi with (24% positive) and Plateau (with 35% positive) have largest numbers of abbatoir animals with VIA antibodies whereas Niger state (3%) had the lowest.

Among the private ranches tested, samples from Galambi ranch in Bauchi (62%) was highest as against ranches in Kano state and Gongola state with 4% positives. Benue-Carney ranch (60%) is an exceptional case. There was an active infection when the sample was collected at the farm.

High percentage positives were recorded among government ranches in Kaduna state (20%) and Bauchi state (40%). Samples obtained from migrant Fulani herds were generally low, although those in Kano and Plateau state (20.3%) and (17.3%) respectively were the highest. Samples from Sokoto migrant herds with 7.4% was lowest.

Serological Survey using 146S antigen in an Indirect ELISA

The result of serological survey using 146S antigen is shown in table 17a and 17b. About 55.9% of the 5,200 cattle sera examined had antibodies to whole virion (146S) particle indicating that about 56% of the animals had FMD infection in the past.

Plateau state (82.6%) had the highest positive while Gongola and Sokoto States had the lowest (46.3% and 47.8% respectively). The 100% rate recorded for Benue is peculiar because samples were taken from only the Benue-Carney ranch where an active outbreak of the disease was reported at the time of investigation. Table 18 summarises the anti 146S antibody profile according to Farm or husbandry method. The frequency of positivity was high in abbatoir samples. The abbatoir samples from Jos, Plateau State with (83%) stand out to be exceptionally high. High percentage positives were also recorded

for samples from some private ranches. Bauchi (100%) and Sokoto (20%) had the highest and lowest rates respectively among private ranches. Bauchi and Kaduna states with 76% each led the government ranches, whereas Gongola (30%) had the lowest reactor rate among government ranches.

Detection of 146S antibodies against four types of FMD Virus

Table 19 illustrates the result of titration of 8 field serum samples against 146S antigen. The samples were from a collection of Sudanese sera known to have all the four types of the virus. It was obvious from the result that though some amount of crossreactivity was exhibited by each sera sample against the four antigens, the homologous reaction was always higher as indicated by higher OD readings. Hence the test system was adopted to detect from Nigerian cattle sera which of the four virus types was most active in particular areas of the country.

Determination of levels of 146S antibodies against three types of FMD Virus

Table 18 illustrates the result of the serological survey with 146S whole virion antigen. About 56% of the 5,200 cattle sera examined reacted positively with 146S purified antigen. Figures 8 - 14 show the distribution of antibodies to three types A, SAT_1 and SAT_2 FMD virus. From the state wide distribution of

antibodies to the three types, Borno and Sokoto states showed an indication of recent activity of type A FMD virus. In Gongola state, Type SAT_1 and SAT_2 were recently active. In Kano, Bauchi and Kaduna states on the other hand reaction to the antigens of the three types seem equal in amplitudes.

TABLE 13

NO. OF SERUM SAMPLES OBTAINED FROM CATTLE IN EACH STATE

STATE	ABBATOIR	FARMS	TOTAL
Sokoto	500	291	791
Bauchi	500	310	810
Kano	500	179	679
Kaduna	300	345	645
Plateau	200	155	355
Borno	500	355	855
Gongola	500	315	815
Niger	*200	-	200
Benue	-	*50	50
Total	3,200	2,000	5,200

TABLE 14a

THE DISTRIBUTION OF ANTIBODIES TO VIA ANTIGEN
BY FARM OR LOCATION OF SAMPLES

STATE	FARM OR LOCATION	NO. SAMPLE	NO. POSITIVE	% POSITIVE
Kano	Kano Abbatoir	500	31	6.2
	Alh. Bala Herd	25	1	4
	Kano Cattle Mkt.	50	6	12
	Tiga Ranch	50	8	16
	Alh. Zogarwa	39	11	28
	Alh. Sumaila	15	0	0
Kaduna	Kaduna Abbatori	300	24	8
	Kaduna Cattle Mkt.	50	5	10
	Alh. Buruku Herd	50	7	14
	Manchok Ranch	45	9	20
	Shuaib Maikidi Herd	50	20	48
	Zaria Abbatoir	150	10	6
Borno	Maiduguri Abattoir	500	18	3.6
	Maiduguri Cattle Mkt.	100	8	8
	Borno Ranch	30	2	6.6
	Borno LIBC.	25	0	0
	Potiskum Mkt.	200	26	13
Gongola	Yola Abbatoir	500	22	4.4
	Jalingo Dairy	25	3	12
	Jalingo Abb.	20	6	30
	Mubi LIBC	80	4	5
	Gongola Valley Ranch	50	2	4
	Kofare Dairy	30	0	0
	Mambilla LIBC	50	2	4
	Alh. Inuwa	30	4	13
	Gongola LIBC	30	3	10
Niger	Minna Abbatoir	200	6	3
Benue	Benue/Carney	50	30	60

TABLE 14b

THE DISTRIBUTION OF ANTIBODIES TO VIA ANTIGEN BY FARM OR LOCATION OF SAMPLE

STATE	FARM OR LOCATION	NO. SAMPLE	NO. POSITIVE	% POSITIVE
Sokoto	Sokoto Abbatoir	500	31	6.2
	Shagari Farm	30	3	10
	Furdi Farm	30	5	10
	Dabagi Dairy	25	0	0
	Alh. Asara	100	5	5
	B/Kebbi Dairy	75	3	4
	Alh. Jeppi	11	2	18.1
Bauchi	Bauchi Abbatoir	500	31	6.2
	Bala Jatau Herd	25	1	4
	Galambi Ranch	50	31	62
	Darazo LIBC	30	12	40
	Alh. Shaib Herd	200	15	7.5
	T/Waja	30	6	20
Plateau	Jos Abbatoir	200	70	35
	Alh. Chiroma	86	6	6.9
	Alh. Sabo	50	18	36
	Alh. Subairu	20	3	15
	Total	5,200	510	9.8%

TABLE 16

DISTRIBUTION OF ANTIBODY TO VIA BY FARM WITHIN EACH STATE

STATE	ABBATOIR	PRIVATE RANCH	GOVT. RANCH	MIGRANT HERD
Sokoto	6.2	10	3	7.4
Bauchi	24	62	40	9
Kano	6.2	4	12	20.3
Plateau	35	NT	NT	17.3
Borno	3.6	NT	3.6	13
Gongola	4.4	4	6	12
Niger	3	NT	NT	NT
Benue	NT	60	NT	NT

NT - Not tested

TABLE 17a

DISTRIBUTION OF ANTIBODIES TO 146S ANTIGEN

STATE	FARM OR LOCATION	NO. SAMPLED	NO. POSITIVE	% POSITIVE
Sokoto	Sokoto Abbatoir	500	227	45.4
	Shagari Farm	30	6	20
	Furdi Farm	50	18	36
	Dabagi Dairy Farm	25	21	84
	Asara Fulani	100	58	58
	B/Kebbi Dairy	75	40	53.3
	Alh. Jeppi	11	8	72.7
Bauchi	Bauchi Abbatoir	500	280	56
	Galambi Ranch	50	50	100
	Darazo Ranch	30	30	100
	Alh. Shaib Herd	200	104	52.0
	T/Waja LIBC	30	16	53.3
Kano	Kano Abbatoir	500	286	57.2%
	Alh. Bala Herd	25	16	64%
	Kano Cattle Mkt.	50	29	58.0
	Tiga Ranch	50	20	40.0
	Alh. Suleiman Zogaruwa	39	28	71.7
	Alh. Balarabe Sumaila Herd	15	8	53.3
Kaduna	Kaduna Abbatoir	300	173	57
	Kaduna Cattle Mkt.	50	21	42
	Buruku Fulani	50	38	76
	Manchok Ranch	45	30	66.6%
	Shuaib Maikidi Herd	50	24	48
	Zaria Abbatoir	150	86	57
Plateau	Jos Abbatoir	200	167	83
	Alh. Chiroma Herd	86	71	82
	Alh. Sabo Herd	50	39	78
	Alh. Subairu Herd	20	17	85

TABLE 17b

STATE	FARM OR LOCATION	NO. SAMPLED	NO. POSITIVE	% POSITIVE
Borno	Maiduguri Abbatoir	500	260	52
	Maiduguri Cattle Mkt.	100	17	17
	Maiduguri Dairy Farm	30	30	100
	Borno LIBC	25	14	56
	Potiskum	200	150	75
Gongola	Yola Abbatoir	500	261	52
	Jalingo Dairy	25	8	32
	Jalingo Abbatoir	20	12	60
	Mubi LIBC	80	21	26.2
	Gongola Valley Ranch	50	28	56
	Kofare Dairy	30	5	16
	Mambilla Fulani	50	8	16
	Gongola LIBC	30	15	50
	Alh. Inuwa Herd	30	19	63
Niger	Minna Abbatoir	200	132	66
Benue	Benue Carney Ranch	50	50	100

TABLE 18

DISTRIBUTION OF ANTIBODIES TO 146S BY FARM WITHIN EACH STATE

STATE	ABBATOIR	PRIVATE RANCH	GOVT. RANCH	FULANI HERD
Sokoto	45%	20%	61%	52%
Bauchi	56%	100%	76%	52%
Kano	57%	58%	48%	67%
Kaduna	57%	67%	76%	48%
Plateau	83%	NT	NT	81%
Borno	52%	NT	59%	75%
Gongola	52%	56%	30%	34%
Niger	66%	NT	NT	NT
Benue	NT	100	NT	NT

NT = Not Tested

% = Positive to 146S Antigen

TABLE 19

TITRE (OPTICAL DENSITY READINGS OF EIGHT SERA SAMPLES TITRATED AGAINST FOUR TYPES OF FMD VIRUS 146S ANTIGEN

SERUM NO.	TYPE O	TYPE A	TYPE SAT_1	TYPE SAT_2
1	.35*	.34	.32	.18
2	.60	.68	.47	.61
3	.18	.37	.48	.00
4	.17	.29	.70	.62
5	.47	.22	1.08	.00
6	.42	.61	.33	.33
7	.13	.02	.17	.00
8	.32	.22	.39	.56

*Arithmetical mean of readings from 3 wells.

CHAPTER 6

COMPARISON OF RECENT NIGERIAN ISOLATES WITH STANDARD VACCINE STRAINS

Introduction:

One of the principal characteristics of FMD virus is its serological diversity. There are seven distinct serotypes which demonstrate no cross-protection and over 60 subtypes which demonstrate only partial crossprotection (Pereira, 1977). Intratypic serological differentiation of virus strains, therefore constitutes a major activity of FMD laboratories engaged in epidemiological studies and also those involved in vaccine selection and production.

Differences between two isolates of the same serotype may be such that vaccines prepared from one isolate may fail to confer adequate protection against the heterologous virus, sometimes even when the two isolates have been considered to belong to the same subtype (Pay, 1972). FMD viruses belonging to the same subtype undergo variation continuously probably as a result of immunological selection and other selection pressures giving rise to continuous antigenic variation with progressively greater differences as

successive variant move away from the parent virus. Evidence from experimental infections (Hyslop and Fagg, 1965; Fagg and Hyslop, 1966; Federer et al., 1968) and natural diseases (Arrowsmith, 1975) have been obtained in support of such variations. For epidemiological purposes, it has been suggested that a comparison of new isolates with reference vaccine strains relevant to the particular geographical area is more important to the control of an outbreak rather than simple classification into subtype groups (Pereira, 1977 and Rweyemamu, 1984).

It is therefore the aim of this study to examine recent isolates of FMD virus (1981-84) with existing and current vaccine strains applicable to Nigeria in order to determine whether or not new isolates have departed from current vaccine strains and whether these vaccines would protect against current field strains.

MATERIALS AND METHODS

Microtitre Plates

Rigid tissue culture polystyrene plates (Nunc.).

Buffer Phosphate buffer M/25 consisted of 6.10gm Na_2HPO_4 $2H_2O$ and 0.78gm KH_2PO_4 in one litre of distilled water, pH 7.6.

Phosphate buffered saline (PBS) consisted of 8.0gm Nacl, 0.2gm KH_2PO_4, 2.9gm Na_2PO_4, $12H_2O$, 0.2gm Kcl in one littre of water pH 7.4.

Veronal Buffered Saline was freshly prepared from oxoid tablets. (Oxoid Ltd., UK).

Red Blood Cells (RBC) A working suspension of sheep red blood cells (SRBC) was prepared (for use in CFT) on the day of the test. The SRBC (supplied by Tissue Culture Services Limited in Alservers solution) were washed three times in buffer and packed at 700g for 5 minutes using a bench centrifuge (MSE superminor at 2000 rpm). An approximately 3% cell suspension was prepared and adjusted to 2.8%.

Haemolytic Indicator System

Rabbit anti - SRBC serum (haemolysin) was obtained from Burroughs Welcome. The haemolysin was titrated in a chequered board format against complement. Aliquots of 2.8% SRBC Suspension were mixed with equal volumes of varying (2 fold) dilutions of haemolysin. Sensitisation was for 30 minutes at room temperature. Complement dilutions were prepared in a two fold series from 1/10 and held at 4°C until used. 50ul of each complement dilution was dispensed into a microtitre plate followed by 50ul of buffer (in lieu of antigen and antibody - 25ul each). The reagents were incubated for 1 hour at 37°C prior to the addition of sensitised SRBC suspension.

The plates were sealed with adhesive tape, incubated for 30 minutes at 37°C with shaking midterm, and subsequently centrifuged at 3000g for 5 minutes to sediment unlysed cells before recording the result.

Guinea Pig Complement

Serum from male guinea pigs taken after 24 hours starvation was used as a source of complement and stored in small volumes at -70°C.

Media were supplied by the tissue culture preparation unit of AVRI, Pirbright.

(a) Eagles medium (Eagle, 1955).

(b) Eagles overlay medium - Eagles medium containing 0.65% Noble Agar (W/V), final concentration.

(c) Versene - Trypsin solution obtained from the tissue culture unit of AVRI and consisting of 8.0gm Nacl, 1.0gm glucose, 0.1gm trypsin, 0.1gm Versene, 0.55gm $NaHCO_3$ and distilled water to make one litre, 0.1% phenol red and antibiotics (100 units per ml Penicillin and 100 units per ml. polymixin).

Tissue Culture

Baby Hamster kidney (BHK), strain 21, clone 13 cells (Macpherson and Stoker, 1962) were grown in Eagles medium with 10% tryptose phosphate broth,

10% bovine and antibiotics (100 units per ml penicillin and 100 units per ml. polymixin). Cells were grown in Roux flasks, incubated at 37°C, until confluent.

Polyethylene Glycol (PEG Molecular weight 6000) 7% W/V was used in the precipitation of virus from tissue culture suspensions. It was obtained from Koch-Light Laboratory Limited.

Acetylethyleneimine (AEI) was obtained from Welcome Research Laboratories, Bekenham, U.K.

Viruses

A total of 13 FMD isolates were used in this study. Eight type SAT_1 strains were used. NIG.4/81, NIG. 5/81, NIG. 10/81, NIG. 11/81 and NIG. 13/81 were strains isolated from outbreaks in Nigeria recently. BOT 1/68, BOT 1/77, TAN 155/68 are standard vaccine reference SAT_1 strains with which the Nigerian strains were compared. Five SAT_2 strains were used, NIG 6/81, NIG 8/81 and NIG 2/82 representing recent SAT_2 strains isolated from outbreaks in Nigeria. ZAM 3/81 and TAN 5/68 strains are standard reference SAT_2 strains with which the Nigerian strains were compared. All viruses had undergone 2 passages in BTY cells.

METHODS

Antisera

Hyper-immune guinea pig reference typing sera used in the test were supplied by the FMD World Reference Laboratory, Pirbright. Anti - 146S sera were prepared by the method of Ferris,et al., (1984). Live 146S antigen was used for the production of antiserum against the SAT_2 strains used in the serum neutralisation test. While inactivated 146S antigen was used for antiserum production against Nigerian SAT_1 strains used in the test.

Guinea pigs (5 per virus strain) were inoculated subcuteneously with 15ugm of either live or inactivated 146S antigen mixed with equal volume of Freund's complete adjuvant. The guinea pigs were test bled at 25 days post infection by cardiac puncture and resulting serum were used in SNT against homogolous virus. Individual guinea pig serum with an SNT titre of $2Log_{10}$ $TCID_{50}$ or more were regarded as good for the SNT while those with lower than 2 Log_{10} $TCID_{50}$ titre were given a single booster dose of the antigen. At 30 days the guinea pigs were killed and exsanguinated. The sera were separated and heat inactivated at 56°C for 30 minutes, pooled, seitz-filtered and stored at -20°C. The type specificity of each antiserum was investigated using

a microneutralisation test.

Preparation of 146S Antigen

The procedure for producing inactivated 146S antigen was based on those proposed by Rowland et al., (1976) as modified by Ferris et al., (1984). Each strain of FMDV used in the tests were grown in BHK-21 cells monolayers (5 Roux flask per strain). The cultures were harvested when they showed maximum CPE, pooled and clarified by centrifugation at 2500 rpm for 30 minutes.

The inactivation of infectivity was carried out by adding 1:10 volume of acetylethyleneimine (AEI) 0.05% V/V final concentration at 37°C for 24 hours with stirring (Rowland, et al., 1972). The inactivation reaction was stopped with excess of sodium thiosulphate 2% final concentration. Virus inactivation was verified by the method of Anderson et al., (1974). Concentration of virus was carried out by precipitating with polyethylene glycol 7% (W/V). The resulting suspension was allowed to stand at 4°C for 24 hours. It was later clarified and the precipitate resuspended with Tris buffer. Lipid materials were removed by using Trichloroethylene (TCE) (Doel and Baccarini, 1981). The resulting aqueous suspension from TCE treatment was centrifuged

at 25,000 rpm for 3 hours. Pellets were resuspended in phosphate buffer or 0.05M tris buffer containing 0.01M sodium chloride, samples were layered on to 15-45% linear sucrose gradients buffered with tris buffer and centrifuged at 30,000 rpm for 3 hours. The gradients were then run through a Unicam SP 1800 ultraviolet spectrophotometer using a method similar to that described by Berteling and Meloen (1974). The antigens were quantified by the method of Bachrach et al., (1964), dispensed in glass vials treated with a water repellant (Repelcote) and stored at -70°C as concentrated sucrose gradient materials. These were later diluted to contain 15 ugm/ml for innoculation of guinea pigs.

Virus Isolation

Field samples (Bovine epithelium in glycerin/ phosphate buffer pH 7.6) were transported in double container (Brooksby et al., 1964) to the World Reference Laboratory, Pirbright. The epithelium was ground up in sterile sand in 0.04M phosphate buffer (pH 7.6) - (1mg of epithelium + 9ml 0.04M buffer) with a pestle and mortar. The suspension was clarified by centrifugation at 2300g (4,000 rpm for 10 minutes). The supernatant fluid was used to innoculate tissue culture and for CFT. Virus

containing fluid from tissue culture sources (in case of samples containing virus) was used undiluted in CFT after clarification at 2300g. The routine virus isolation was carried out in primary bovine thyroid cells (BTY) (Snowdon, 1966) or porcine renal cell line (IB-RS-2) (deCastro, 1964) on rolled 4oz bottles. Cultures which were negative or with less than 40% CFE were given a second blind passage in (IB-RS cells). Those cultures which remained negative after a second passage (58 hours) were regarded as devoid of detectable virus. Virus isolated were typed and stored away with equal volumes of glycerol at -20°C.

Assay of FMD Infectivity in BHK-21 Cells

Virus infectivity was assayed in BHK-21 cells in microtitre plates. Serial ten fold dilution of each virus suspension (glycerinated virus) were prepared using Eagles medium with tryptose broth, antibiotics but no serum. A row of microtitre containing 8 wells was used for each virus dilution step. 25ul of Eagles medium was layered into each well followed by 25ul of the virus dilution and 25ul of BHK cells containing 1.5×10^6 cells/ml. The plates were sealed with adhesive tapes and incubated at 37°C in a humidified chamber. Corresponding control plates

were set up along with each virus strains. Control plates contained, medium control and cell control.

Plates were stained after 72 hours incubation by immersing the plates in a solution of napthtalene black and citric acid for one hour. Excess stains were washed off with tap water and the plates dried in the incubator at 37°C. Plates were read for end point ID_{50} using the method of Reed and Muench, (1938).

Serum Neutralisation Test

Antisera against each of the virus strains for the test were diluted 1 in 4 in serum free cell culture medium consisting of Eagles medium with 10% tryptose phosphate broth, 100 i.u/ml each penicillin and polymixin. Diluted sera were inactivated at 56°C for 30 minutes and 50ul amounts were used to prepare a two fold dilution series. Pretitrated virus, also diluted in serum free cell culture medium in half log steps at 50ul dose per well were then dropped into each well using 50ul dropper pipette. Following incubation of virus/antiserum mixture at 37°C for 1 hour, 50ul of BHK cell suspension, containing 1 x 10^6 cells/ml, in cell culture overlay medium containing 12% normal ox serum, was added to each well and the

plates sealed with pressure sensitive adhessive cellulose tape. Controls were set up in each test and it included a homologous antiserum of known titre, a cell control, medium control and a virus titration from which the actual amount of virus used in the test was calculated.

Plates were incubated at 37°C and monolayers were confluent within 24 hours. At 48 hours the plates were examined for CPE using inverted microscope. On the morning of the third day, at about 72 hours, the plates were unsealed and without removal of the culture fluid, were immersed in a solution of citric acid and napthalene black thus achieving simultaneous disinfection and fixation. Plates were removed from the stain after one hour and were rinsed in a bath of tap water and dried before being read microscopically.

Wells with 50% or greater evidence of CPE were considered to be infected and neutralisation titres were expressed as the reciprocal of the final dilution of serum present in the serum virus mixture at the 50% end point estimated according to the Kaber method (1931).

Tests were considered valid when the actual amount of virus used per well in the test is between

$10^{1.5}$ and $10^{2.5}$ $TCID_{50}$ and when the titre of the reference serum is within two fold of its expected titre estimated from the mode of previous titrations.

Complement Fixation Test (CFT)

Reagents were added in the following sequence:-

(1) Veronal buffer (25 ul)
(2) Antiserum (25 ul)
(3) Complement (50 ul)
(4) Antigen (25 ul)
(5) Haemolytic indicator (RBC) system (25 ul)

Initially, buffer was added to the plates as required. Antisera was added in serial 1:1.5 dilution steps by successive transfer of 50ul loops charged with a 1/16 starting dilution into wells containing 25ul of diluent. Complement was then added in 50ul volumes containing the appropriate number of 3 units. This was followed by the addition of 25ul of diluted antigen to each well. Primary fixation took place for 1 hour at 37°C in the presence of 3 units of complement. The plates were sealed, given a second incubation at 37°C for 30 minutes, shaking midterm, before centrifugation and recording of results. Controls for detection of abnormal behaviour of any

of the reagents were performed in each test. CF titres were expressed as the reciprocal of the serum dilution producing 50% haemolysis.

RESULTS

Virus Isolation

From a total of ten samples collected from fresh outbreaks in the field, only two samples contained viruses. An SAT_2 virus was isolated from a sample obtained in Benue State designated NIG 2/82 and a SAT_1 virus isolated from a sample obtained in Gujba in Borno state - designated NIG 13/81. Other viruses previously isolated at AVRI, Pirbright from outbreaks in Nigeria during the study period were also included. These were NIG 6/81, NIG 8/81, NIG 4/81, NIG 10/81 and NIG 11/81.

Assay of FMD virus infectivity in BHK Cells

The Nigerian SAT_2 strains especially NIG 6/81 gave very low titre in BHK-21 cells during assays in microtitre plates. Consequently the viruses were propagated in RS and BK cells in microtitre plates and in test tubes. Titres in RS and BK cells in microtitre plates were slightly higher than in BHK cells. Virus titre assay in test tubes were on the average higher than in microtitre plate system.

Table 5 shows the result of titration of the SAT_1 viruses in the 3 cell systems (BHK, BTY and IBRS), while table 6 shows the titre of the SAT_2 viruses in the 3 cell systems.

Preparation of 146S Antigen

Quantities of live 146S antigen yield varied according to strain. On the whole, the SAT_2 Nigerian strains produced less 146S antigen than the SAT_1 strains. Table 7 illustrates the quantities of antigen produced before and after inactivation with AEI.

Inactivation reduced drastically the quantity of 146S antigen produced and in the case of the SAT_2 strains the 146S antigen recovered after inactivation process was insignificant. This led to the decision to immunise guinea pigs with live purified 146S antigen.

Preparation of antiserum in Guinea pigs

Table 8A illustrates the titres of antiserum produced by individual guinea pigs and the mean titre of pooled guinea pig antisera. Table 8B illustrates the mean titre of pooled antiserum from 5 guinea pigs.

Subtyping of Nigerian SAT_1 and SAT_2 Strains

Tables 9 and 10 show the 'r' values and 'R'% values for the SAT_1 strains used in the test. Tables 11 and 12 show 'r' and 'R'% values for the SAT_2 strains. The Nigerian SAT_1 strains have 'R'% values above 77% showing that they are probably identical virus - i.e. belonging to the same subtype.

The Nigerian SAT_2 strains also show close relationships NIG 6/81 seems more closely related to NIG 2/82 than to NIG 8/81 but all three could be regarded as belonging to the same subtype.

In terms of the ability of the reference vaccines strains to protect against an infection by the Nigerian strains, the invitro tests suggest that the reference strains would protect against infection by any of the Nigerian field strains tested.

TABLE 5

TITRE OF SOME SAT 1 STRAINS OF FMD IN BTY AND IBRS BHK-21 CELLS

VIRUS STRAIN	TITRE IN BTY CELLS LOG $TCID_{50}$	TITRE IN IBRS CELLS LOG $TCID_{50}$	TITRE IN BHK-21 CELLS LOG $TCID_{50}$
NIG. 4/81	*6.44	6.00	3.24
NIG. 5/81	6.31	6.41	3.39
NIG. 10/81	6.00	6.56	3.41
NIG. 11/81	6.37	6.49	4.12
NIG. 13/81	6.00	6.12	4.56
TAN. 155/68	6.82	6.44	4.49
BOT. 1/77	6.80	6.52	4.37
BOT. 1/68	6.87	6.48	4.38

*Mean of 3 titrations.

TABLE 6

TITRE OF SOME SAT_2 STRAINS OF FMD IN BTY AND IBRS AND BHK CELLS

VIRUS STRAIN	TITRE IN BTY CELLS LOG $TCID_{50}$	TITRE IN IBRS CELLS LOG $TCID_{50}$	TITRE IN BHK-21 CELLS LOG $TCID_{50}$*
NIG. 2/82	6.2	6.87	2.87
NIG. 6/81	4.8	4.44	5.31
NIG. 8/81	5.4	5.31	3.44
ZAM. 3/81	6.0	6.44	3.44
TAN. 5/68	5.2	6.0	3.0

*Mean of 3 titrations

TABLE 7

QUANTITIES OF 146S ANTIGEN PRODUCED BY SOME STRAINS OF FMD GROWN IN BHK CELLS

STRAINS	NOT INACTIVATED*	INACTIVATED	MEAN 146S** CONCENTRATION
SAT 1			
NIG 4/81	411.30 ugm/ml	62.8 ugm/ml	20.096
NIG 5/82	460.71 ugm/ml	81.2 ugm/ml	25.96
NIG 10/81	396.12 ugm/ml	54.4 ugm/ml	17.40
NIG 11/81	380.04 ugm/ml	50.49 ugm/ml	16.15
NIG 13/81	420.5 ugm/ml	59.4 ugm/ml	19.18
SAT 2			
NIG 6/81	59.4 ugm/ml	Nil	19.08
NIG 8/81	310.0 ugm/ml	29.84 ugm/ml	99.20
NIG 2/82	131.3 ugm/ml	8.24	42.01
ZAM 3/81	158.34 ugm/ml	8.51	50.66

* Quantity of 146S per 1ml of purified sucrose density gradient material.

** Mean concentration ugm of inactivated and purified 146S antigen per 10^8 BHK cells.

TABLE 8A

IMMUNE RESPONSE OF INDIVIDUAL GUINEA PIGS TO SOME STRAINS OF FMD VIRUS

ANTIGEN TYPE	A	B	C	D	E	MEAN TITRE
SAT 2						
NIG 2/82	3.16	2.41	2.56	1.95	2.25	2.40
NIG 6/81	2.11	2.46	2.86	1.66	1.66	2.13
NIG 8/81	2.66	2.25	1.66	2.86	1.95	2.27
ZAM 3/81	1.95	2.86	2.71	2.41	2.11	2.35
SAT 1						
NIG 4/81	3.16	2.86	2.25	3.16	2.56	2.79
NIG 5/81	3.16	3.16	2.86	3.31	2.86	3.07
NIG 10/81	3.61	2.86	3.46	3.46	3.20	3.31
NIG 11/81	2.41	1.95	2.11	2.71	2.86	2.40
NIG 13/81	2.86	3.31	3.01	2.41	2.46	2.82

TABLE 8B

TITRES OF ANTIBODY PRODUCED BY GUINEA PIGS TO DIFFERENT STRAINS OF FMD VIRUS

ANTIGENS	TITRES*	
	RANGE	MEAN (GEOMETRIC)
NIG 2/82	32-64	57
NIG 6/81	128-512	256
NIG 8/81	128-512	288
ZAM 3/81	64-512	345
NIG 4/81	32-128	51
NIG 5/81	128-512	332
NIG 10/81	32-128	56
NIG 11/81	64-256	153
NIG 13/81	32-128	70

* Titre expressed as the reciprocal of the serum dilution that neutralised 100 $TCID_{50}$ of the homologous virus.

TABLE 9

'r' VALUES FOR SAT 1 STRAINS OF FMD FROM NIGERIA AND STANDARD SAT 1 VACCINE STRAINS*

VIRUS / SERUM	NIG 4/81	NIG 5/81	NIG 10/81	NIG 11/81	NIG 13/81	BOT 1/68	BOT 1/77	TAN 155/68
NIG 4/81	1.0**	0.95	0.92	0.97	0.84	0.67	0.69	0.68
NIG 5/81	0.91	1.0	0.91	0.97	0.83	0.66	0.77	0.84
NIG 10/81	1.00	1.00	1.00	0.96	0.94	0.75	0.78	0.89
NIG 11/81	0.95	0.98	0.63	1.00	0.85	0.75	0.88	0.87
NIG 13/81	0.97	0.95	0.94	0.96	1.00	0.84	0.88	0.89
BOT 1/68	0.65	0.79	0.75	0.69	0.73	1.00	0.70	0.85
BOT 1/77	0.69	0.69	0.69	0.71	0.68	0.82	1.00	0.77
TAN 155/68	0.84	0.91	0.92	0.91	0.79	0.82	0.77	1.00

*'r' values derived from cross neutralisation test.

** Mean values of three tests.

TABLE 10

'R' VALUES FOR SAT 1 STRAINS OF FMD FROM NIGERIA AND STANDARD SAT 1 VACCINE STRAINS*

VIRUS / SERUM	NIG 4/81	NIG 5/81	NIG 10/81	NIG 11/81	NIG 13/81	BOT 1/68	BOT 1/77	TAN 155/68
NIG 4/81	100	92.8	100	95.9	90.2	65.6	69.0	75.5
NIG 5/81		100	91.0	97.5	88.8	72.2	72.0	76.4
NIG 10/81			100	80.9	94.0	75.0	73.4	76.0
NIG 11/81				100	90.3	71.4	79.0	88.9
NIG 13/81					100	78.3	77.3	83.8
BOT 1/68						100	84.0	83.2
BOT 1/77							100	77.0
TAN 155/68								100

*'R' values derived from cross neutralisation tests.

** Mean values of 3 tests.

TABLE 11

'r' VALUES FOR SAT 2 STRAINS OF FMD FROM NIGERIA AND STANDARD SAT 2 VACINE STRAINS*

VIRUS / SERUM	NIG 2/82	NIG 6/81	NIG 8/81	ZAM 3/81	TAN 5/68
NIG. 2/82	1.0**	0.77	0.59	0.66	0.81
NIG. 6/81	0.93	1.0	0.50	0.47	0.87
NIG. 8/81	0.97	0.87	1.0	0.89	0.73
ZAM. 3/81	0.67	0.97	0.73	1.0	0.90
TAN. 5/68	0.76	0.46	0.67	0.53	1.0

* 'r' values derived from cross neutralisation tests.

** Mean values of 3 tests.

TABLE 12

'R' VALUES FOR SAT 2 STRAINS OF FMD FROM NIGERIA AND STANDARD SAT 2 VACCINE STRAINS*

VIRUS / SERUM	NIG 2/82	NIG 6/82	NIG 8/81	ZAM 3/81	TAN 5/68
NIG 2/82	100**	84.6	75.6	66.5	61.5
NIG 6/81		100	65.9	67.5	63.2
NIG 8/81			100	80.6	69.9
ZAM 3/81				100	69.0
TAN 5/68					100

* 'R' values derived from cross neutralisation test.

** Mean values of 3 tests.

FIG 1

'0' PACHEKO VIA SUCROSE DENSITY GRADIENT PROFILE

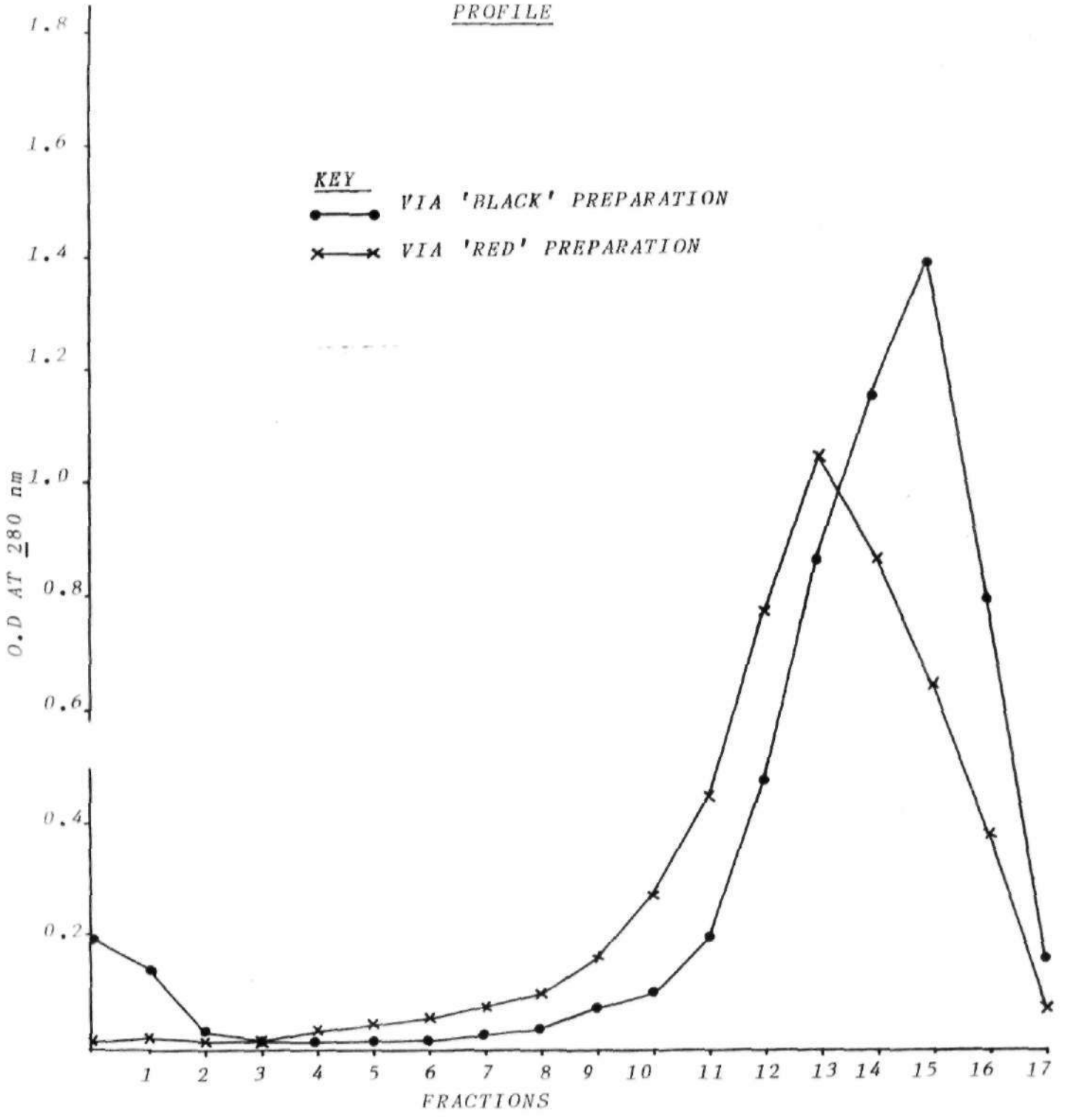


FIG 3

TITRATION OF VIA ANTIGEN AGAINST PRE-VACCINATION POSTVACCINATION AND RECONVALESENT SERA

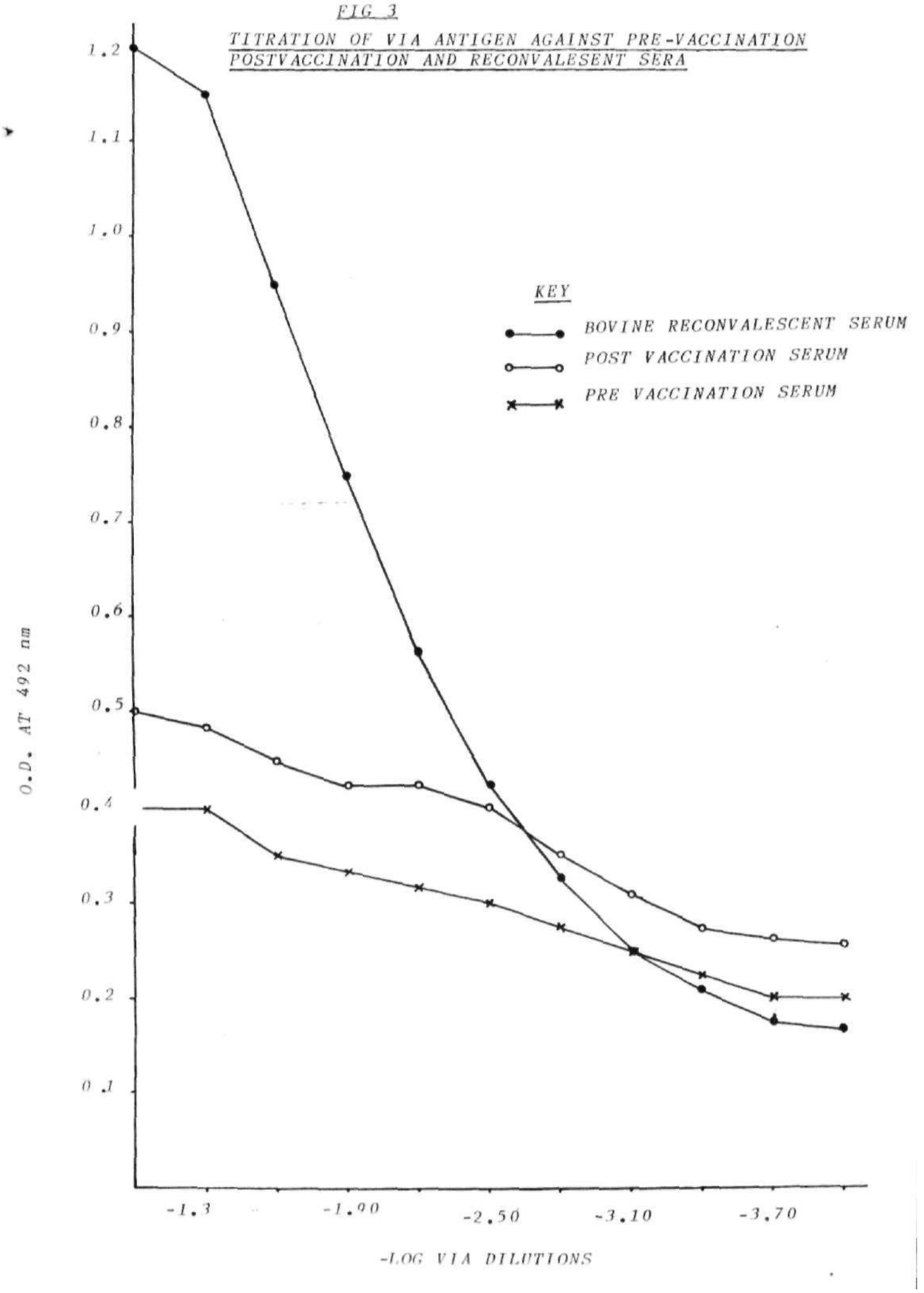


FIG 3

TITRATION OF VIA ANTIGEN AGAINST PRE-VACCINATION POSTVACCINATION AND RECONVALESENT SERA

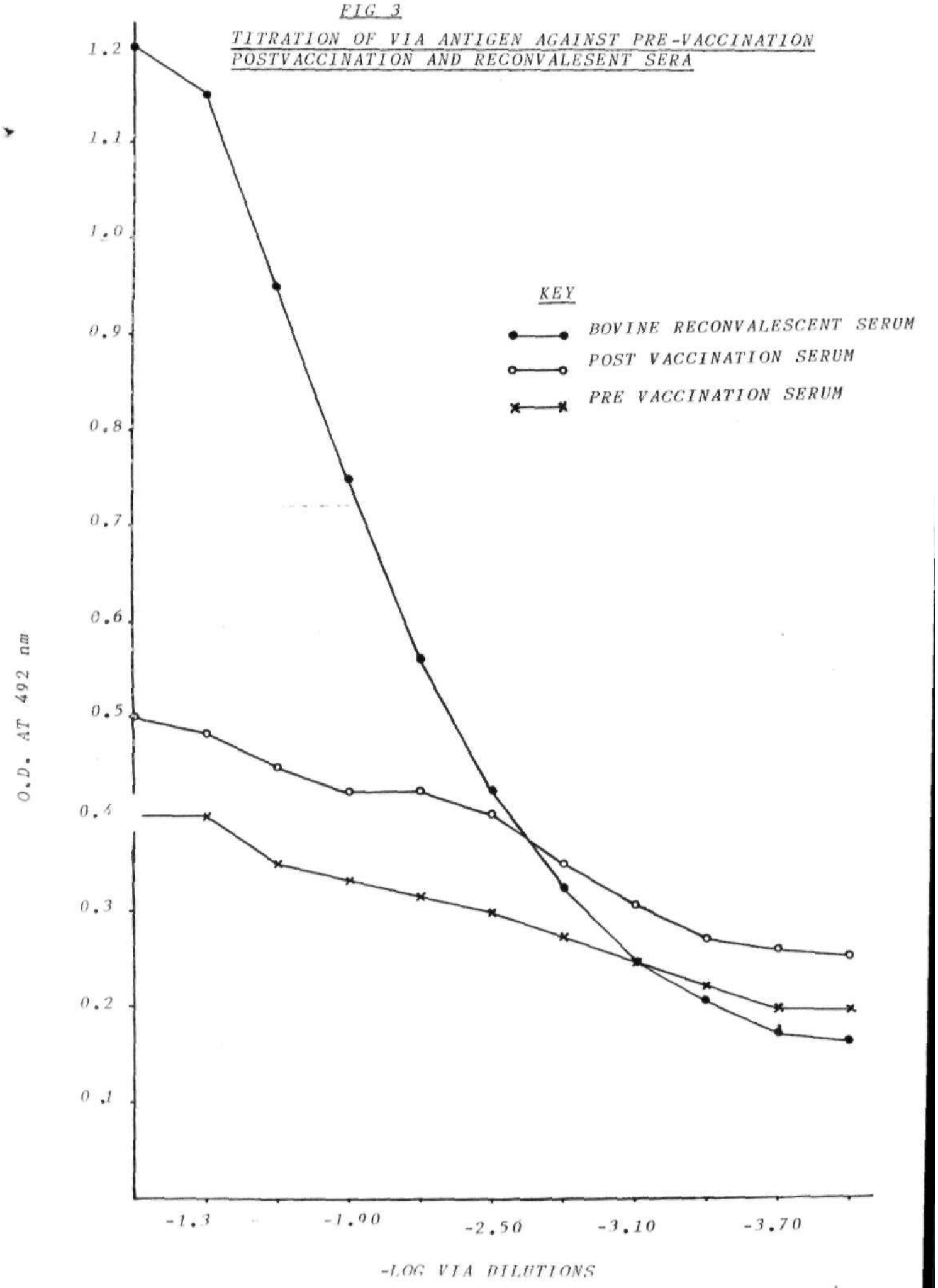


FIG. 4

STANDARDISATION OF CONJUGATE

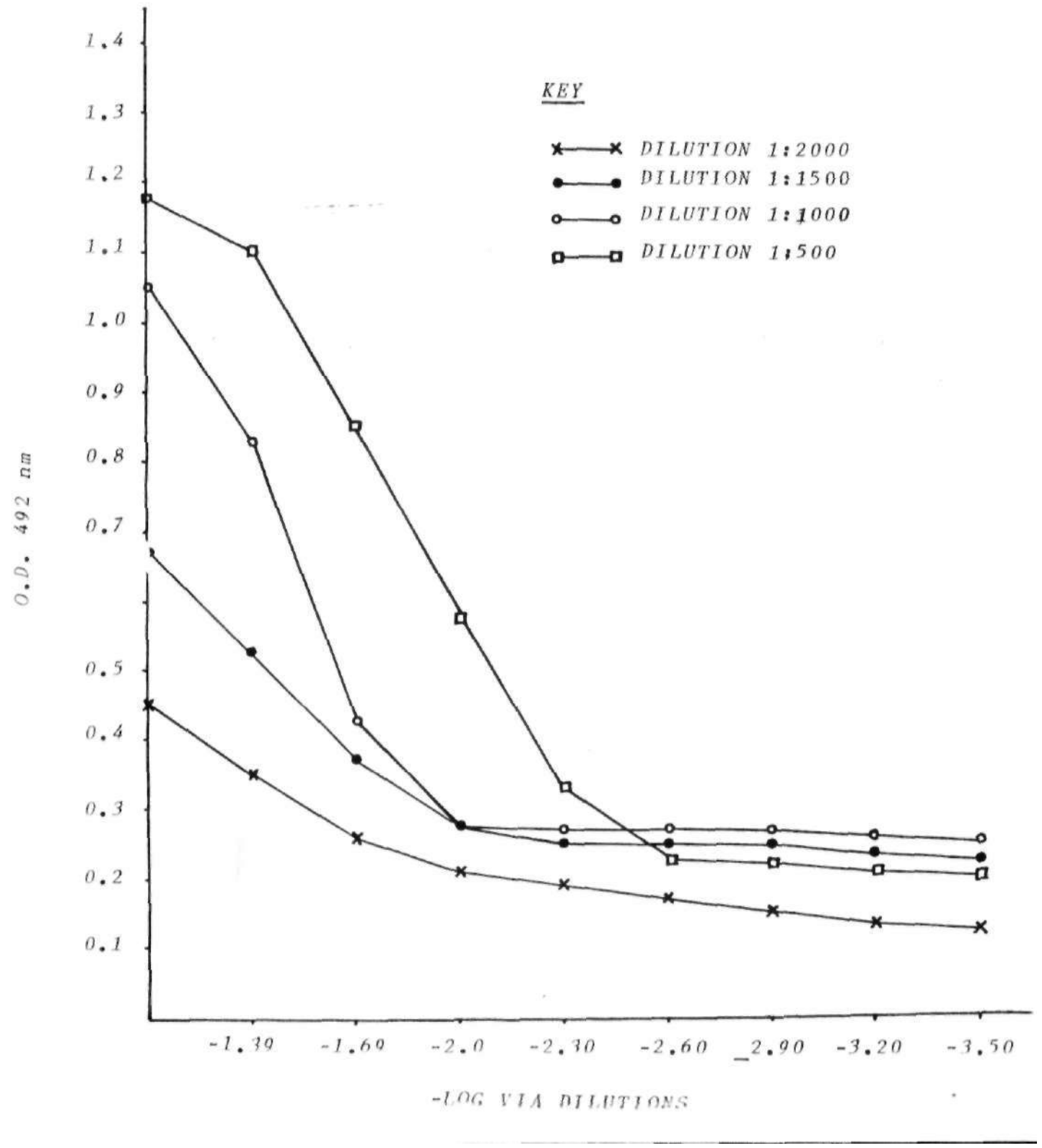


FIG 5

TITRATION OF NORMAL AND HEAT INACTIVATED NIGERIAN BOVINE SERA

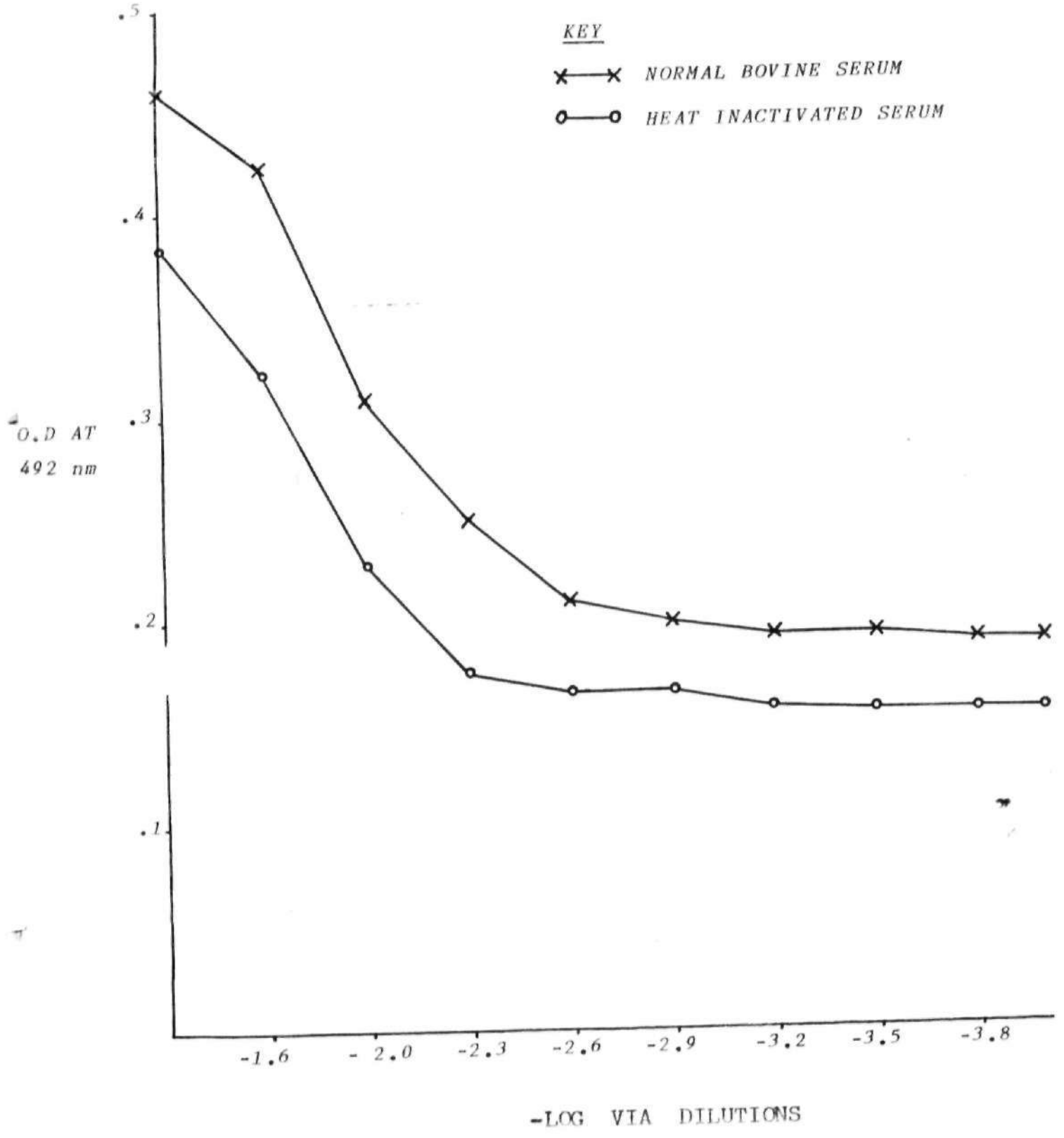


FIG 6

DETERMINATION OF THE EFFECT OF SERUM PROTEINS ON THE ELISA PLATES

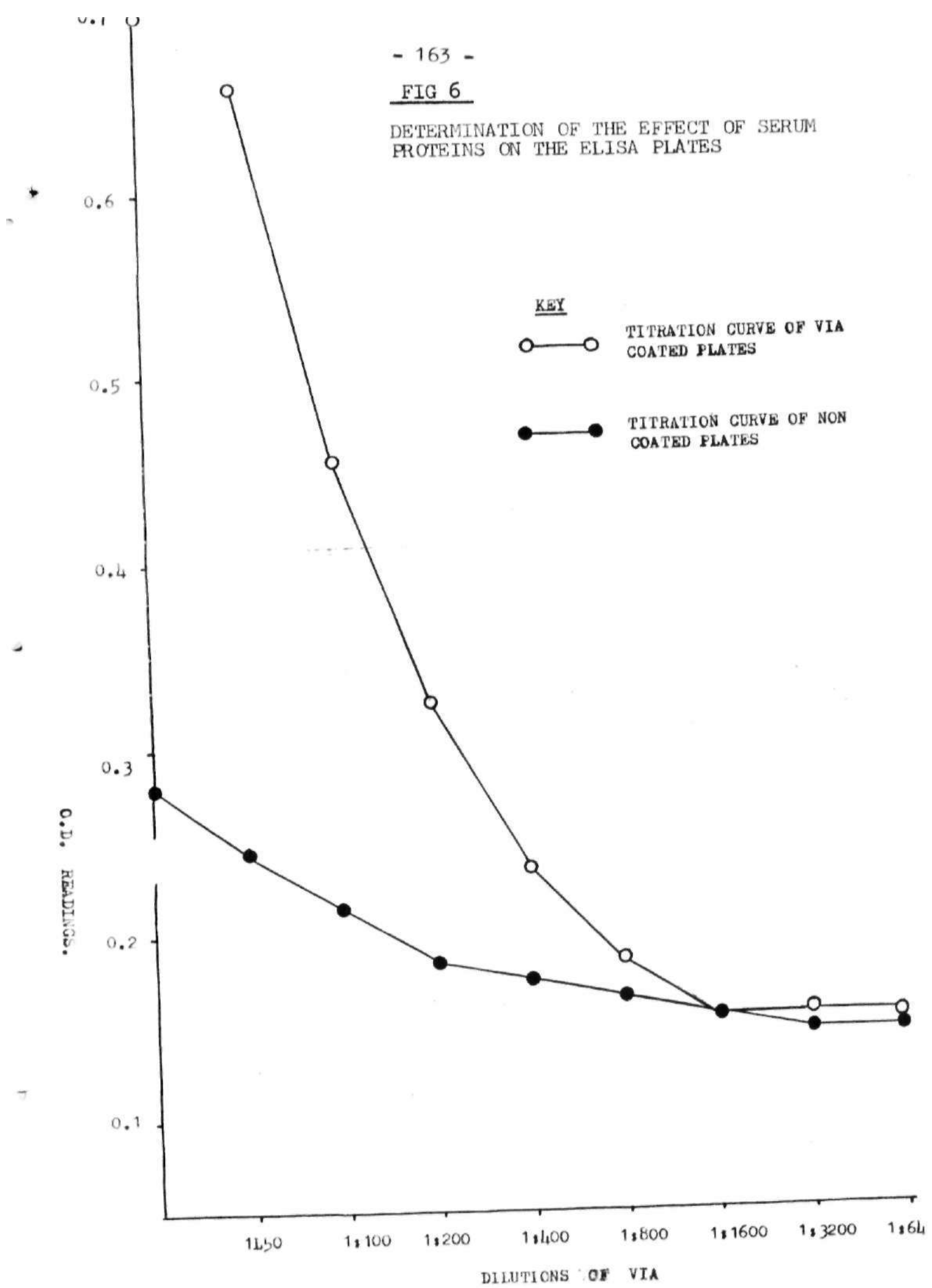


FIG. 7

STANDARDISATION OF NIGERIAN BOVINE SERA

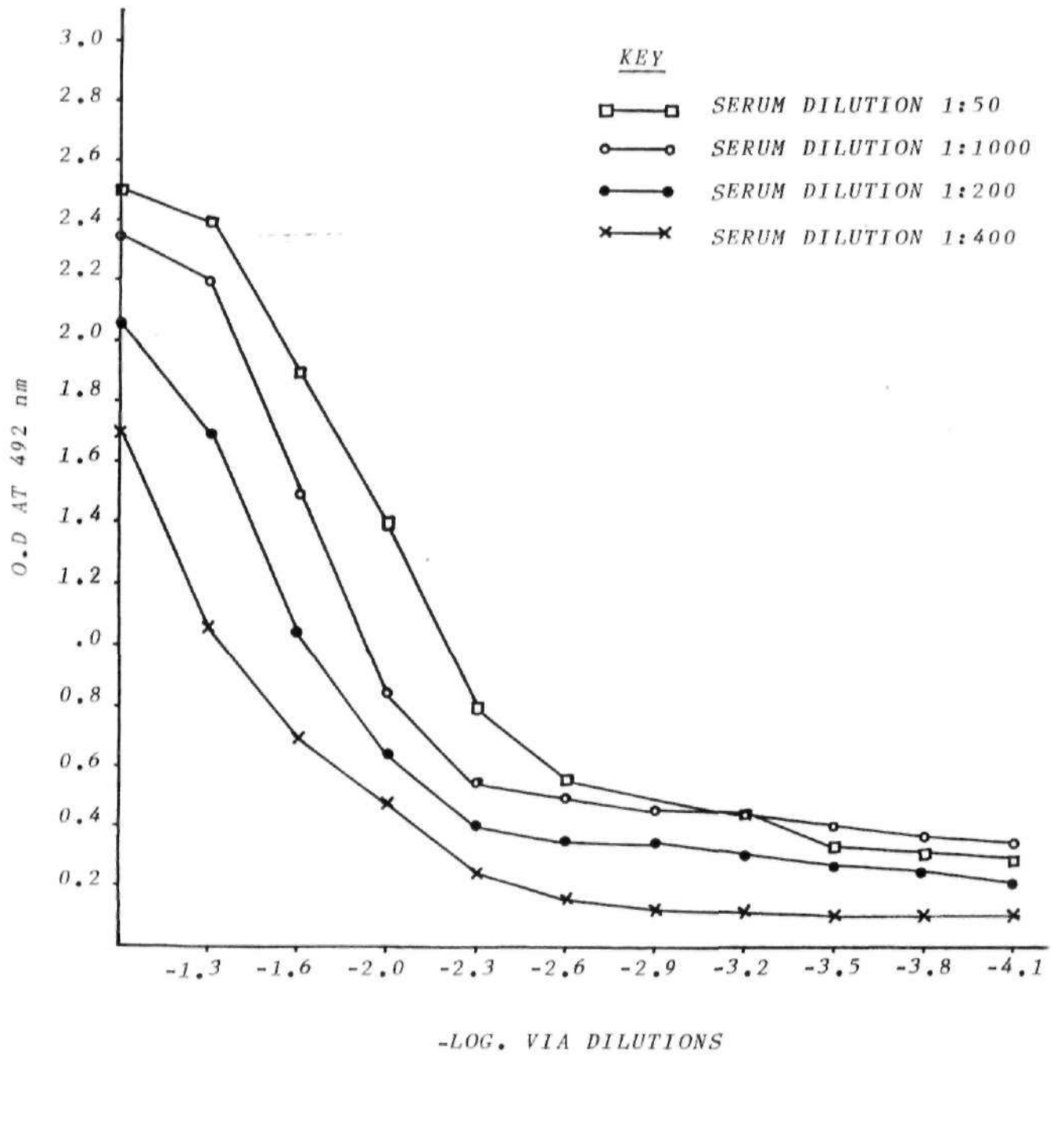


Fig. 8

DISTRIBUTION OF ANTIBODIES TO 3 TYPES FMD VIRUS IN (A) KADUNA STATE (B) BORNO STATE

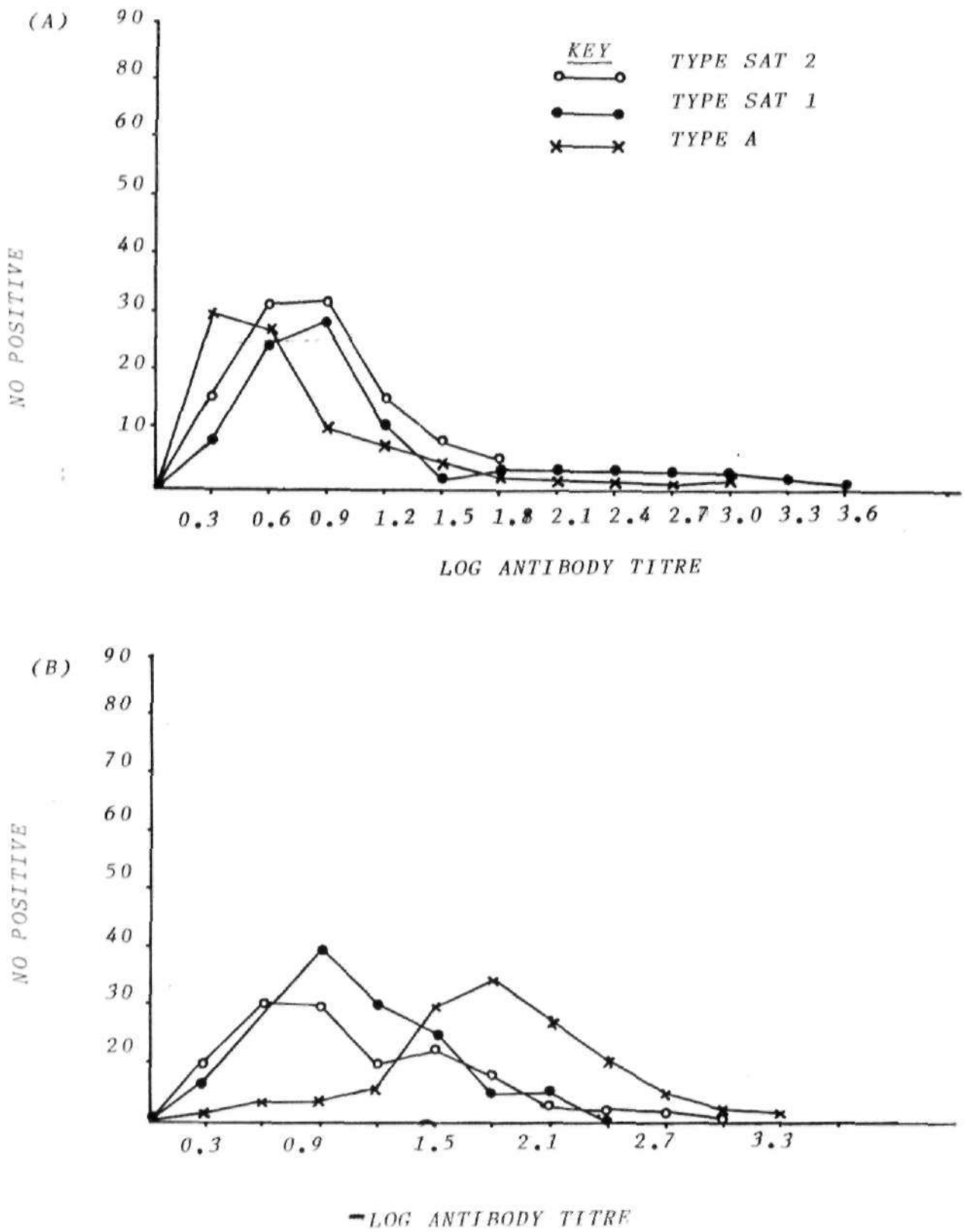


Fig.9

DISTRIBUTION OF ANTIBODIES TO 3 TYPES FMD VIRUS IN (A) KANO (B) BAUCHI STATE.

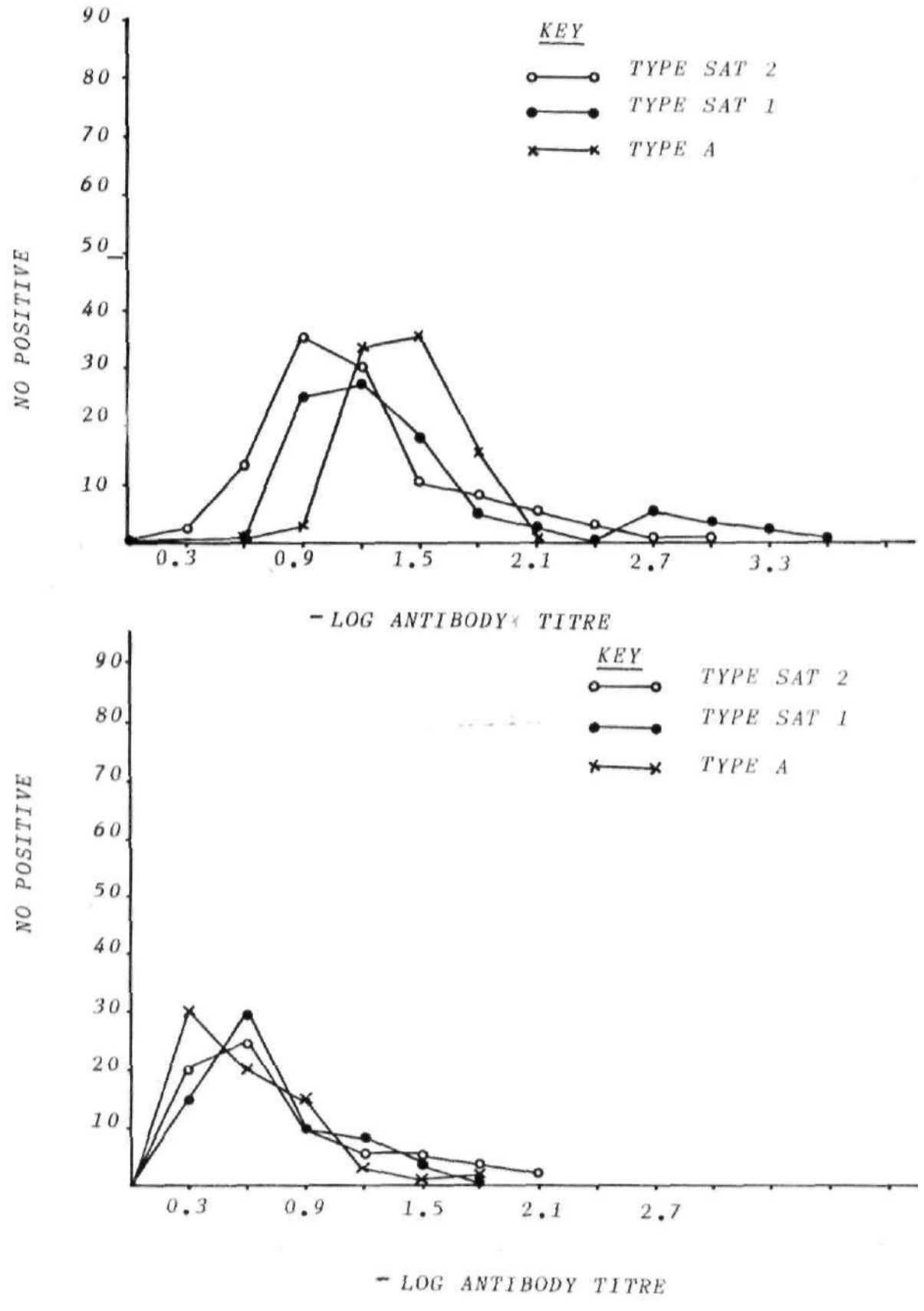


Fig. 18

DISTRIBUTION OF ANTIBODIES TO 3 TYPES FMD VIRUS IN SOKOTO STATE

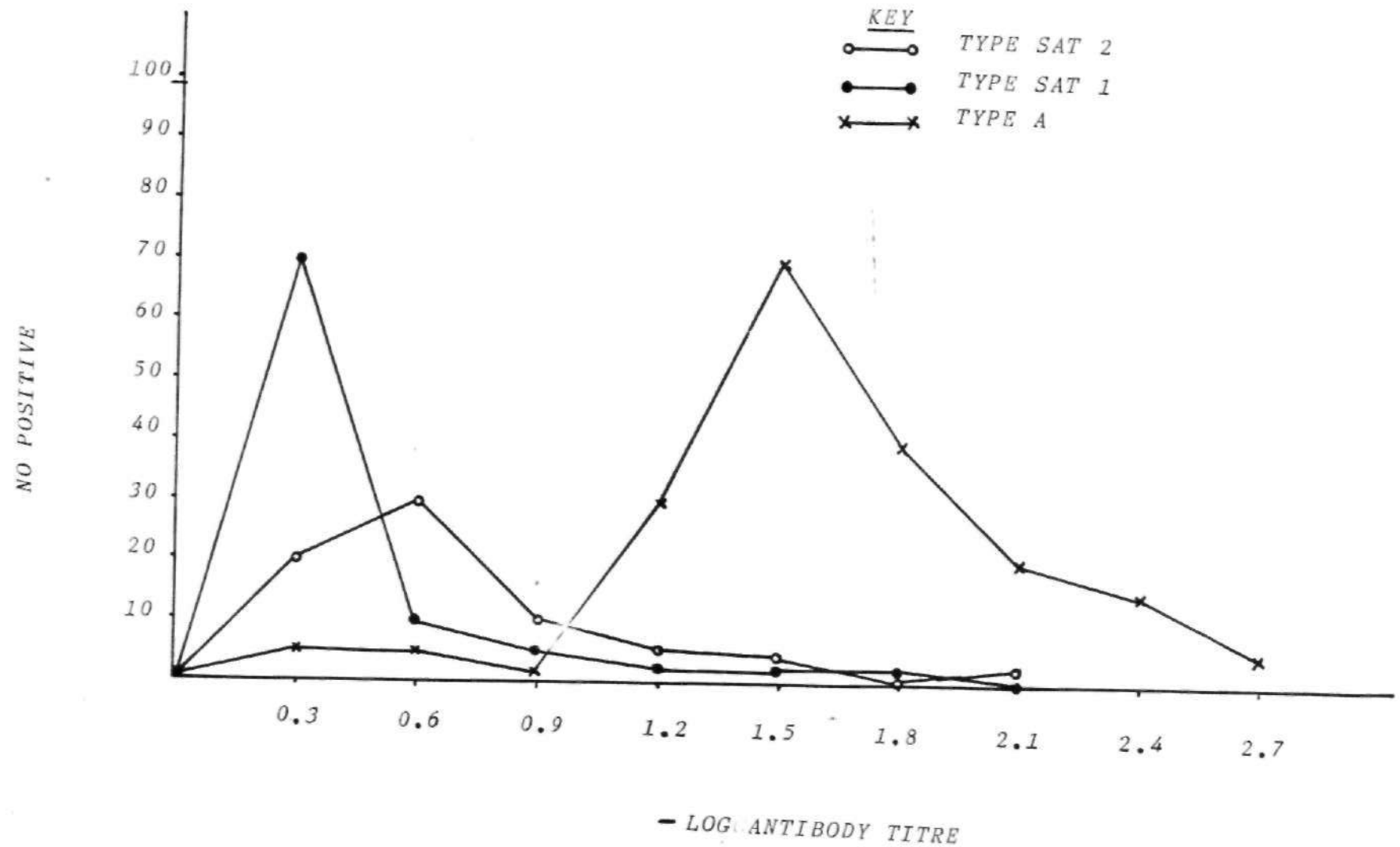


Fig. 11

DISTRIBUTION OF ANTIBODIES TO 3 TYPES FMD VIRUS IN GONGOLA STATE

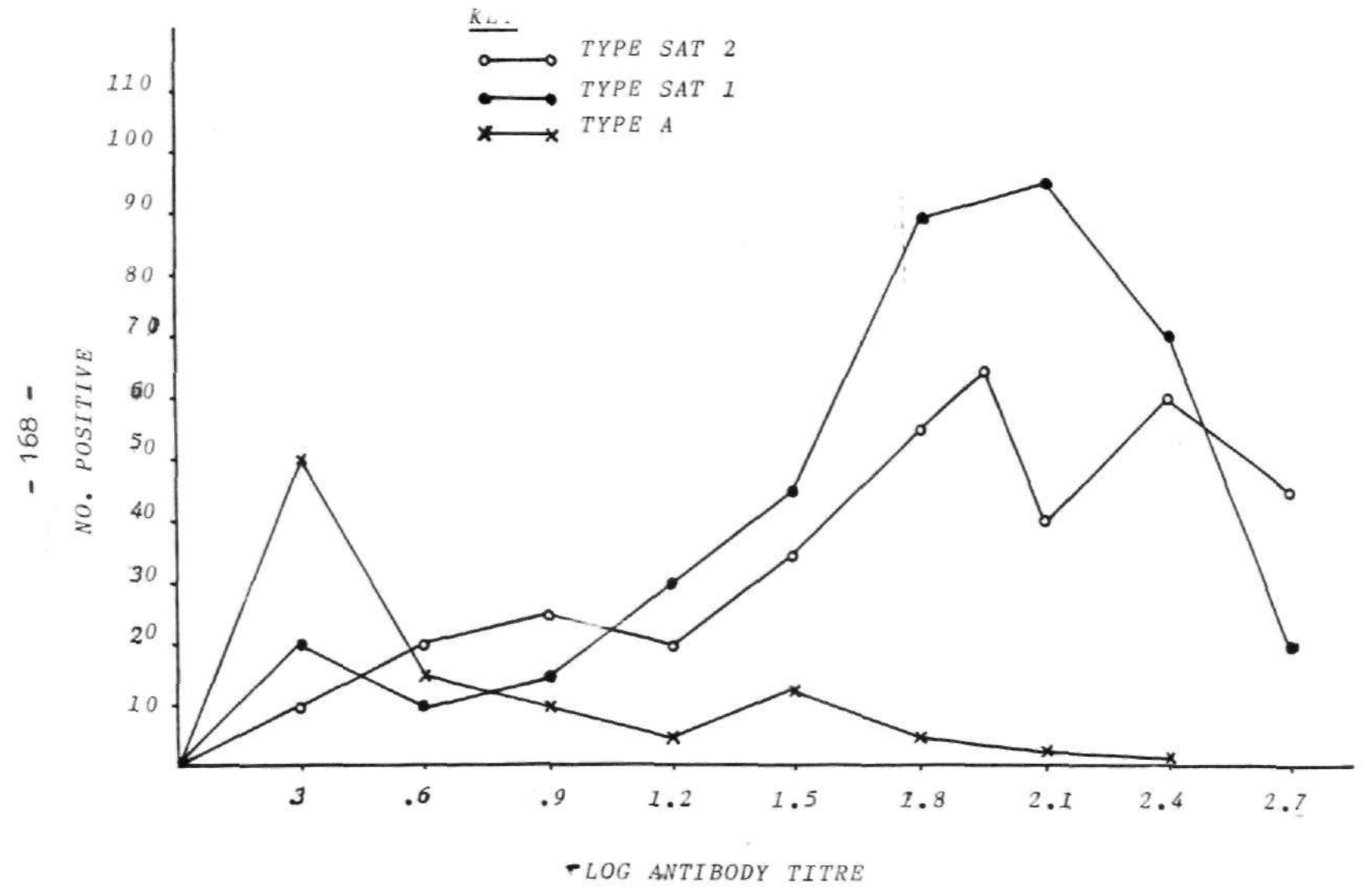


FIG. 12

PROFILE OF CATTLE INVOLVED IN FMD OUTBREAKS 1964 - 1984.

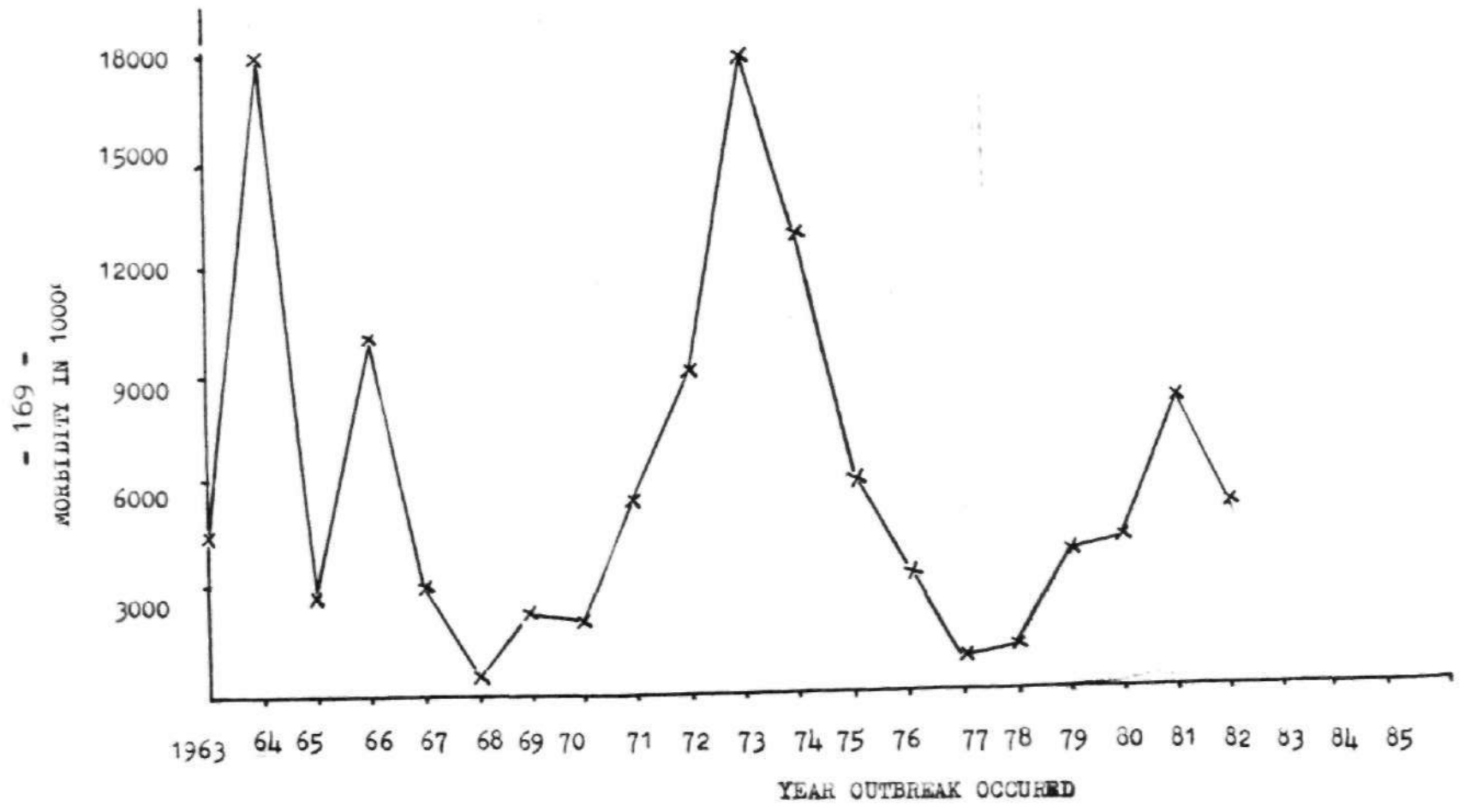


FIG 18

PROFILE OF FMD OUTBREAKS 1963-1982

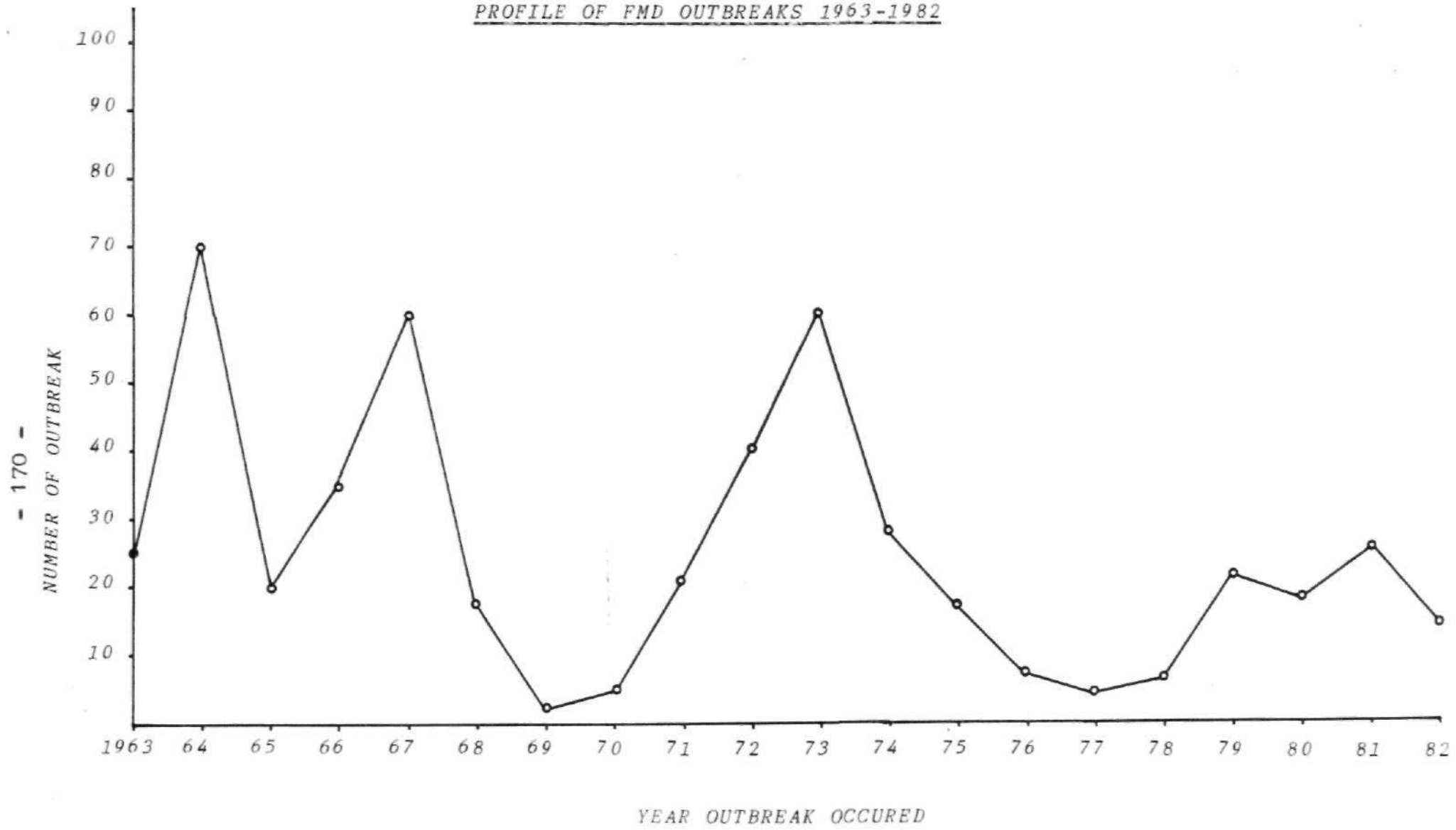


Fig. 14

SUCROSE DENSITY GRADIENTS

PROFILE OF SAT 1 STRAINS.

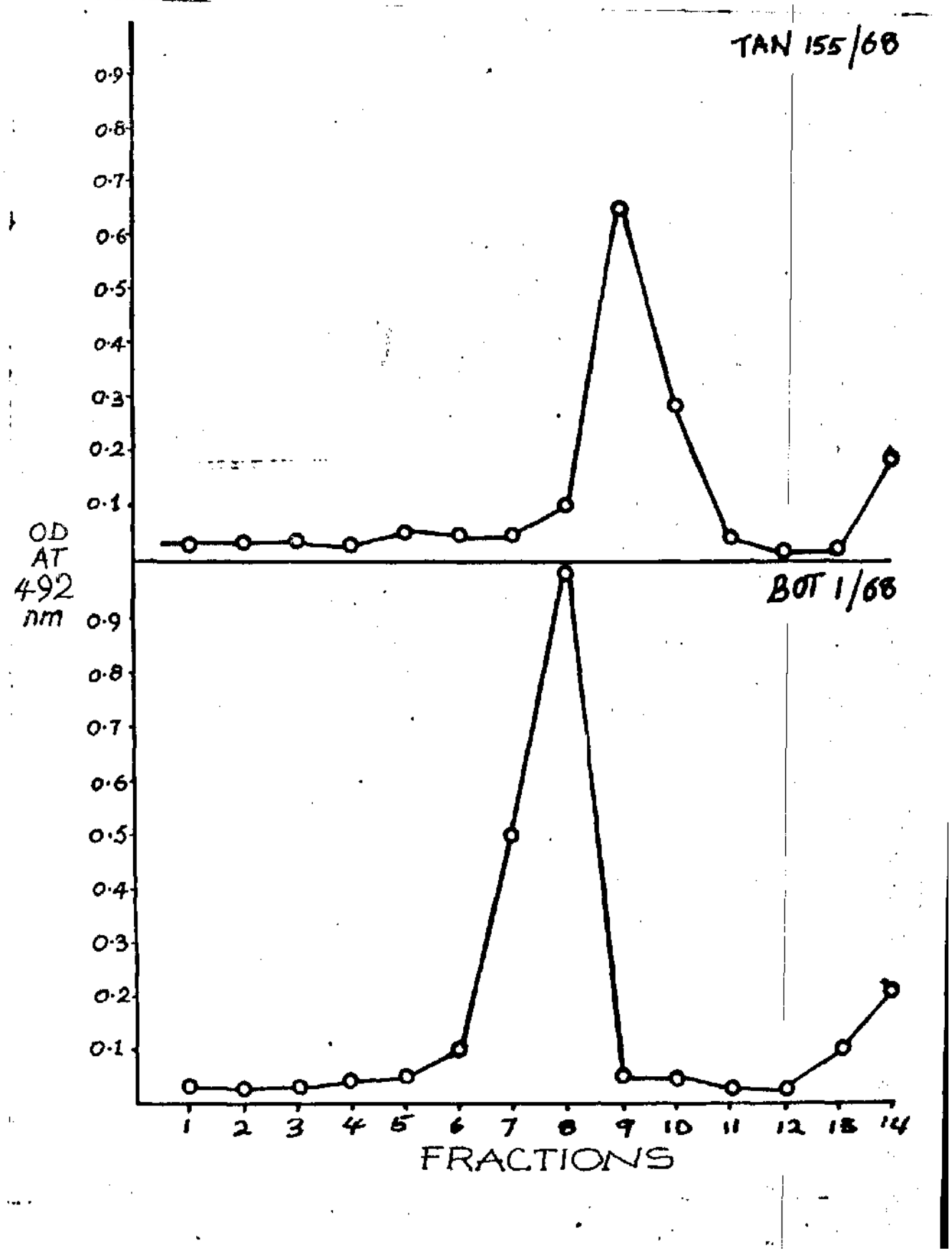


Fig. 15

SUCROSE DENSITY GRADIENT PROFILES

OF SAT 1 STRAINS

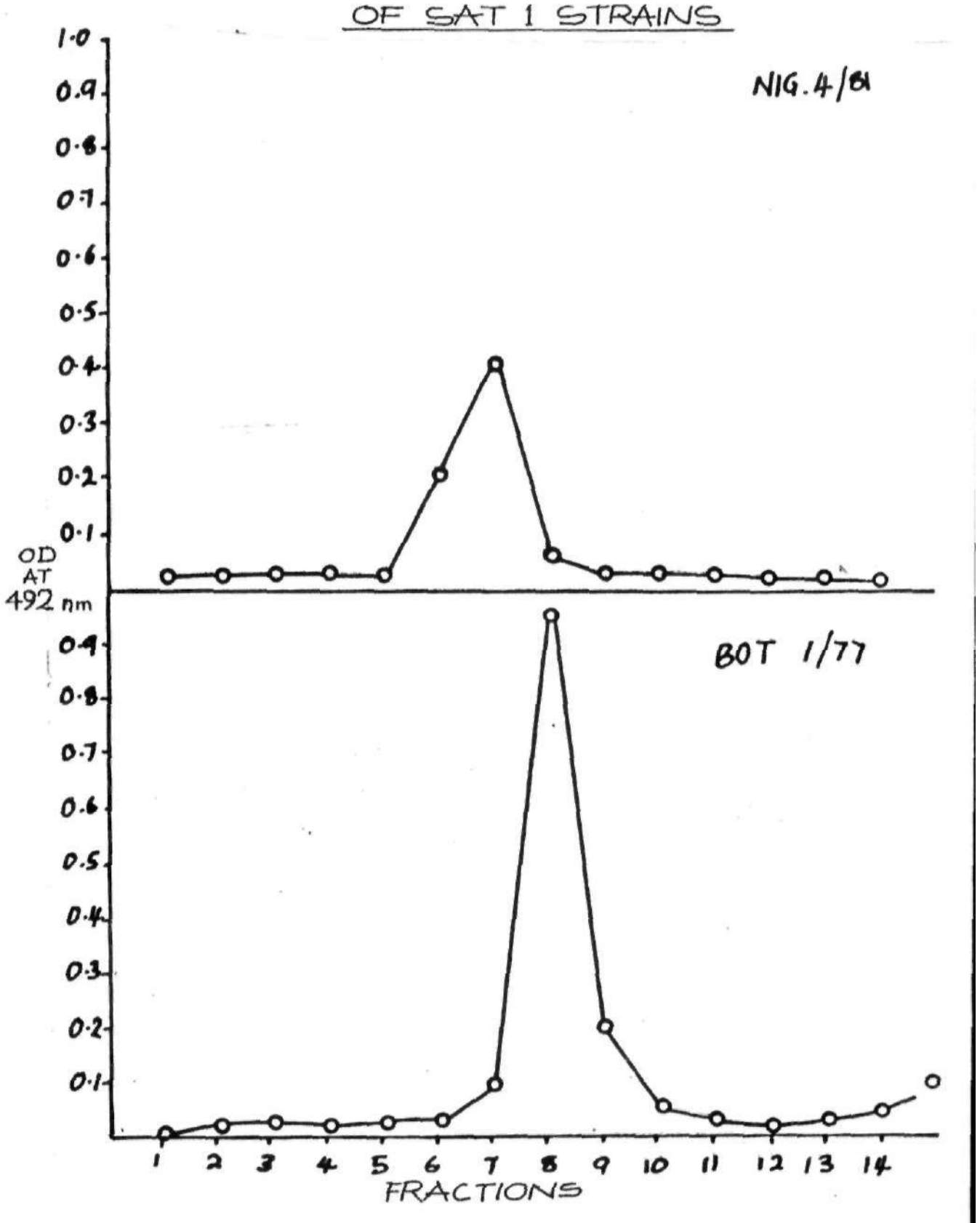


Fig.16

SUCROSE GRADIENT PROFILES OF NIGERIAN SAT 1 STRAINS

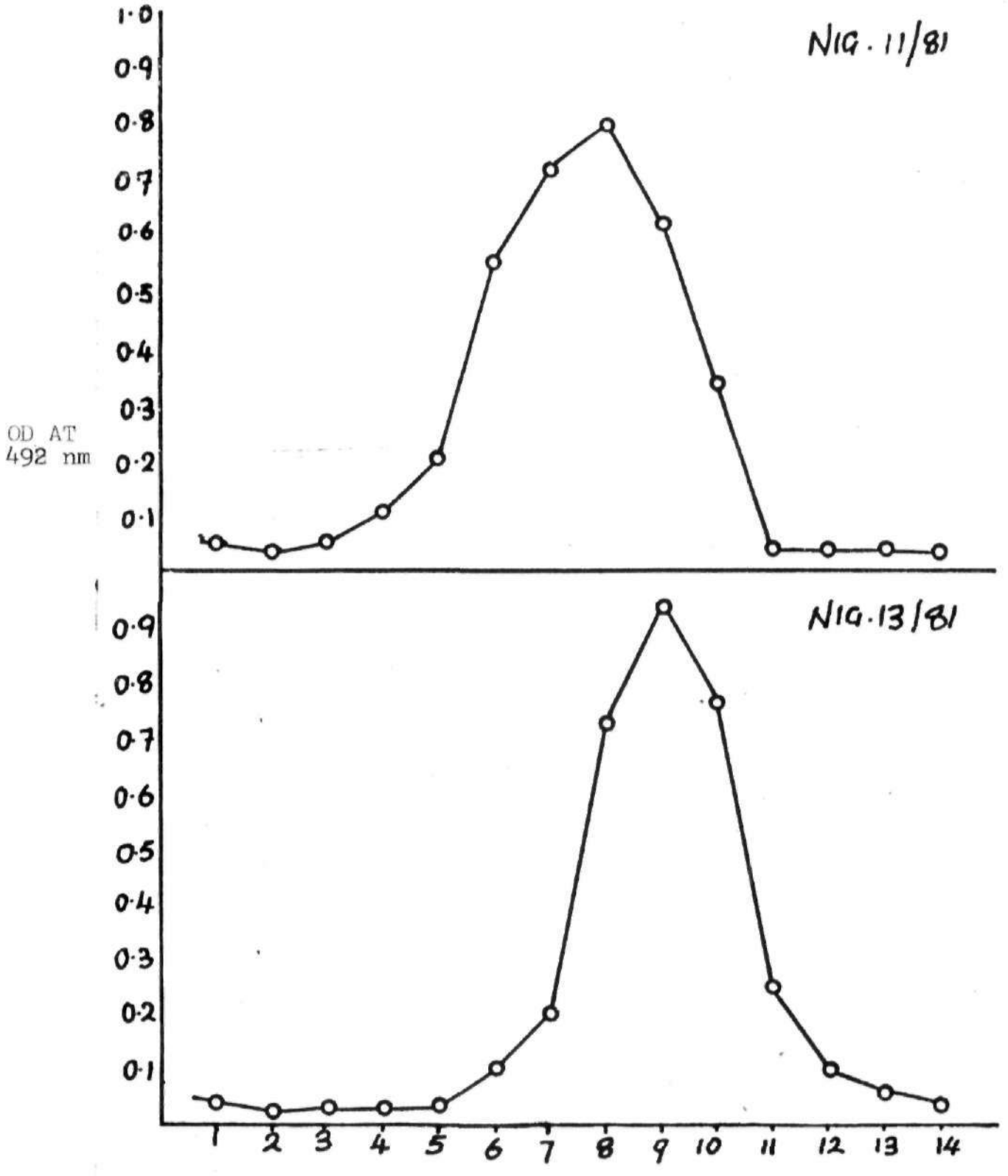


Fig. 17

SUCROSE DENSITY GRADIENT PROFILE OF SAT 1 STRAINS.

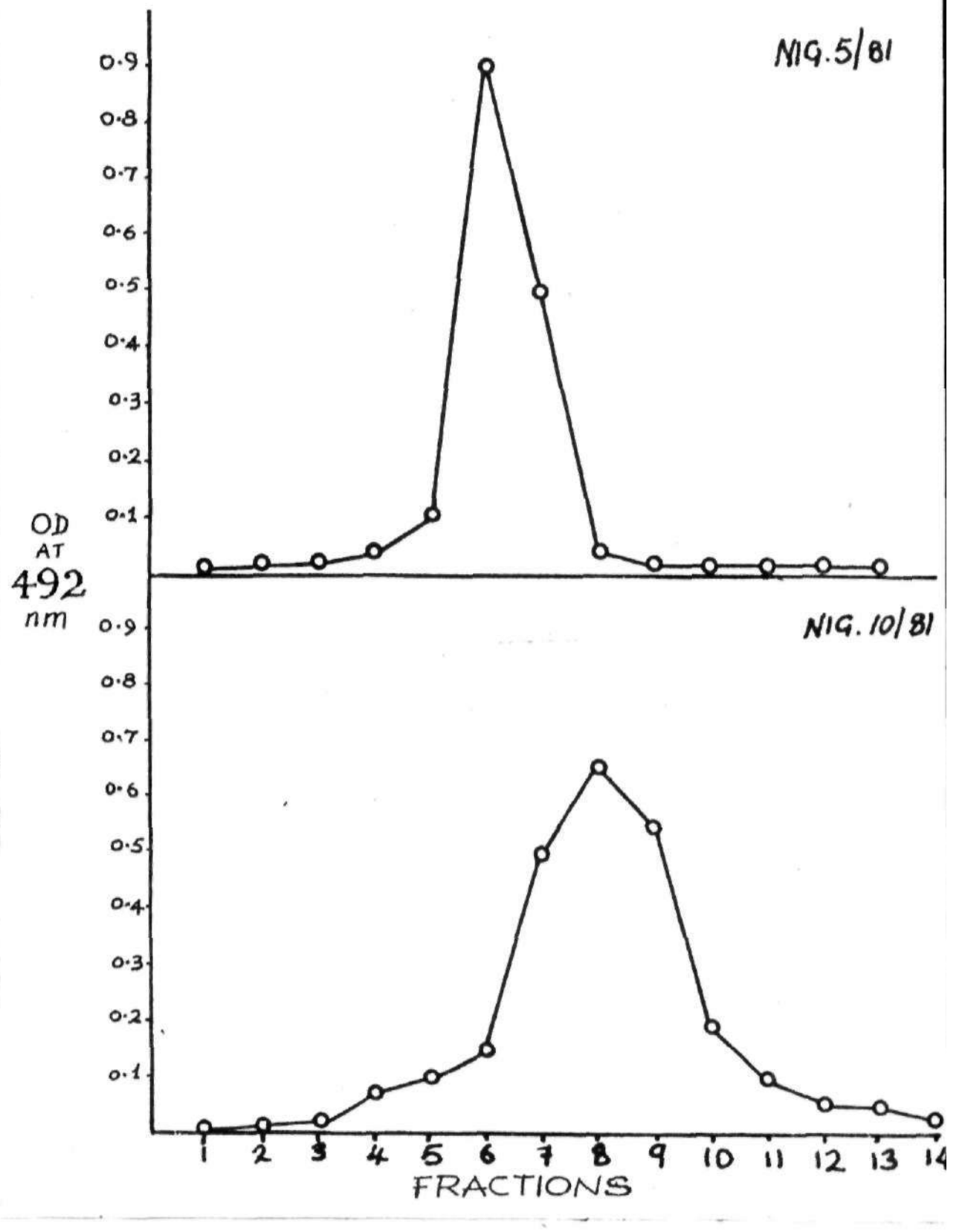


Fig. 18

SUCROSE DENSITY GRADIENT PROFILES SAT 2 STRAINS

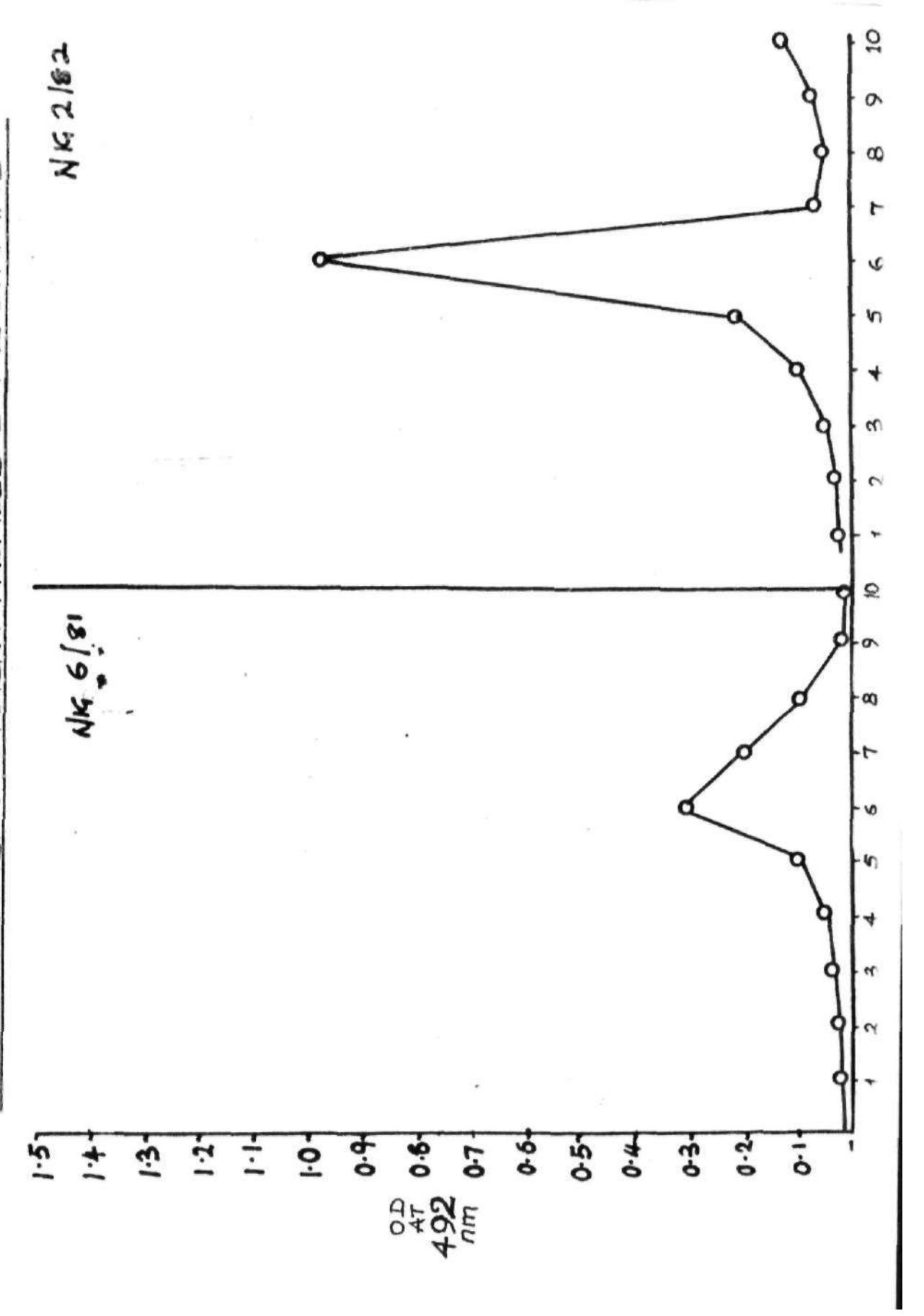




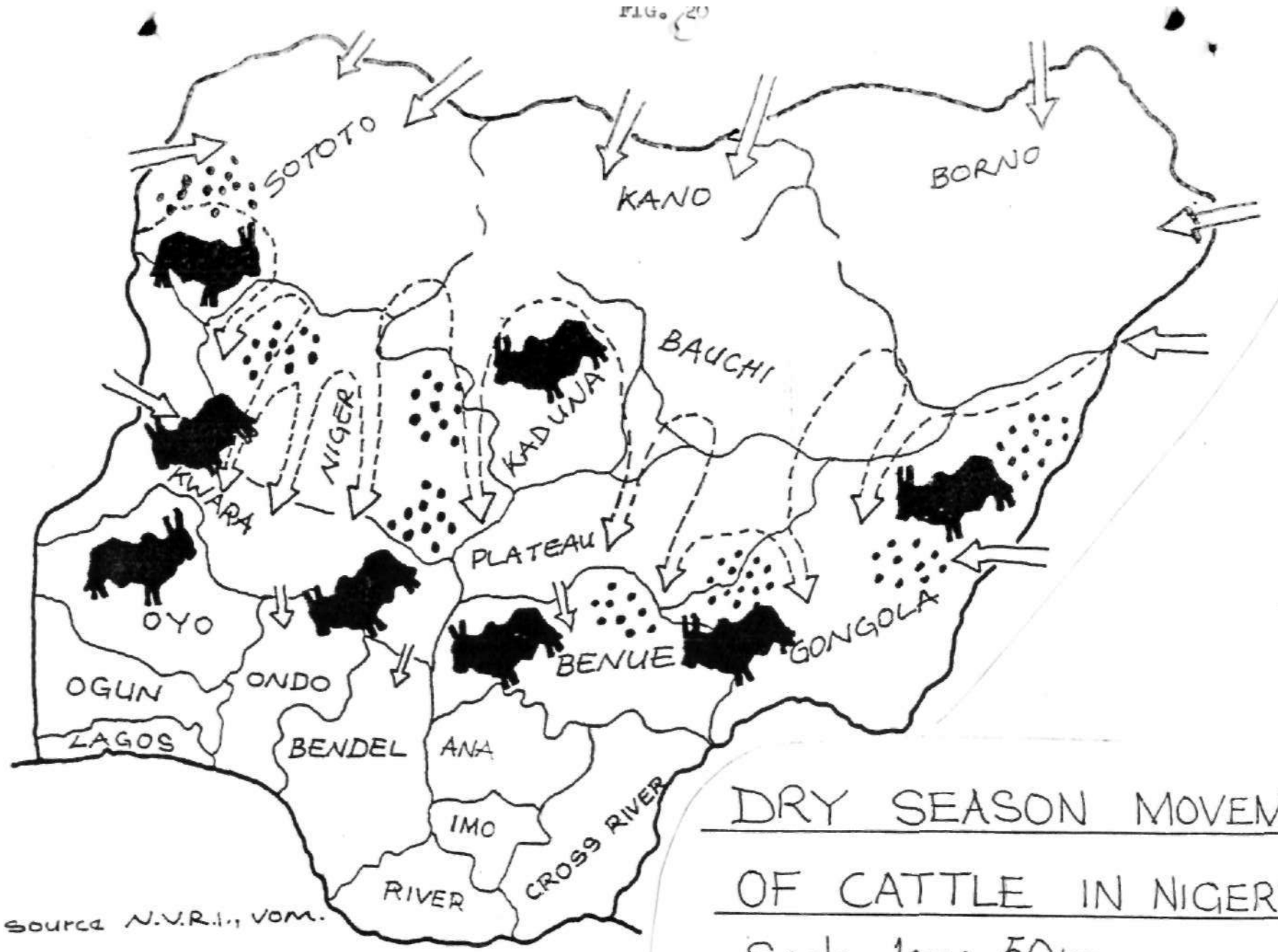
Fig 19

MAP OF NIGERIA

SHADED AREA INDICATES AREA COVERED BY THE SURVEY.

Scale 1cm = 50 Km.

FIG. (20)



Source N.V.R.I., Vom.

DRY SEASON MOVEMENT OF CATTLE IN NIGERIA

Scale 1cm: 50km.

DISCUSSION

Epidemiological Investigations

Epidemiological studies are essential to increasing our understanding of disease, its reservoirs, mode of spread and perhaps most important of all, to monitor the resistance of the national herd to the multiplicities of types of FMD on the African continent and to enable policy makers decide on the best strategies for the control of the disease.

Type O virus was isolated in Northern Nigeria between 1951 and 1961 (Owolodun, 1971) but in recent years, Type SAT_1, SAT_2 and Type A viruses have been isolated (Asagba, 1982). No evidence was obtained concerning the activity of type O virus in recent years. During the course of the study, only Type SAT_1 and SAT_2 FMD viruses were isolated. This was in agreement with what had been the trend since 1964 when Type SAT_1 first appeared in Nigeria and spread from Adamawa province to other provinces in Northern Nigeria. Out of a total of 55 outbreaks reported between 1981 and 1985, 41 samples were submitted for virus isolation. Viruses were isolated from 8 samples. The rate of virus isolation from samples is a function of the timely collection of the samples and timely reporting of an outbreak.

Evidence collected during the investigation of specific outbreaks show that contact and animal movement (trade cattle and tranhumance) were the major method of dissemination of the disease. This was in agreement with what had been officially documented as the most common method of dissemination of FMD in Nigeria.

Retrospective study of official data on FMD in Nigeria showed a disease affecting cattle, sheep and swine with low mortality (mean mortality rate in cattle over a 25 year period was 0.3%, range 0.006 - 2.2%), which compares favourably with mortality rate obtained during the investigations. The study also revealed tremendous rise in numbers of reported outbreaks, numbers of animals involved and mortality when new major epidemiological types were reported. The source of most of the new epidemiological types were traceable to neighbouring countries of Chad and Niger (Owoloodun, 1971). It appears that epidemic strains of FMD are carried into Nigeria by trade cattle from Chad, Niger and Cameroon. On entry, the virus spread rapidly via cattle trade routes and nomads to the south of the country. The prevalence rate stabilises after a year or two,

then declines to low level simering outbreaks during interepidemic periods.

Serological Survey

The serological survey revealed that the disease is more widespread than reported. The officially reported incidence rate was 0.01%, while prevalence rate was 0.05%. The use of VIA antigen to detect VIA antibodies to FMD which is an indication of recent or current infection shows an incidence rate of 9.8% while the use of 146S antigen revealed a prevalence rate of 55%. The prevalence rate contrasts sharply with officially reported prevalence rate of less than 1%. The figure also differs widely from official statistics but is in agreement with oral reports by nomadic Fulani herdsmen and others (Durojaiye 1981).

Incidence and prevalence of FMD as revealed by the serological survey varied from State to State. The border States of Borno, Gongola, Kano and Sokoto have suprisingly low incidence (less than 10%). It was expected that the border state through which most epidemic strains appear to enter Nigeria should have had higher incidence and prevalence. A possible explanation may be that FMD outbreaks are closely linked with trade cattle

and migrant Fulani movements. FMD infection which enters Nigeria from Borno State, for example, via trade cattle from Chad is rapidly dispersed southwards to the middle belt and southern markets (centres of consumption). The greater proportion of the cattle population in the Sahelian region of Borno, Sokoto and Kano migrate southwards to Plateau, Bauchi, Abuja and Niger states during the dry season where they tend to concentrate in watering areas and places where grasses could be found. This yearly event creates the proper conditions for the spread of FMD.

When the animals sampled are broken down into groups based on animal husbandry methods, a similar trend of high incidence of FMD in Bauchi and Plateau states becomes evident. Among the abbatoir samples, Plateau (35%) and Bauchi (24%) were highest. It is not suprising to find high percentage of reactors among abbatoir samples. Slaughter animals are in general old (more than 3 years) and are mostly fulani animals.

Within the private ranch group, Galambi ranch in Bauchi state has the highest incidence rate (62%), while Benue/Carney (60%) was next. In the case of Benue-Carney ranch sampling was

carried out during a period of ongoing outbreak of FMD. With the exception of Benue-Carney ranch which had a recent outbreak of FMD, other private and government ranches sampled did not have any record of an outbreak of FMD in the three years preceding the sampling. High percentage positive reactors among such animals therefore is an indication of inapparent (Subclinical) FMD infection.

Identity and Purity of VIA Antigen

With the additional one step sucrose density gradient purification of the antigen, it was possible to prepare sufficient quantities of VIA antigen free of other viral sub particle contaminants. The antigen preparation used in the serological survey were clearly VIA as indicated by reaction of identity with reference anti VIA serum and its non reaction with reference anti 146S serum.

The possibility of preparation of pure (uncontaminated) VIA antigen and the ease and speed of testing large quantities of serum samples cheeply and accurately recommends VIA testing as a viable tool in epizootiological surveilance of FMD. None of the animals serum tested except Benue

has had any previous vaccination or active clinical disease in the previous 3 years hence the result could be interpreted to indicate a true incidence rate of FMD infection. Mcvicar and Suttmoller (1970) found that as much as 90% of all non immunised cattle, as well as those immunised with inactivated vaccines, respond to FMD virus infection with the production of antibodies to VIA antigen. The observation of (Lobo et al., 1976) in Colombia corroborates the findings of Mcvicar and Suttmoller.

Detection of anti VIA antibodies in the absence of obvious clinical infection is a pointer to the existence of FMD virus carrier animals. It has been shown that exposure of susceptible cattle to minimal amount of virus could establish a carrier state with a subclinical infection with or without fever and viraemia Suttmoller et al., (1968). Evidence of subclinical infection shown by Suttmoller has been supported by field observations (Hedger, 1968, 1970). The detection of VIA antibodies in cattle older than 1 year is an indication of infection with FMD.

The indirect ELISA test method used has been shown to be more sensitive than AGDP Test, Radial

Immunodiffusion (RID) and Immunoelectro-osmophoresis (IEOP) in the detection of VIA antibodies (Pinto et al., 1976). Because of its simplicity, time and material economy, reproducibility and specificity, it has advantage over Radio-immunoassay (RIA). Lombard and Pirord (1981) showed that correlation exists between results of ELISA test and serum neutralisation test in the estimation of antibodies to VIA antigen. The test system was therefore chosen because it could answer the dual question of trying to ascertain the incidence and prevalence of FMD in Nigerian cattle, especially when large quantities of sera samples are involved.

On the possibility of using the test system to detect virus type that has caused a particular infection or have been active in a particular area, the result showed a certain level of cross reactivity of serum antibodies with the different types of 146S antigens used. Homologous reaction of antibody and antigen was generally higher than heterologous reactions. This agrees with the findings of Franz et al., (1982) and Piroirad and Lombard, (1980) that possible cross reactions between individual FMD virus types are small and

that reactions of homologous systems are markedly stronger. The cross reactivity observed can be explained by the multiple exposure to infection which animals in endemic areas experience regularly. When an animal is exposed the first time to a particular type FMD virus, it only makes antibodies against that very type. Future exposure to other types of FMD virus elicites the production of antibodies of broad specificity to that new type and to other types. Hedger (1970) found that although type ASIA 1 and C was never reported or isolated in Botswana among wild beasts, yet their sera reacts with type ASIA 1 and C antigen.

When the sera tested are examined individually against different FMD 146S antigens, no significant differences could be shown between the reactivity of the sera against the different type 146S antigens used. But when a group of sera are examined, their group reactivity against the different 146S antigens show a clear pattern. Thus, in the group of sera from Sokoto state, type A virus reactivity predominates. In Gongola state, type SAT_1 and SAT_2 predominate. The findings in Gongola state are in agreement with recent virus isolations from

the area. In Sokoto state however, no virus type has been isolated from the area in the past four years. The test shows that Type A virus has been active recently in Sokoto state. This test therefore can be useful in situations like Nigeria where inadequate reporting and virus isolations makes it difficult to identify which type of virus has been causing outbreaks.

Comparison of Nigerian Isolates with reference vaccine strains

The Nigerian strains of FMDV grown yielded high titres in the range of $\log_{10}$ 5.8 - 6.0 $TCID_{50}$/ml in bovine thyroid and bovine kidney cell cultures in roller tubes but the same titres was not achieved in BHK cell cultures. Nigerian SAT_1 and SAT_2 strains yielded fair to good titres (range $\log_{10}$ 2.87 $TCID_{50}$/ml - 5.31 $TCID_{50}$/ml) in BHK cells. Inactivation of SAT_2 strains with AEI without formaldehyde pretreatment yielded concentrations of 146S particles which were generally low compared with SAT_1 strains. The 146S particle yield after inactivation varied considerably between the different strains tested. This finding is in agreement with that of Ferris et al., (1984) and Arrowsmith (personal communications) that SAT_1

and SAT_2 strains generally yield lower quantities of 146S particles after inactivation than the A, O and C types.

The response of guinea pigs to the inoculation of inactivated and live 146S particles was generally good. High homologous titres of antiserum was attained with repeated inoculation with inactivated antigens and single inoculations with live antigens.

Subtyping results of the Nigerian SAT_1 strains revealed a close relationship between them. Although the virus strains were isolated at various times during a calender year and from different locations in the country the R% values ranged from 90-100%. It would therefore seem that during 1981, all FMD outbreaks involving SAT_1 was caused by the same strain of virus. These SAT_1 strains however differed from the standard vaccine strains with which they were compared - R% values ranged from 66 - 89%. Of the three standard vaccine strains used TAN 155/68 would appear to provide the widest coverage against the Nigerian SAT_1 strains. While these standard strains would elicit immune response against the Nigerian strains the NIG 4/81 appeared to be the best candidate for a local vaccine. The NIG 6/81 was

more closely related to NIG 2/82 than to the NIG 8/81. NIG 6/81 and NIG 8/81 were isolated from different locations during 1981. They are close enough to be regarded as the same subtype. However all three isolates differ substantially from the standard vaccine strains, ZAM 3/81 and TAN 5/68 with which they were compared. The standard vaccines strains would therefore not offer enough broad protection against any of the Nigerian SAT_2 strains.

The Nigerian viruses studied showed relatedness in serological tests. They however exhibited differences in their ability to grow to high titre in BHK cells, their yield of 146S antigen after inactivation, their ability to stimulate immune response in guinea pigs and their sucrose density gradient profiles.

CONCLUSIONS

The following conclusions could be drawn from this study:

(1) That the incidence rate of 9.8% and prevalence rate of 55% in the states investigated is far much higher than officially reported incidence 0.01% and prevalence 0.05%.

(2) That recent SAT_1 isolates of the FMD are closely related. A candidate vaccine strain

from among them would give protection against these strains. Recent type SAT_2 strains are less closely related. A candidate vaccine strain from among these isolate would give broad protection against current field strains. Other standard vaccine strains used in the African region could protect against Nigerian field strains but would be less effective than vaccine prepared from local strains.

(3) That FMD remains endemic in Nigeria with low level subepizootic outbreaks taking place regularly between epizootic peaks initiated by the introduction of new epizootic strains, or the evolution of more virulent variants of prevalent types.

(4) That an effective disease reporting system is a necessary prelude to an effective control programme. That the disease will become more important and prominent in future when the control of rinderpest and CBPP have been achieved.

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APPENDIX 1

F.M.D. EPIDEMIOLOG

INTERVIEW OF CVO'S AND ANIMAL HEALTH OFFICERS.

1. Date of interview 5th September 1982
2. Officer interviewed/Rank Chief Veterinary Officer
3. State Kano
4. History of FMD in the State Outbreaks occur every year particularly during the dry season months of December, January February and March. Most of the outbreaks are not reported by herdsmen. All known cases by Veterinary officials are promptly reported to Vom and FLD.
5. Outbreaks per Year officially reported: 1980 - 6 outbreaks 1981 - 4, 1982 5 outbreaks
6. Outbreaks not reported:- estimate Estimated 20 other outbreaks occur which are not reported.
7. Reason for non reporting movement restriction, Non availability of vaccine etc
8. Estimated Morbidity rate ~~Absent~~ 5% elsewhere
9. Estimated Mortality rate Less than 0.1%
10. Seasonality of outbreaks More outbreaks occur in dry season
11. State FMD control programme if any Quarantine and vaccination of imported animals occasionally.
12. Means of disease dissemination Disease are carried into Kano from Niger Republic → Animal movements or market
13. Animal Movement Patterns and Routes ~~Names of routes~~ Places given to investigator.
14. Other informations (from field, Veterinarians, Herdsmen etc).

It was the opinion of veterinarians interviewed that outbreaks are rampant during the dry season but they do occur to lesser extent during the rainy. Some herdmen say that they experience FMD outbreaks every three years.

APPENDIX II

Kwara State

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To:
The Director,
National Veterinary Research Institute,
P.O. Vom.

DISEASE OUTBREAK REPORT

Further to my Telegram/Radio Message Ref. No.....Dated.....
I have to report an outbreak of Disease as Detailed Below:—

1 (a) Disease Foot & Mouth (b) State Ref. No. KW/Fab-85/2

Species Bovine

First	by L.A.	by L.S.	by V.O.
10/10/84		✓	

(c) Dates on which Symptoms seen

(d) Means of Diagnosis

	Clinical	P.M.	Laboratory Test
Date	10/10/84	—	—
By Whom	L. S.	—	—

2. LOCATION

Hamlet	Village	District	Province	Map Ref.	Nearest Town
	Ogidi	Ijumu	Kogi LGA	5° 58" E 7° 47 N	AYEGE

3. ANIMALS INVOLVED (as on.....10/10/84.....)

Original No. of Cattle			Deaths to Date		Symptoms		Alive		In-contacts	
Herds/ Flocks	Adults	Young Stock	Adults	Young Stock	Adults	Young Stock	Adults	Young Stock	Herds/ Flocks	Animals
1	247	85	—	—	127	70	247	85	—	—
Totals	332		—		197		332			

4. ACTION TAKEN

(i) Quarantine: Yes/No (ii) No. of Vaccinations already carried out .....

(iii) Vaccine ordered: Yes/No (iv) Slaughter out complete/started/proposed

(v) Other action taken or proposed.....
Animals were covered with antibiotic and streptopen applied to foot lesions. Movement stopped and all surrounding herds alerted.

Signed [signature]
Rank PLSI (JF)
Date 25/2/85

Note.—Additional information and slaughter out details overleaf.

APPENDIX 3

FMD VIRUSES USED IN THE STUDY

1. O1 Pacheko: Type O FMD virus isolated in South America.
2. OBFS 1876: Type O FMD virus (British field strain) sample No. 1876.
3. BOT 1/68: Botswana type SAT_1 FMD virus isolated in 1968.
4. BOT 1/77: Botswana type SAT_1 FMD virus isolated in 1977.
5. TAN 5/68: Tanzanian type SAT_2 FMD virus isolated in 1968.
6. TAN 155/68: Tanzanian type SAT_1 FMD virus isolated in 1968.
7. ZAM 3/81: Zambian type SAT_2 FMD virus isolated in 1981.
8. NIG 4/81, NIG 5/81, NIG 10/81, NIG 11/81, NIG 13/81 } Nigerian type SAT_1 FMD viruses isolated in 1981
9. NIG 6/81, NIG 8/81 } Nigerian SAT_2 FMD virus isolated in 1981
   NIG 2/82 } Nigerian SAT_2 FMD virus isolated in 1982
10. NIG 4/79 - Nigerian type A FMD virus isolated in Niger State in 1979.

APPENDIX IV

F.M.D. EPIDEMIOLOGY

DISEASE INVESTIGATION FORM

1. Date outbreak was reported to investigator:- 16th Nov. 1982.
2. Date investigation was conducted: November 18th 1982
3. Name of farm or herd: Benue - Carney Ranch - Ikyogen
4. Owner of animals: Benue - Carney Ranch.
5. Location of farm/herd: Town Ikyogen via Gboko
   L.G.A. Gboko, State Benue
6. No. of animals in the herd/farm 642 made up of 204 Santa Gertrudes, 340 Sokoto Gudalis and and 198 White Fulanis
7. No. of animals Affected 42 mostly Santas. 34 Santas and 8 Gudalis.
8. Mortality: NIL
9. History of herd movements before outbreak 28 sokoto gudalis were purchased, quarantined and transported to Benue State. After 7 more days quarantine, they were mixed with herd on the ranch.
10. Previous history of FMD in herd NONE
11. Observations of Present outbreak (symptoms, Management practices etc) Typical FMD signs in the Santas. Vesicles on the gum a palate in the gudalis
12. Material for laboratory examination epithelial tissue vesicular fluid.
13. Examination required FMDV isolation CFT, Typing etc.
14. Any other relevant Information:

On the 4th day of noticing the outbreak, 42 animals have been affected and is likely to spread further. The majority of the santas have been isolated on a hill top to prevent spread of the disease to them.