EPIDEMIOLOGY OF RABIES IN KADUNA OYO AND LAGOS STATES OF NIGERIA

BY

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DECLARATION

I hereby declare the originality of this piece of work which was carried out by me in the Department of Surgery and Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University under the supervision of Professor I. A. Abdulkadir and Drs. J. U. Umoh and N. E. Gomwalk.

The work of other investigators is duly acknowledged and reffered to. No part of this thesis has previously been submitted for a degree or diploma.

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THESIS APPROVAL

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DEDICATION

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This work is dedicated to my beloved wife Dr. (Mrs.) Yetunde Ogunkoya and the family who have been devoted and selfless throughout the hard times both in Nigeria and in the United States of America.

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ABSTRACT

Attempts to establish the status and the public health importance of rabies in Nigeria have been undertaken using three states in Nigeria as models: Kaduna State which is centrally situated in the northern part of the country, Oyo and Lagos states in the South-west.

Questionnaire interviews, traceback investigations and examination of hospital and health centre records revealed that in Kaduna state, between 1971 and 1978, 1,495 people were bitten by dogs of which 79% were males and 21% females. Male children. 10-14 years of age, appeared to be at relatively higher risk of being bitten by dogs than female children in the same age group. Monthly distribution showed a high incidence in September, November and February. Thirty-seven point six percent and sixty five percent of the reported cases of biting dogs were diagnosed in differert laboratories as being rabid and the incidence of bite was higher in Soba areas and Southern part of Kaduna State than in the northern part. All the 15 people recorded in 3 hospitals as dying of rabies were males and the death incidence was highest among teenagers.

In Oyo state, 3,374 people were recorded as having been bitten by animals between 1978 and 1981. Males also appeared to have been at greater risk of being bitten by animals than females. Among those bitten, 57.0% of males and. 49.3% of the females were 14 years of age or younger. The rate of bite exposure continued to diminish with increasing age. Ibadan and Iwo areas had the greatest number of bates. About 65% of the biting dogs were diagnosed rabid. Between 1971 and 1979, 22 human deaths due to animal bites were recorded in the state. Of these, 5(4?%) were adult females, 2(25%) were male children in the 10-14 year-old-age group and two victims, both of them females, were victims of cat transmitted rabies.

In Lagos state between 1979 and 1983, 1,849 people were recorded as having had bites from animals. Of these, 57.8% were males and 42.2% were females. Young people up to the age of 14 were at the greatest risk, making up 51% of the bite victims. Within this period, 27 deaths were recorded in the state. Of the 15 fatal cases with complete information, 11(73.3%) were males and 4(26.6%) were females. Of the 11 males 4(36,4%) were children 14 years or younger while 2(50%) of the 4 females were children

14 years of age or younger. In 3 fatal cases, the biting animals were not known; the rest were bitten by dogs. The highest number of deaths, were recorded in 1967 and. 1969.

A survey of dog-*owner* relationship in all 3 states revealed that. 65,*2%* of dogs were kept for security purposes, and 15.2% as pets. About 91% were fed. on table scraps. About 76.6% roamed free, and more than 60% had never been given any previous medical attention.

Serological surveys for serum neutralizing (SN) antibodies against rabies and related viruses in dogs and in humans in Nigeria revealed that out of 857 dog serum samples tested at 1:16 dilution, 311(36.3%) were found to contain antibodies against rabies. Of 463 unvoccinated dogs, 142(30.7%) had neutralizing antibodies. Also 39.2% of stray dogs and 59.5% of known vaccinated dogs had SN antibodies at 1:16 dilution. Two hundred and forty-one dog serum samples were tested against Mokola and Lagos bat viruses at 1:16 dilution. Of these, 17.4% showed antibodies against Mokola virus and 5.8% against Lagos bat virus.

Also, 350 human serum samples were tested for SN antibodies at 1:16 dilution, 28.6% were found to have SN antibodies against rabies virus. Age distribution showed 37% positive within the 40-44 year age group and 40% in people above 59 years of age.

Also out of 158 human serum samples tested against Mokola and Lagos bat viruses at 1:16 dilution, 12(7.5%) had SN antibodies against Mokola virus and 4(2.5%) had antibodies against Lagos bat virus. These findings have established an evidence of infections by groups of lyssa-viruses of the dogs and the people of Kaduna, Oyo and Lagos states.

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INTRODUCTION

According to Fagbami and his co-workers (1981) 99% of rabies victim contracted the disease from dogs. Nawathe and his co-workers (1981) in supporting Fagbami and his group stated that rabies in cows, horses, pigs, cats and goats etc. is a spill-over from dog rabies. In 1932, an apparently healthy dog was reported to have bitten other dogs; the biting dog remained healthy for 6 months whereas the dog bitten came down with rabies (Anon, 1932). Another apparently healthy dog in Kaduna bit two other dogs that later became rabid while the biting dog lived for over a year (Anon, 1936). Since then, there had been a strong feeling that latent rabies exists in this country.

The diversification of lyssavirus species which had been detected in Nigeria and in some other parts of Africa seemsto suggest a long period of speciation and adaptation of the viruses to animals, (Beran, 1981). This diversification and adaptation further strengthen the idea of latency in Nigeria and complicate the vaccination, control and eradication programme for the country because vaccination against rabies does not necessarily protect against

rabies related viruses (Wiktor et al., 1983). So far, it has not been possible to assess serological response to Low Egg Passage (LEP) vaccine in the dog population nor any studies have been undertaken to investigate if chronic rabies exists in Nigeria as it does in India and Ethiopia. Nawathe et al., 1981. Uzebuiro and his co-workers (1980) have outlined these aspect as areas of research that needs urgent attention for a meaningful control and eradication programme to be worked out for the country.

The aims of this study are:-

1. To delineate the epidemiology of dog bites and rabies in the three state's (Kaduna, Oyo and Lagos), by questionnaire survey, review of hospital records, interviews and discussions with health and veterinary workers, villagers and their heads and traceback and interview with selected family members and acquaintances of victims.
2. To accummulate basic information on the presence or absence of latent rabies and rabies-related viruses in dogs population of the 3 states. Serological evidence of infection with lyssavirus group will be based on the presence of neutralizing antibodies (NA) against lyssaviruses in apparently

healthy unvaccinated dogs of the 3 states.

3. To determine the antibody prevalence in dogs in the three states, reported to have been vaccinated with avianized low egg passage (LEP) antirabies vaccine produced in Nigeria.

An apparently healthy dog that lived for over a year after infecting other two dogs with rabies was reported in Kaduna (Anon, 1938); Mokola virus was isolated in a shrew in Ibadan, Capital of Oyo state (Shope et al., 1970) and Lagos bat virus was isolated in Lagos from the pool of fruit bat (Boulger and Porterfield, 1958). It would therefore be interesting to study the rabies situation in these areas.

LITERATURE REVIEW

The Virus

Rabies and several rabies related viruses belong to a group of more than 75 viruses called Rhabdoviruses because of their bullet-shaped morphology. The virus is about 180 nanometers long and 75 nanometers in diameter. It is enveloped, lipid-containing and has single stranded RNA (Hummeler et al., 1967). The virus particles contain RNA (1-3%), lipids, carbohydrate (15-25%) and five proteins. The glycoprotein G , forms the spikes on the surface of the virions. (Murphy, 1985). The spikes are important in attachment of virions to susceptible cells. They also carry the key antigenic deteminants which elicit the production of neutralizing antibodies when they are contained in a vaccine (Murphy, 1985).

The virus is fragile, easily inactivated by disinfectants and does not exist free in the environment (Kaplan et al., 1966).

Susceptible host All warm-blooded animals are susceptible to rabies (Kaplan, 1977), but not all animals are equally susceptible. The most highly susceptible are foxes, coyotes, jackals and wolves, kangaroo-rats, cotton rats and common field wolves.

The next most susceptible group contains many species which are important in maintaining reservoirs of infection in wildlife in many parts of the world. Dogs are listed as being only moderately susceptible but they are the animals most likely to spread the infection to human being (Kaplan, 1977).

Transmission The most effective way of transmission is through bite wound and abrasion of the skin inflicted on the victims by the transmitting host. In specialized situation, aerosol, (Winler, 1968, Winler et al., 1972, 1973), oral routes (Correa-Giron et al., 1970; Fischman and Schaeffer, 1971) and corneal transplantation have been reported (Baer et al., 1982).

Pathogenesis Following inoculation into the muscle, the virus can only be recovered from the site for a brief period, usually not over 24 hours (Baer, 1975, Murphy et al., 1974). The virus enters an eclipse phase three hours after inoculation (Baer et al., 1968). The initial virus replication occurs in the striated muscle cells at the site of inoculation (Murphy et al., 1973). This serves as an amplifying source of the virus for assault upon exposed endings of the peripheral nervous system (Murphy et al., 1973). The exposed sensory nerve endings in the

Clinical Signs and Symptoms

Precise and consistent topographic locations of rabies infection in the brain is the most likely explanation for the specific signs and symptoms at various stages of clinical illness in people and animals.

The incubation period has been reported to be as short as 9 days and although unconfirmed, as long as 19 years (Hattwick, 1974). The incubation period is usually between 20 and 90 days and is shorter when the site of the bite is on the heads (25-48 days) than when it is on an extremity 46-78 days (Held et al., 1967).

Although occurring in less than one percent of the cases, well documented incubation periods have occurred 1-5 years after exposure, (Hattwick, 1974; Held et al., 1967 and Rubin et al., 1969. The incubation period depends on the size of the viral inoculum, the proximity of the wound to large nerves, and the length of the neural path from the wound to the brain (Beran, 1981). In the dog, rabies can be manifested as furious or dumb. The illness is ushered by a prodromal period with irritability, abnormal sensations about the wound site and hyperesthesia of the skin. Animals may claw and chew at the skin until it is dehaired and abraded.

Dilatation of the pupils and a nonfocusing stare may give the eyes a squinting, frightening appearance (Beran, 1981). Intense conjunctival secretions and salivation with the head characteristically held at a downward angle causing drooling or frothing of tenacious saliva could often be noticed. Frothing at the mouth and the holding of the tail tightly between the rear legs have been described as characteristics of canine rabies through the ages (Beran, 1981) as the clinical disease becomes apparent. Rabid dogs characteristically develop a threatened or aggressive pattern of behavior. There may be alertness, loss of natural timidity, aberrant sexual behavior and aggressiveness (Johnson, 1971).

Snapping at or attacking people or other animals which seem threatening, biting cages or buildings after being confined, or aggressively seeking to attack real or even imaginary objects within range,are characteristic. During the attacks, the animals apparently feel no pain and may lacerate their mouths or knock out their teeth in their frenzy. Animals may wander and may attack a large number of people and/or animals. Beran (1979, 1981) observed that one frenzied dog in the

Philippines attacked 41 people and 9 other dogs during a 4 hour period before it could be killed The vicious behaviour may be intermittent and each attack may last only a few minutes followed by essential calmness. Intense conjunctival congestion has been observed as a frequent feature (Miner, 1977).

During the furious period, some dogs may show increased thirst and may drink or try to drink. In spite of these behavioral aberrations, most dogs continue to recognize their owners and to respond in a quieting way to their voices (Kaplan, 1977). They may snap at those whom they know if they feel threatened but they do not commonly attack their owners. Rabid dogs generally recognize their homes and know the way to them (Beran, 1981).

As the clinical disease progresses, there is a development of increased muscle tone and difficulty in swallowing, owing to painful and spasmodic contractions of the muscles of deglutition when fluid comes in contact with the face. Often, the mere sight of liquids will induce such contractions; hence, the common name hydrophobia or "fear of water." This, of course, does not seem to

develop in all cases of rabies. The final stages of the disease result from extensive damage in the CNS. Varying degrees of CNS problems are demonstrated (Baer et al., 1977).

In the paralytic or dumb stage of canine rabies, the animals become progressively paralyzed. Paralysis commonly begins at the head and neck, then one or both hind legs causing the animal to sway or fall. The jaw falls open and muscles of deglutition become paralyzed, causing drooling of saliva and inability to swallow food and water even if the dog tries to eat. Paralysis continues to develop, though not always uniformly bilaterally. The affected dog falls, ultimately becomes comatose, and death ensues, usually from respiratory failure (Beran, 1981).

Pathology

At necropsy, grossly visible congestion of meningeal vessels of the brain and spinal cord are commonly seen. (Dupont et al., 1965). Negri bodies within affected neurons are the most characteristic and, indeed, are the only pathognomic microscopic findings. These cytoplasmic

inclusions are sharply defined, spherical or oval, eosinophilic Feulgen-negative bodies 2-10nm in diameter and containing a central mass of basophilic granules (Matsumoto, 1963). Immunofluorescent studies have demonstrated specific viral antigens in the Negri bodies and electron micrographs have shown that they consist of large matrices of viral nucleocapsids (eosinophilic material) and budding virus (the basophilic granules) (Matsumoto, 1962, 1963, Matsumoto and Miyamoto, 1966, Mujamoto and Matsumoto, 1965).

Negri bodies occur in about 75-95% of animals which have died of rabies in the field (Dupont et al., 1965). Large neurons of the hypocampus are the favored area of search for Negri bodies, although the midbrain, cerebellum, various ganglia, and motor nuclei have frequently been reported as important sites of Negri bodies, in some studies showing higher prevalence than in hypocampus (Beran, 1981). Other histopathologic findings include a variable degree of cerebral edema, perivascular mononuclear inflammatory infiltration, neurophagia, and neuronal degeneration. These changes are usually sparse in relation to the extent of the terminal infection (Murphy, 1977).

In the brain, nerve cells in the grey matter are most affected. In the spinal cord, histological lesions are most marked in the nerves and posterior root ganglia corresponding to the sites of exposure. Subependymal and subcortical demyelination may be observed (Perl, 1975).

Rabies Virus Antigenic Relationships

There are seven rabies related viruses of which four (Lagos bat, Kotonkan, Mokola, Nigerian horse virus) were isolated in Nigeria. Other rabies related viruses are Obodhian Klamath and Duvenhage. (Beran, 1981, WHO, 1984).

Experimentally, it has been indicated that some of the rabies related viruses are apparently as pathogenic to dogs as classical rabies virus and that these viruses are also excreted in the saliva of experimentally infected dogs (WHO, 1984). Apart from this Mokola virus has been isolated on two occasions in Nigeria from patients with central nervous system disease (Familusi and Moore, 1972; Familusi *et al*., 1972), and recently there has been a report on a rabies-like disease outbreak among cats in Zimbabwe caused by Mokola virus which was horizontally spread among kudu (*Tragelaphus scriptis*) and also

Kudu and cattle (Foggin, 1983).

The pathogenesis is immediately extendable to domestic animals and to man. There is no difference in pathogenetic behaviour of the virus in its victim. The difference in rabies infection in reservior host species and the "deadend" host species has more to do with different biting tendencies of each species when rabid than with the nature of the infection, per se. (Murphy, 1985).

Epidemiology of Rabies in Nigeria

Rabies has long been recognized in Nigeria. The evidence of the long history of the disease is contained in the fact that every major Nigerian language has a name for rabies in dogs (Umoh and Belino, 1979) "chiwon kare" in Hausa, "digbolugi" in Yoruba, "ginnaji" in Fulfulde, "idat ebua" in Efik/Ibibio (Umoh and Belino, 1979) and "Ara nkita" in Ibo language (Fagbami et al., 1981)

The first available record of human rabies in Nigeria is contained in the Annual Report for Southern Nigeria, 1912 Boulger and Hardy, (1960), which reported two cases, one in Eket and the other

in Bonny. On October 21, 1933, a Porto-Novo woman with a history of dog bite on the chin 13 days previously was admitted to the African Hospital, Lagos (Savage, 1934).

Bisseru (1972) quoted the record of rabies which occurred in 1936 in a school-boy who developed the disease 40 days after a bite on the foot by a suspected rabid dog in Ibadan. Between 1928 and 1959 the number of reported human deaths due to rabies in the country was 120 (Boulger and Hardy, 1960). During the same period, the laboratory in Yaba, Lagos examined 79 human brains of patients with clinical rabies. Analyzing 61 of these 79 cases into two age categories, children and adults, Boulger and Hardy (1960) observed that a definite history of dog bites could be obtained in 82% of the 50 cases in which information on history of exposure was available.

Prior to 1960 human rabies continued to be reported sparingly. In 1951, three human cases, in 1952, two and in 1953 eleven human cases were reported for the country (Bisseru, 1972).

Starting from 1962, there seems to have been improvement in the reporting of humen rabies. The data on reported human rabies from 1962-1974 compiled by World Health Statistical Annual shows (excluding 1966 in the calculation) the yearly average number of deaths in the country due to rabies was 17.67 and the range was from 8-28. Data collection for 1966-1970 was probably affected by the civil war (Umoh and Belino, 1979).

Apart from this, few other workers have reported cases of humen rabies in various parts of the country. Ezeokoli and Schnurrenberger (1977) indicated that the annual deaths from rabies in East Central State (now Imo and Anambra States population 6,152,823) averaged 9.0 between 1973-1974 while between 1962-1974 averaged annual death for Nigeria was 17.67 (Umoh and Belino, 1979), whereas the national 10 year survey conducted by Ezebuiro et al. (1980) revealed a mortality of 16.9 per annum in 37% of the Nigerian population from whom reports were available.

In 1935, a dog brain was found positive for Negri bodies when examined by Medical Research Laboratory in Yaba (Annual Report of Nig., 1935), Between 1928 and 1929, 1,100 cases of rabies were reported, 1,046 (95.1%) of the cases were in dogs (Boulger and Hardy, 1960). The annual report of the Veterinary Division of Northern Nigeria recorded outbreaks of the disease in canine species from Kano, Zaria, Katsina, Borno, Ilorin and the Cameroons (Anon, 1946). In 1943, outbreaks occurred in Katsina and Kano and in 1946 there were reports of outbreaks in Kano, Plateau, Makurdi and Enugu (Anon, 1946).

The annual reports of Veterinary Services, Northern Nigeria (1956-1957) reported an outbreak in Sokoto involving 16 dogs. Between 1948 and 1953, Thorne (1954) reported a total of 256 laboratory confirmed animal rabies out of which two cases each were in horses and cattle, one in a cat and the remaining 257 (98%) occurred in dogs. In the Lagos area between March, 1956 and December, 1959, the laboratory at Yaba confirmed 86 cases of canine rabies accounting for 91.5% of the total laboratory diagnosed cases. The Federal Department

of Veterinary Research, Vom confirmed 64 cases of canine rabies in 1964, 48 cases in 1965 and 35 cases in 1966. Between May, 1967 and January, 1969, (Banerjee and Blogbo, 1970) reported 83 confirmed canine cases.

Nuru (1973) conducted a ten year survey (1963-1972) in Kano State. During the period there were 59 confirmed cases and 56 unconfirmed suspects. The highest number of cases (25) were recorded in 1966.

In 1971, 53 cases of animal rabies were reported and in 1972, a total of 44 cases of which 42 (95.5%) were in dogs. In 1974-1975, [illegible] rabies was confirmed in 79% of the specimens examined but during 1977-1978, only 57 (23.5%) out of 242 brains examined by the Federal Veterinary Research Centre, Vom (Annual Report, 1976) were positive.

Between 1962 and 1973, a total of 2,064 persons were treated for dog bites at the University College Hospital in Ibadan (Kale, 1977). Fagbami et al (1981) reported that a total of 7,549 victims of dog bites received post-exposure antirabies treatment from 24 hospitals over a 10 year period.

The results of questionnaire surveys conducted in 1975 however, indicated that the frequency of canine rabies has been increasing in most of the states except Rivers State which recorded only one case of rabies between 1970 and 1975 despite the fact that the dog population was said to be increasing (Umoh and Belino, 1979).

In Nigeria, however, it is not unusual for puppies to have rabies even before they reach the age they could be vaccinated against rabies. Such cases in puppies have been reported by Adeyanju and Addo (1977) and Anon (1976).

Next to dogs in order of importance in relation to bite and transmission of rabies are cats (Umoh and Belino, 1979). In the country, between 1928 and 1959, there were 48(4.4%) confirmed cases reported in cats (Boulger and Hardy, 1960). Eight cases (8.5%) were reported for Lagos between March, 1956 and December, 1959. For the four year period, 1965-1968, there were only four confirmed feline cases and all occurred in Plateau Province (Owolodun, 1969). Between

1974-1975, rabies was confirmed in 5(41.7%) of 12 specimens examined in National Veterinary Research Institute, Vom and between 1977-1979 one of the five cat brains examined was positive. Reports on human exposures to feline rabies are more scanty than for canine. Many people have not actually associated cats with rabies (Umoh and Belino, 1979). Death as a result of cat transmitted rabies in man was found to be relatively low. Out of 169 cases recorded in hospitals between 1969 and 1978 only in one case (0.5%) was a cat involved (Fagbami et al., 1981).

Rabies has also been reported in other domestic animals. From 1944 to 1956, one case in a cow and one in a horse were reported by Libeau (1960). There have been two reported serious outbreaks in farm animals. One outbreak in Butu, near Minna affected a herd of 55 cattle and a flock of 15 sheep (Elegbe et al., 1970). The other outbreak was in Fresian cows imported from England by Kano State Government for livestock improvement in which rabies was confirmed in four of the cows (Nuru et al., 1974).

Rabies has been reported in Nigerian dwarf goat (Elegbe et al., 1970) a donkey (Banerjee et al., 1970); Porras et al., 1976), and a pig (Boulger and Hardy, 1960).

Between 1968 and 1977 rabies diagnostic laboratory of NVRI, Vom confirmed rabies in 2 pigs, 11 cows, 5 sheep, 3 goats, 2 donkeys and 1 horse (Ezeubuiro et al., 1980).

The existence of wildlife rabies has not yet been well established in Nigeria. Rabies virus could not be isolated from the brains and salivary glands of 1,913 rats and 220 shrews trapped or found dead in Lagos in 1959 (Boulger and Hardy, 1960). But there is a belief among the Hausa people that a bite from the ground-squirrel (Xerus erythropus) results in madness and death about one to six months after the bite (Mcmillan et al., 1960). However, there have been occasional reports of laboratory confirmed rabies by mouse inoculation tests and Negri bodies in a caracal lynx that died in the University of Ibadan zoo. Okoh (1982) also reported a case of rabies in the same species.

Kasali (1977) confirmed rabies in one civet cat. All the animals involved were in captivity and this has raised questions on the sources of infection. Ezebuiro et al (1980) speculated that rodents may be involved in the transmission.

Apart from these animals in captivity what is happening in the natural range is yet to be reported.

Control of Rabies

The control and prevention strategy of rabies in human beings, livestock and other non-resevoir ("Deadend") hosts are based primarily on breaking the cycles of transmission in reservoir hosts and secondly on protection of these alternate hosts from exposure or infection (Beran, 1981).

The basic principles of control of animal rabies as recommended by World Health Organisation (WHO, 1982, 1984) include:

1. Elimination of stray dogs
2. Canine vaccination
3. Wildlife control.

In addition, the Committee recommended the following specific measures in areas affected with rabies:-

1. Registration, licensing and taxation of dogs.
2. Elimination of stray animals other than dogs.
3. Restrain of dogs while control campaigns are underway.
4. Mass vaccination of dogs free of charge
5. Provisions of adequate facilities for diagnosis
6. Reduction of wildlife species where these are reservoirs of the disease.
7. Continual and energetic publicity campaign.

Implementation of these basic principles and specific measures are often very difficult to achieve in most countries, especially in developing countries (WHO, 1984).

In Nigeria, attempts to control rabies have been directed towards controlling the disease in dogs and cats (Fagbami et al., 1981) by stray dog control and by vaccination with modified live virus chick embryo origin low egg passage (LEP) Flury strain vaccine prepared by the National Institute of Veterinary Research, (NVRI) Vom (Umoh and Belino, 1979).

The NVRI is the sole supplier of canine and feline anti-rabies vaccine to the Veterinary clinics in the country. Annual vaccine production and issuance by this Institute has been found wanting for the past 10 years (Okeke, 1981). There has been a rising demand which could not be met due to dependence on imported items. (Okeke, 1981).

Fagbemi _t al_ (1981), however, found that up till 1978, antirabies vaccination is given free in 75% of the Veterinary clinics with the other 25% charging 50k - ₦3.00. They also found out that pets are licensed in some few local governments areas, but this is not practised in many areas. Even where practised, there is no uniformity in the practice and it is the few enlightened members of the society who are likely to license their dogs.

Ezebuiro and his colleagues (1980) revealed that vaccination of dogs is not generally practiced. It was noted that only 27.7% of dogs they surveyed had received antirabies vaccination. Stray dog control has not been seriously implemented. There have been sporadic efforts when outbreaks were reported or a dog bite involved an "Important Personality". (Personal observation).

Latency and Abortive Rabies

The question of latency and abortive rabies especially the secretion of rabies virus by healthy-appearing dogs, has been raised from time to time. As a rule rabies in dogs is always considered a fatal disease. It is now known that dogs can survive an experimental rabies infection with or without sequelae (WHO, 1984). Such dogs are subsequently shown to be immune (Pasteur, et al., 1882, Martin, 1963; Nilsson, 1970; Fekadu et al., 1982).

In rabies enzootic areas, serum neutralizing (SN) antibodies have been detected in apparently healthy animals. A survey of SN antibodies in mongoose in Grenada (Everard et al., 1981) was reported. Yasmuth et al. (1974) found 18% of the 49 dogs tested for pre-immunization evaluation had SN antibodies. In Nigeria antibodies against Mokola virus was found in a bat and bird (Ruegsegger et al., 1961; Kemp et al., 1972; Bell, 1966, 1975; Bell et al., 1972) found heat-stable antibodies prior to vaccination in 6% of 230 students and Faculty members of a Veterinary School.

The conclusions generally reached from these findings are that rabies is not necessarily fatal to man and animals, that there are subclinical infections probably due to attenuated strains of rabies virus (Ruegsegger et al., 1961) or as yet unidentified related viruses (Shope et al., 1970).

MATERIALS AND METHODS

Materials and Methods

Study Areas

The estimated population of Kaduna state is 6,535,400 with a land area of 70,245 km^2. The state falls within the Sudan savanna in the extreme north and Guinea Savanna in the south.

Oyo state has an estimated population of 8,306,400 and a land area of 37,705 km^2. To the north of Ibadan, the state capital, there are seven cities that have populations of not less than 150,000 each. The cities are usually surrounded by clusters of villages which lie within a few kilometers. Climatically, Oyo state is defined as tropical hinterland and receives from 100-150 cm of rainfall annually. The northern part of the state bordering Kwara state is Guinea Savanna, but the south is a forest area.

Lagos state is situated in the southern part of Nigeria. It is a very small state but densely populated. It has an estimated population of 2,644,800 and 3,345 km^2 of land. This state is partly composed of sea water lagoons stranded behind sandbars and partly of islands separated by creeks.

It is low-lying and marshy. It is on intermediate forest hinterland. Figure 1 shows an outline map of Nigeria with the location of the three states, and of the places visited.

Serum Samples

(I) Canine Sera: Serum samples were collected from:

1. owned dogs without history of antirabies vaccination,
2. owned dogs with history of vaccination,
3. owned dogs with unknown antirabies vaccination status,
4. impounded stray dogs.

(II) Human Sera: Serum samples were collected from:

1. persons who visited the hospital for various complaints,
2. persons previously bitten by dogs but did not receive post exposure vaccination,
3. persons who could not recollect any history of exposure and has never been given any preexposure prophylaxis.

(III) 1. Specific anti-Mokola and anti-Lagos bat viruses sera were obtained from Dr. Wiktor of the Wister Institute, Philadelphia, Pennsylvania.

2. Known rabies positive and negative sera used in the Rapid Florescent Focusing Inhibition Test (RFFIT) were obtained from NVSL.

Viruses (1) BHK-21 adapted CVS strain of rabies virus with a titre of $10^{5.3}$ $TCLD_{50}$ which was obtained from National Veterinary Services Laboratory, Ames, Iowa was used.

(2) BHK-21 adapted Mokola and Lagos bat viruses obtained from Dr. T. Wiktor of Wistar Institute Philadelphia, Pennsylvania with titres of $10^{8.0}$ for Mokola and $10^{1.7}$ for Lagos bat virus were used.

Monoclonal Antibody

Monoclonal antibody 422-5 prepared as mouse ascitic fluid specific for rabies-related viruses was obtained from Dr. T. Wiktor, Wistar Institute, Philadelphia, Pennsylvania.

Cell lines Baby hamster kidney cells (BHK-21) clone 13a obtained from the National Veterinary Services Laboratory (NVSL) Ames Iowa were used in the Rapid Fluorescence Focusing Inhibition Test (RFFIT) as previously described by Smith et al (1973).

Growth Medium

Glasgow minimum essential medium ($G.MEM_{10}$) supplemented with 10% fetal calf serum (FCS) and antibiotics penicillin and streptomycin (1%) were used to propagate the BHK-21 cells in 150cm^2 plastic tissue culture flask, Corning Glass Works, Corning, New York.

Histoplates

a. The eight well disposable plates obtained from Lux Scientific Corporation, Newbury Park, California and 72 well flat bottom Terasaki Histoplate from Cooke Laboratory, Alexandria, Virginia were used.

General Survey

Three questionnaire surveys were carried out.

1. The first was aimed at obtaining information on the epidemiology of dog bites, clinical rabies, treatment given to exposed people and the general reactions and understanding of the people to rabies. Questionnaire I (appendix I) was used and this was distributed to health centers, hospitals and

epidemiological units in the 3 states. Subsequent visits were made to these different health establishments and completed questionnaires were collected. Whenever possible on such visit, physicians were questioned on points that were not clear in the questionnaires or on points that needed further clarification.

2. The second questionnaire (appendix II) was used as previously described. Among the data requested were (a) number of canine rabies outbreaks per year, (b) number and species in which rabies was diagnosed and methods of diagnosis, (c) number of animals vaccinated against rabies per year, source of the vaccine and the methods of storage. The completed questionnaires were also collected during a subsequent visit to the C.V.Os. or D.V.Os.

3. The third questionnaire (appendix III) was administered to answer questions on the purpose of keeping dogs in the 3 states. The questionnaires were distributed to dog owners who visited the Veterinary clinics in the 3 states and to some owners in the villages. For the owners who could not read and write, questions were asked directly or through an interpreter to complete the questionnaire.

4. The fourth questionnaire (appendix IV) was used in hsopitals and villages to obtain information on previous exposure, type of treatment or nonexposure from people whose blood were obtained for serum.

Oral Interviews and Discussions

As previously indicated above, the DVO, CVO livestock superintendents, the health workers and physicians were interviewed during visits made after the distribution of questionnaires. During visits to rural areas, arrangements were made to interview many village or hamlet heads. Most of the questions asked were contained in the questionnaire (1-3). The livestock officer or other employees of the Divisional Veterinary Office or Health Office generally arranged the interview with village heads and usually also acted as interpreter.

In villages, attempts were made to estimate the human, canine and feline populations, the incidence of "mad" dogs or cats, the incidence of animal bites, human rabies, the local reaction to rabies outbreaks and treatments for bites. Human population was estimated as a product of the number

of households and the estimated average number of persons per household. The dog population was estimated by using the average number of dogs per household in the area. The village heads were asked for their recollections or incidences of "mad" dogs during the past two to three years, and subsequently asked to describe the behaviour of the "mad" dogs.

Discussions were held with physicians, health superintendents, nurses and other traditional healers on rabies problems.

Case Records

Case records were provided by the University of Lagos Teaching Hospital the University Teaching Hospital, Ibadan and Teaching Hospitals of Ahmadu Bello University in Zaria, Kaduna and Malunfashi. At the state hospitals and health centers, the investigator searched the records to extract information on the daily or weekly incidence of animal bites reported for treatment and the number of cases of human rabies encountered.

Trace-back visits

This was usually stimulated by cases from records in the hospitals. Efforts were usually made to obtain missing information.

Apart from this, attempts were made to discover the understanding people had about rabies, specifically what they knew about the maintainance and transmission of the disease, how they recognized outbreaks and what measures were taken on the victims and the animals involved.

Serum Collection

Blood samples from humans and dogs were collected by venous puncture in vials without anticoagulant. Sera were obtained from the blood by centrifugation or following clotting, and stored at -20°C until used.

Treatment of serum Samples

Both human and dog sera collected were heat inactivated at 56°C for 30 minutes in a waterbath as recommended for use in RFFIT test, Smith et al (1973). All dog sera were treated with 25% kaolin to absorb non-specific inhibitors (Hierholzer et al., 1969). Briefly, an equal volume of 25% kaolin in distilled water and the serum sample after being allowed to stand for 20 minutes and then centrifuged at 1800 rpm for 10 minutes. The supernatant was stored at -20°C until used.

Cell Growth

When monolayer was formed, the medium was decanted and the cells washed with the trypsin-versene (ATV). The cells were then trypsinized, resuspended in fresh growth medium and dispensed (0.4ml) to each well of a single-use eight well plastic plates. The plates were incubated in a humid chamber at 37°C and 5% CO_2 atmosphere.

RFFIT for serum neutralizing (SN) antibody

All sera were subjected to RFFIT as previously described by Smith, et al (1973). A 0.1ml of dilutions of test sera were mixed with an equal amount of rabies virus (50-50% Fluorescing Foci Doses ($50FFD_{50}$/0.1ml) in a multi-chambered slides, flat-bottom microtitration plate in test tubes. After allowing the mixture to react in a carbon dioxide (4-5%) incubator for 90 minutes when the mixture was incubated in a multi-chambered slides, a 0.2ml of $5x10^5$ cells/ml suspended in the growth medium was added to each chamber of all the slides. When incubated in a microtitration plates, with micropipette a 0.01ml of virus-serum is transfered into corresponding wells of the histoplates and 1 drop of $1x10^6$/ml BHK-21 cells suspended in growth medium was added to each well, and when incubation

of the mixture was done in test-tubes, 0.2ml of the incubated serum-virus mixture was introduced to a monolayer of BHK-21 cell and allowed to absorb for few minutes in a CO_2 incubator. After absorption, excess serum-virus was removed and 0.4mls of growth-medium was added to each chamber.

The resultant presence or absence of viral infection in both cases was examined after 24 hours of incubation in a 4-5% carbon dioxide atmosphere at 35-37^{o}C by fluorescent antibody (FA) staining. (see appendix 18).

Known rabies positive and negative sera obtained from NVSL were set up similarly and used as controls in the same manner. This method was used to screen 857 dog and 350 human sera. Some positive sera were titrated using 2 or 5 fold dilutions.

Serum Neutralizing (SN) Antibody Test Against Mokola and Lagos Bat Viruses

Detection of antibodies to Mokola and Lagos bat viruses was done by RFFIT, but the staining was done by an indirect FA using monoclonal antibody (422-5) prepared as a mouse ascitic fluid. After the mixture of the test serum and the virus had been

incubated at 37°C and 5% CO_2 atmosphere for 24 hours, the histo-plates were removed, washed in physiological buffered saline (PBS) pH 7.2-7.4 and fixed in cold 80% acetone for 30 minutes. After drying, 5ul monoclonal antibody (422-5) were dispensed in each well and incubated for 30 minutes.

The plates were again washed twice and 5ul anti-mouse globulin conjugate was placed in each well and incubated for 30 minutes. The plates were washed and read by FA microscopy while still wet.

Known positive and negative sera against Mokola and Lagos bat virus were used as controls on each test.

R E S U L T S

Results

Kaduna State

Questionnaire Survey

The results of the questionnaire survey carried out in Kaduna state are summarised in Table (1). Kaduna and Zaria reported the highest number of dog bites as well as of rabies vaccinations. The rate of dog bites was higher in the large cities than in rural areas. Funtua, Katsina and Daura reported very few dog bites and further investigations showed that the ownership of dogs in these areas was confined to the non-indigenous population, usually residing in the Government Reservation Areas (GRA).

Interviews

The information obtained from the oral interviews with villagers and chiefs in Kaduna state is summarized in Table (2).

In Kaduna state, the dog population was neligible in villages around Katsina and Funtua, (1 dog to 5,000 person on the average) and consequently, there were no recollections of dog bites or rabies. The dog population increased progressively towards the South and so did recollection of dog bites and rabies. Villages in the Zonkwa and Kafanchan areas had the highest density of dogs (1 dog to 6 people) on the average.

Doctors of St. Louis Hospital, Zonkwa, recollected that there were three deaths due to rabies within the previous year. Although other hospitals had inadequate records and recent personnel changes, there were still some stories of death associated with dog bites. Of the 9 physicians interviewed in Kaduna state, 3 gave very useful information. A few of their observations were: (1) that patients showing clinical rabies were often asked to return home because of inavailability of beds and accommodations in hospitals, and/or that

it is futile to try to treat clinically rabid patients (2) that shortage of vaccine and antiserum have been so acute that physicians have been, in most cases, helpless even where there was an obvious danger to life of the exposed persons, (3) that traditions and religion have made autopsies on patients that died of suspected rabies almost impossible.

Records

Between 1971-1978, 1,495 people were recorded bitten by dogs. Out of these the information on the site of bites were available on 119 cases and are summarized in Table 3. The most common sites were legs and feet (27.7%) while the head was the least (0.8%). Of the 1,495 exposed people, only 186 had complete information on age, sex and period of bite which could be analyzed. (Fig. 2). Seventy-nine percent were males and 21% females. Male children, (10-14 years), represented 14.5% and male adolescent (20-24 years), accounted for

16.1%. The two groups appeared to be at greater risk of being bitten by dogs in Kaduna state than other age grouns. Female children below 5 years of age and 5-9 years were at higher risk among females. Generally, there were more people recorded as being bitten between 1971-1973 than between 1974-1978. Occurrence of dog bites for the different age grouns was dependent on sex ($P<0.01$).

Monthly distribution of dog bites of 195 victims (Fig. 3) showed a high prevalence for the months of September and November.

Between 1971 and 1980, 21 people were thought to have died of rabies. Data (Table 5) for 6 of the cases was not sufficient for tabulation. Fatality was highest in 1973 followed by 1979 and no record was available for 1976. The 15 fatal cases in which date were available occurred in males.

Table 5 shows the result of laboratory examinations on 59 dogs between 1971-1973 at the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. Thirty-one brain samples (53%) were positive by one or more tests. Eighteen of the positive cases (58%) were owned dogs while 13 (42%)

were stray dogs. Approximately 50% of the cases in which clinical signs were recorded showed the furious form and the other 50% showed the dumb form of rabies. Rabies was also diagnosed in 1 cat, 1 horse, 3 cows and 3 people. The three methods of diagnosis agreed quite closely, although the mouse inoculation test was not used as consistently as the others. There were only two cases which were positive by mice inoculation tests but negative by both FA tests and sellers staining for Negri bodies.

Oyo State

Questionnaire Survey

The results were summarized in Table 7. Between 1976 and 1979, 291 outbreaks of rabies were recorded and the highest outbreaks occurred in Ibadan and its districts. Of the 414 dogs captured as rabies suspect 39(9.4%) were found to be actually rabid whereas 38 dogs brought to the veterinary clinic for quarantine when showing abberant behaviour, such as biting occurred 26(68.4%) were rabies positive. Three dogs previously vaccinated against rabies died of rabies in 1980.

Oral Interviews

Information obtained from oral interviews is summarized in Table 8. It was observed that dog and cat populations in Oyo state were large though it was difficult to make an estimate.

People in both the cities and rural areas were aware of the disease called "digbolugi" (rabies). Sixty percent of all individuals who were interviewed could describe the clinical manifestation of rabies in dogs.

Records, Hospitals, Health and Veterinary

Three thousand, three hundred and seventy-four animal bites were recorded between 1978 and 1981. Of these 1,196 (35.4%) occurred in 1980 and exceptionally large number of these were recorded during the period January-April, 1980 (Figures 4 and 5). The number of animal bites for the period (1978-81) averaged 66 persons per month. The sites of bites are summarized on Table 4. The most common site of bites was legs and feet (48.1%).

Among the people bitten by animals, 57% were males and 49% were females of 14 years of age or younger. The rate of bite exposure continued to diminish with increasing age. An exception to this trend occurred in females aged 33-39 years who accounted for 9.1% of animal bite-exposures experienced by females. Occurrence of dog bite in this state for the different age groups is also dependent on sex ($P < 0.001$).

There were 137 brain specimens examined at the virus Research Laboratory of the University of Ibadan between 1971-1976, 87% of the total diagnosed rabies positive were in dogs (Table 10). Five of the seven human brain specimens examined were positive for rabies. All five were bitten by dogs.

Between 1971 and 1979, 22 human deaths were recorded in hospitals. Twelve of these had complete information. Five (42%) of these were adult females who were between the ages of 30 and 55 years. Male children in the 10-14 years age group were 3(75%) of the 4 fatalities. The two victims of cat transmitted rabies were both females. There was one fatal human rabies case recorded annually between 1971 and 1979 except 1975 when four people died of rabies. Table 9.

Lagos State

Oral Interview

From the interviews held with the Chief Veterinary Officer (CVO) of Lagos State and few people from towns surrounding Lagos city, the following information was recorded

(1) that the veterinary division was hardly being contacted for cases of animal rabics.

(2) that both muslims and christians keep dogs alike and there is a high density of dogs in Lagos metropolis.

(3) that dog population is increasing as more people keep dogs for security reasons.

(4) that it seems Badagry area witnessed an epidemic of rabies between 1976-1977.

(5) that between 1969-1980, 370 stray dogs were captured. Vaccinations were done regularly, but no records were available. Antirabies vaccines obtained by the CVO's office between 1979 and 1982 were 8,712 LEP and 1,891 HEP (Table 11).

Records

Between 1979-1982, 1,849 people were recorded as having been bitten by animals (Fig. 6). The occurrence of dog bite at different age group was independent of sex($P>0.05$).

However, young people up to the age of 14 were at relatively greater risk of being bitten, than other age groups. The rate of bite exposure continued to diminish with increasing age. Fig. 7 shows the monthly distribution of dog bites from 1979-1982. Between the months of February and March, 1980, there was a marked upsurge which later leveled out. It appeared that the occurrence of dog bites throughout the years in this state was regular and consistently average 35 human bites per month. Fig. 7.

Through both interviews and records collected in Lagos state, 37 human death due to rabies were recorded. Table 12 shows the distribution of 16 deaths recorded by LUTH between 1964-1983. Of these, 12(75%) were male and 4(25%) were females. Forty-two percent of the fatal cases in males were children 14 years old and younger, while 50% of the deaths in females were in those less than 14 years

of age. In 3 fatal cases, the biting animals were not known; the rest were bitten by dogs. The highest number of deaths due to rabies were recorded in 1967 and 1969.

General Observations

In all the states, persons bitten by suspicious dogs and cats were subjected to a variety of traditional treatments. In Kaduna state for example, wounds were sometimes, covered with hair from the biting animals. Occasionally, bite victims were asked to drink concortion which sometimes included livers, spleen or washings of hair from the offending animals.

In other cases, the victims were regarded as safe from rabies if the biting dogs accepted food.

In Oyo and Lagos states, however, superstitious beliefs were common. These beliefs were usually more exaggerated whenever the biting animals were cats. It appeared that people believe that some people in the society could bedevil dogs and most especially cats to bite people as vengeance or a show of power. As a result, when bites occurred, the interpretation of the immediate families of the

victims, the social status of the victims and the general situation in the area, town or village dictated the mode of treatment.

When bites occurred, there was always a tendency for people to leave the victims and attempt to get rid of the biting animals first. These attempts might take from a few minutes to a few hours. Attention was then paid to the wounds. The wounds were usually washed with water, lime juice etc. and then preparation like "apora" "ipagbe" and "asejo" to mention a few would be applied. These medications were usually made of burnt leaves and head of certain species of snakes.

Educated victims usually sought medical treatment and only few noneducated people who lived in big cities also tended to seek medical treatment in hospitals.

Rabies Vaccine Supplied to the Veterinary Clinics and Hospitals

Antirabies vaccines supplied by the various state governments to health centers and veterinary clinics were grossly inadequate. Almost all the hospitals, health centres and veterinary clinics complained about this inadequate supply. It was

however, difficult to determine the number that was required and actual numbers supplied.

In Kaduna state prior to 1979, the supply of LEP antirabies vaccines for dogs had been satisfactory and the vaccine was given free of charge. By 1981-1983, many clinics had no regular supply.

In Oyo and Lagos states, the supply had always been unsteady. Some private practitioners had to order antirabies vaccine from abroad to supplement government supplies.

Provision of antirabies vaccine and rabies antiserum has been progressively phased out by hospitals and health centers. The last batch of antiserum at the University Hospital in Zaria was in 1972 and in Onireke Health Center, 1969. Since 1973, supply of antirabies vaccines diminished gradually and by 1980, only a very few hospitals could boast of more than 10 vials in store at any period. between 1979 and 1982 the worst shortage of antirabies vaccine for human use was recorded in the 3 state. By 1982-83, most of the chemists stores that had been serving as alternative sources had actually run out of antirabies vaccines. This

situation had actually promoted the unscrupulous sales of false vaccines which were then available in open markets.

Dog-owner Relationship

The summary of the result of the general survey of dog-owner relationships carried out on Table 13.

One hundred and eighty-five (65.2%) of 285 dogs were kept for security purposes and only 43/285 (15.2%) as pets. Ninety-one percent of dogs exist on scraps; 72.6% had never been on leashes at any time and more than 60% had never been given any medical care.

A total of 857 sera were examined which included 463 from owned unvaccinated dogs, 108 from dogs of unknown vaccination status, 212 from stray dogs and 74 from owned vaccinated dogs.

Of the 463 unvaccinated dogs, 142(30.7%) had neutralizing antibody titre at 1:16 screening dilution. The distribution of dogs with antibodies of the screening dilution according to age is shown in Fig. 8.

Of the 105 dogs tested which were of unknown vaccination status, 42(38.8%) had SN titres at 1:16 dilution. Also, 83(39.2%) of 212 stray dogs and 44(59.5%) of 74 known vaccinated dogs had SN titres at the same dilution of 1:16 (Fig. 9).

Table 14, A and B shows the SN titer levels of selected samples from all categories of dogs. Table 15 shows the total number of human serum samples tested as distributed by age and sex. Out of the 350 samples tested, 100(28.6%) were found to contain SN antibodies at 1:16 final dilution. Distribution of the positive samples by age showed 37% positive within the 40-44 year old age group and 40% in people above 59 years of age. (Fig. 10). Antibodies were detected in all the age groups. Distribution by sex showed that 48.0% male and 52% females had antibodies. Table 15.

About 22.8% of positive sera titrated had SN titres greater than 1:32.

Serological Findings Against Rabies-Related Viruses

Table 16 shows the SN titres against Mokola and Lagos bat viruses in dogs and humans at 1:16 dilution. Of the two hundred and forty-one dog serum samples

tested. 42(17.4%) showed antibodies against Mokola virus and 14(5.8%) showed antibodies against Lagos bat virus. Also, out of 158 human serum samples tested 12(7.5%) had antibodies against Mokola virus and 4.25%) had antibodies against Lagos bat virus.

Table 1: Incidence of Human and Canine Rabies from June, 1975 - May, 1980 as Reported by Divisional Veterinary Officers, Kaduna State

Division	Human			Canine			
	Number of Dog Bites		No. of Deaths	No. of Rabies Outbreaks	No. of Animals Confirmed Rabied	No. of Dogs Confined for Observation	No. of animals vaccinated
	Total	Per Year					
Daura	0	0	0	0	0	0	38
Katsina	0	0	0	1	1	5*	7*
Funtua	3	3	1***	0	0	3	185
Zaria	49	16	-	3	3	49	3,506
Kaduna	42	11	2	2	2	69	4,919
Zonkwa	8	-	3***	11	3	-	41
Kafanchan	0	0	0	0	0	1	350**

* Records for 1979-1980 only

** Recorded vaccine failure in 2 dogs, i.e. rabies occurred in these dogs that were previously vaccinated.

*** Information obtained from the local hospital.

Table 2: Summary of Information Obtained in Oral Interviews with Villages and Hamlet Chiefs in Kaduna State

Division/ Village	Estimated Number of Households	Estimated Human Population	Estimated Dog Population	Number of Rabies Cases Per Year in Dogs	Human Rabies Cases in Memory	Date of Latest Human Death
Katsina						
Kamb Arew	1,000	10,000	2	0	0	0
Dan Dakworo	330	3,000	3	0	0	0
Funtua						
Maigenji	1,709	10,000	0	0	0	0
Yartabpi	241	661	0	0	0	0
Zaria						
Sobo		10,000	500	-	Several	1972-83, 1977-78,
Tukur Tukur		7,963	300	0	0	1953-80
Zonkwa						
Semaru Kataf	1,200	12,000	2,000	10		1977
Fedan Sofo						
Jankasa	567	8,000	500	many		
Jankasa Hamlet	35	300	100	2		
Fedan Kofe	306	3,000	600			
Kafanchan						
Fori	120	900	60		1	
Fodin Kagoma	2,372	20,000	1,000	3	1	1965

Table 3: Sites of Animal Bites Victims ir Kaduna State

Bite Site	Number of Victims	Percent
Lower limb		
Leg and foot	33	27.7
Upper limb		
Hand and upper limb	13	10.9
Trunk	11	9.2
Head	1	0.8
Not stated	61	51.3
	119	99.9

Table 4: Sites of Animal Bites in Victims Reporting to Health Centers, Distributed by Age and Sex - Oyo State

Bite Site	Number of Victims	Male Children	Male Adults	Female Children	Female Adults	Percentage of Bites
Legs and feet	13	4	1	6	2	48.1
Thighs, knees and buttocks	6	1	2	2	1	22.2
Fingers, palms or wrists	3	2	-	-	1	11.1
Neck and head	2	1	-	1	-	7.4
Arms and Shoulders	1	-	-	1	-	3.7
Abdomen and Back	1	1	-	-	-	3.7
Multiple Bites	1	-	-	-	1	3.8
Total	27	9	3	10	5	

Table 5: Distribution Of Fatal Human Cases Of Rabies By Age, Sex Biting Animal and Date of Death from 1971-1980 in Kaduna State

Age (years)	Male	Female	Biting Animal	Date of Death
Unknown	1		Dog	24/9/71
Unknown	1		Dog	23/4/73
Unknown	1		Dog	9/10/73
Unknown	1		Dog	21/11/73
Unknown	1		Dog	11/4/73
Unknown	1		Dog	1974
Unknown	1		Dog	1975
28	1		Dog	21/11/76
16	1		Dog	29/4/77
20	1		Dog	11/3/77
Unknown	1		Dog	1979
Unknown	1		Dog	1979
Unknown	1		Dog	1979
10	1		Dog	23/12/80
4	1		Dog	18/6/80
Total	15	0*		

Data for 6 other cases was insufficient for tabulation.
* No fatal case was registered in females.

Table 6: Summary of Rabies Diagnoses, Faculty Of Veterinary Medicine, A.B.U., Zaria

Year	Species	Total Test	Positive[a]	Positive all test	Pos. Negri	Pos. FAT	Pos. MI	Owned (positive	Stray cases	Furious	Dumb	Human Exposur
1971	Canine	10	6	2	6	6	2	4	2	1	1	1
1972		25	18	9	15	16	12	11	6	5	4	6
1973		24	8	2	6	8	4	3	5	3	3	5
	Total	59	32(54)	13	27	30	18	18	13	9	8	12
	percentage	100	54	22	45.7	50.8	30.5					20.3
	percentage	-	100	40.6	84.4	93.75	56.25					
1971-	Feline	4	1	1	1	1	1	4				
1972	squirrel	1	0	0	0	0	0	1	-	-	-	-
	equine	1	1	0	0	-	1	1	-	-	-	-
	bovine	3	3	1	1	2	3	3*	-	-	-	-
	human	3	3**	-	ND	-	2	-	-	2	1	-

[a]positive to one or more tests.

*From Kano State Government Farm

**All died showing signs of rabies

ND = Not done

FAT = Fluorescent antibody test

MI = Mouse inoculation test

Table 7: Animals Quarantined During Outbreaks from 1976-1979 in Oyo State

	Number Quarantined	Number Rabid	Percent
Veterinary Clinics	38	26	68.4
Health Centers	376	13	3.5
Total	414	39	9.4

Table 8: Summary of Information Obtained in Oral Interviews with People in Towns and the Surrounding Villages in Oyo State - 1974-1981

Towns and Villages	*Estimated Population	**Estimated Dog Population	Rabies Cases Per Year in Dogs	Human Rabies Cases in Memory
Oyo town and surrounding villages	22,133,075	184,442	Many	2
Iwo and surrounding villages	842,052	70,171	8 dogs 2 cats	3 induced by cats
Ogbomosho	858,197	71,516	16 dogs	1976
Oshogbo	532,157	44,346	4	2 women 1 man 1 child
Ikire	6,900	575	11	0
Ede	336,375	28.031	27	1974 1977 1980 1981

* Estimated populationsis a product of the population figure for 1963 (Western Region Vol. 1: 56-92) by 2.5

** Estimated dog population was calculated based on 1 dog to 12 people as established by the United Nations Development Programme 1981.

Table 9: Distribution of Fatal Human Cases of Rabies by Age, Sex and Biting Animal From 1971-1979[1] - Oyo State

Age Group	Number	Sex	
		Male	Female
0-4	1	1	-
5-9	0	-	-
10-14	4	3	1*
15-19	1	-	1
20-24	0	-	-
25-29	0	-	-
30-34	1	-	1*
35-39	1	-	1
40-44	2	-	2
45-49	0	-	-
50+	2	1	1
Total	12	5	7

[1]Data for 10 other cases was insufficient for tabulation.

*Bitten by cat, others bitten by dogs.

Note: There was one human rabies fatality each year from 1971 through 1979 except in 1975 when there were four fatal cases.

Table 10: Laboratory Examination of Brain Specimens From 1971-1976
(Virus Research Laboratory, University of Ibadan)

Source of Specimens	Number Examined	Positive	Percent Positive
Dog	108	94	87
Human	7	5	71
Cat	1	1	100
Goat	1	1	100
Sheep	1	1	100
Cow	2	0	0
Horse	1	0	0
Monkey	15	1	13
Total	137	103	76

Table 11: Antirabies Vaccines Obtained by Lagos State Government Between 1978 and 1982

Year	LEP	HEP	TOTAL
1978	2,040	-	2,040
1979	1,198	181	1,379
1980	2,174	1,200	3,374
1981	2,600	-	2,600
1982	700	500	1,200
Total	8,712	1,881	10,593

Table 12: Distribution of Fatal Human Cases of Rabies by Age, Sex, Biting Animal and Date of Death in Lagos State as recorded by LUTH

Age (years)	Male	Female	Biting Animal	Date of Death
3	1		Unknown	1969
5		1	Unknown	1965
5½	1		Dog	1983
7	1		Dog	1965
7		1	Dog	1967
13	1	D	Dog	1968
16	1		Unknown	1969
116	1		Dog	1983
19		1	Dog	1964
22	1		Dog	1971
25		1	Dog	1972
2[illegible]	1		Unknown	1982
40	1		Dog	1969
42	1		Dog	1967
47	1		Dog	1967
-	-	-	-	1973-1980*
-	-	-	--	1981**
Total	11	4		
Percentage	73.3	26.6		

*Date not available

Table 13: Survey Findings of Dog-owner Relationship in Kaduna, Oyo and Lagos State

	Positive Response	Percentage
Why do you keep dogs?		
- as guards for security	185	65.2
- I love them as pets	43	15.2
- both of the above	37	13.0
- for hunting	9	3.2
- given as gifts	5	1.8
- I don't know	6	2.1
Total	285	
What types of food do you give your dogs?		
- Table food (scraps)	180	91.0
- special pet food	3	1.5
- they fend for themselves	3	1.5
- table and occasionally pet food	9	4.9
- I don't know	4	2.0
Total	199	
Where are your dogs kept during the day?		
- outside the house	49	24.6
- within the home compound	96	48.2
- have a special kennel	08	4.0
- they rest anywhere they like	26	13.1
- they lie down in my garage	73	1.5
- I don't know	17	8.5
Total	199	

Table 13 (Continued)

	Positive Response	Percentage
Where are the dog kept during the night?		
- they are let loose to keep guard	121	64.0
- they are within the compound	29	15.3
- they are confined in their kennel	1	0.5
- in the garage	3	1.5
- I don't know	35	18.5
Total	189	
Do you ever keep your dogs on a leash?		
- never	157	72.6
- keep them chained during the day	19	8.8
- keep them chained during the night	2	0.9
- I don't know	38	17.6
Total	216	
Have you visited a Veterinary Clinic?		
- never been before	121	60.8
- yes have been	49	24.6
- I can't remember	6	3.0
- I don't know	23	11.6
Total	199	
Who in your household does not like to see the dog around the house?		
- my husband	22	14.0
- my wife	31	19.7
- my children	3	1.9
- the house boy	15	9.6
- the house girl	83	52.9
- visitors	3	1.9
Total	157	

Table 14A: Serum Neutralizing Antibody Levels of Nigerian Dog Sera Against Rabies Virus by RFFIT Test

	1:10	1:20	1:40	1:80	1:160	1:320	Total
1. Unvaccinated owned dogs							
Number	22	20	19	17	9	3	90
percentage	24.4	22.2	21.1	18.9	10.0	3.3	100
2. Stray Dogs							
Number	6	6	8	8	3	1	32
percentage	18.8	18.8	25	25	9.4	3.1	100

Table 14B: Serum Neutralizing Antibody Levels of Nigerian Dog Sera Against Rabies Virus by RFFIT Test

	1:4	1:8	1:16	1:32	1:64	1:228	1:128	Total
Vaccinated Dogs								
Number	8	6	10	10	10	8	6	16	74
percentage	10.81	8.11	13.51	13.51	13.51	10.81	8.11	21.62	99.9

Table 15: Distribution of Rabies Antibodies in Human in Nigeria by Age and Sex

Age group (years)	Number tested	Total positive	%	Males positive	%	Femeles positive	%
10-14	18	5	27.8	2	40.0	3	60.0
15-19	36	8	22.2	2	25.0	6	75.0
20-24	82	27	32.9	9	33.3	18	66.7
25-29	75	18	24.0	10	55.5	8	44.4
30-34	38	11	28.9	8	72.7	3	27.3
35-39	27	7	25.9	4	57.1	3	42.9
40-44	27	10	37.0	2	20.0	8	70.0
45-49	12	2	16.7	1	50.0	1	50.0
50-54	11	4	36.4	4	100.0	0	0.0
55-59	4	0	0.0	0	0.0	0	0.0
59	20	8	40.0	6	75.0	2	25.0
Total	350	100	-	48	48.0	52	52.0

Table 16: Antibodies to Mokola and Lagos Bat Viruses* In Human Beings in Nigeria

	No. Tested	Total Positive Number	Percentage
A. Dog Sera			
Mokola Bat Virus	241	42	17.4
Lagos Bat Virus	241	14	5.8
B. Human Sera			
Mokola Bat Virus	158	12	7.5
Lagos Bat Virus	158	4	2.5

* Rabies related viruses.

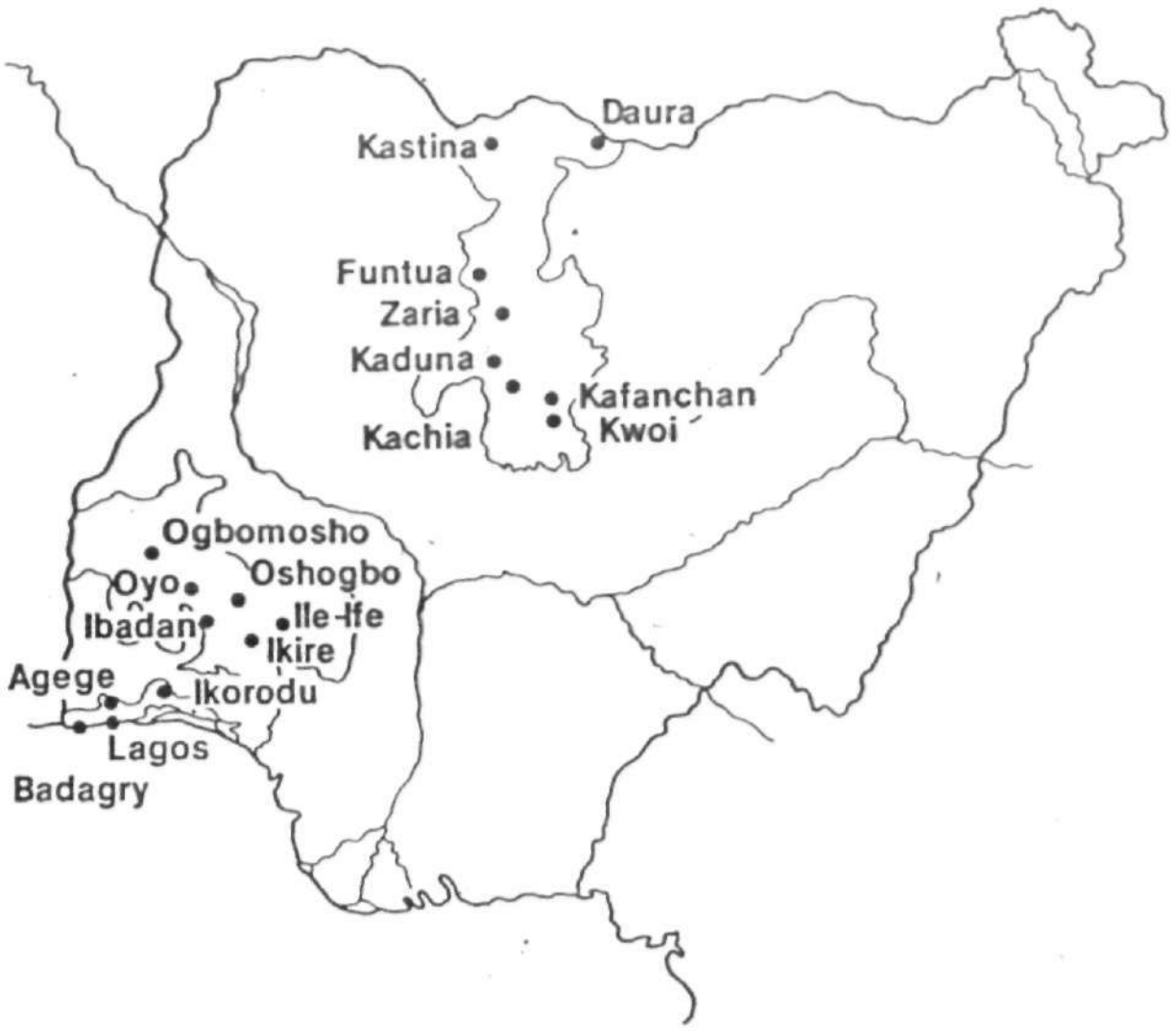


Figure 1. Outline map of Nigeria showing Kaduna, Oyo and Lagos States with places visited in this study.

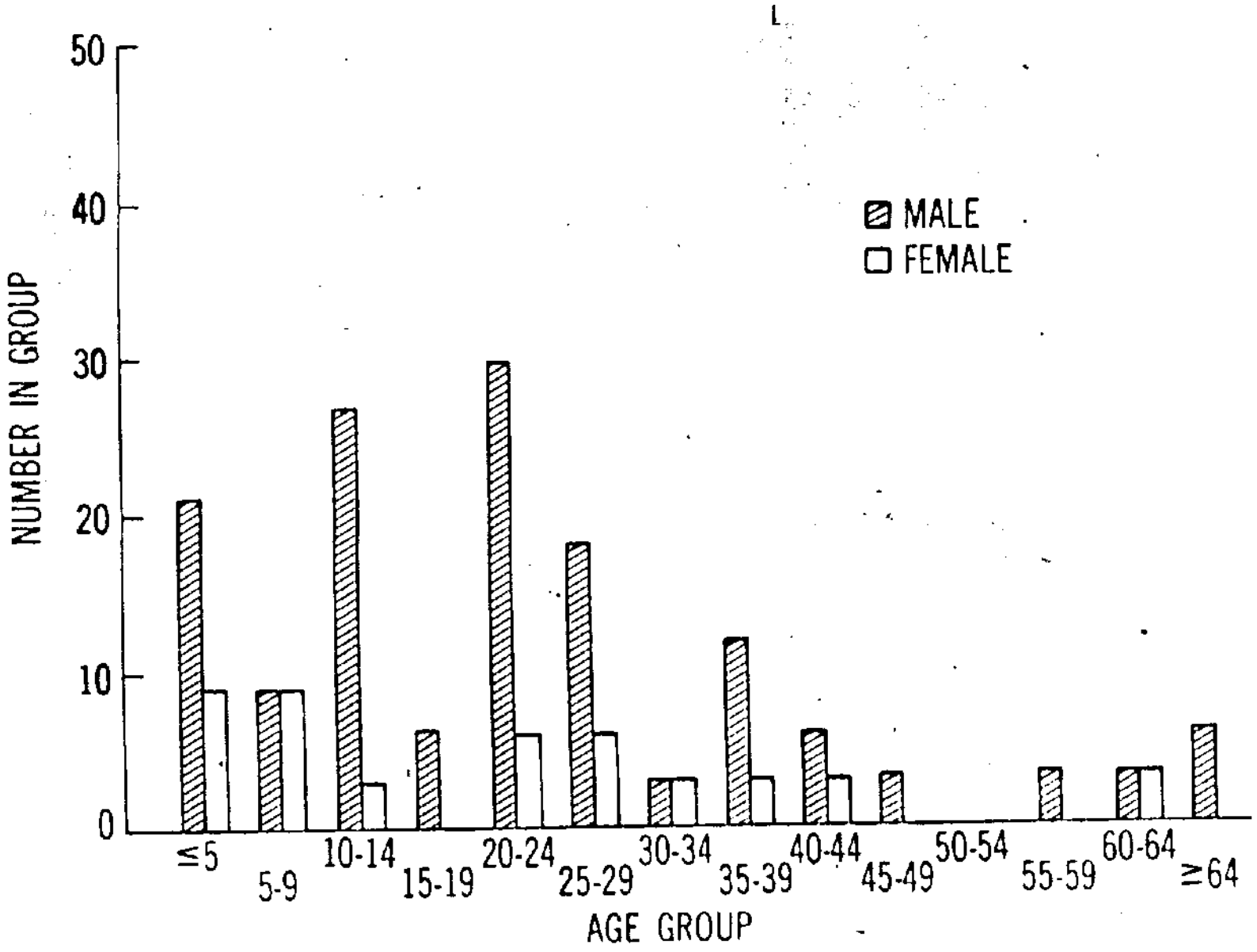


Figure 2.Distribution of Animal Bite Cases in Human Beings by Age and Sex, Kaduna State, Nigeria, 1971—1978.

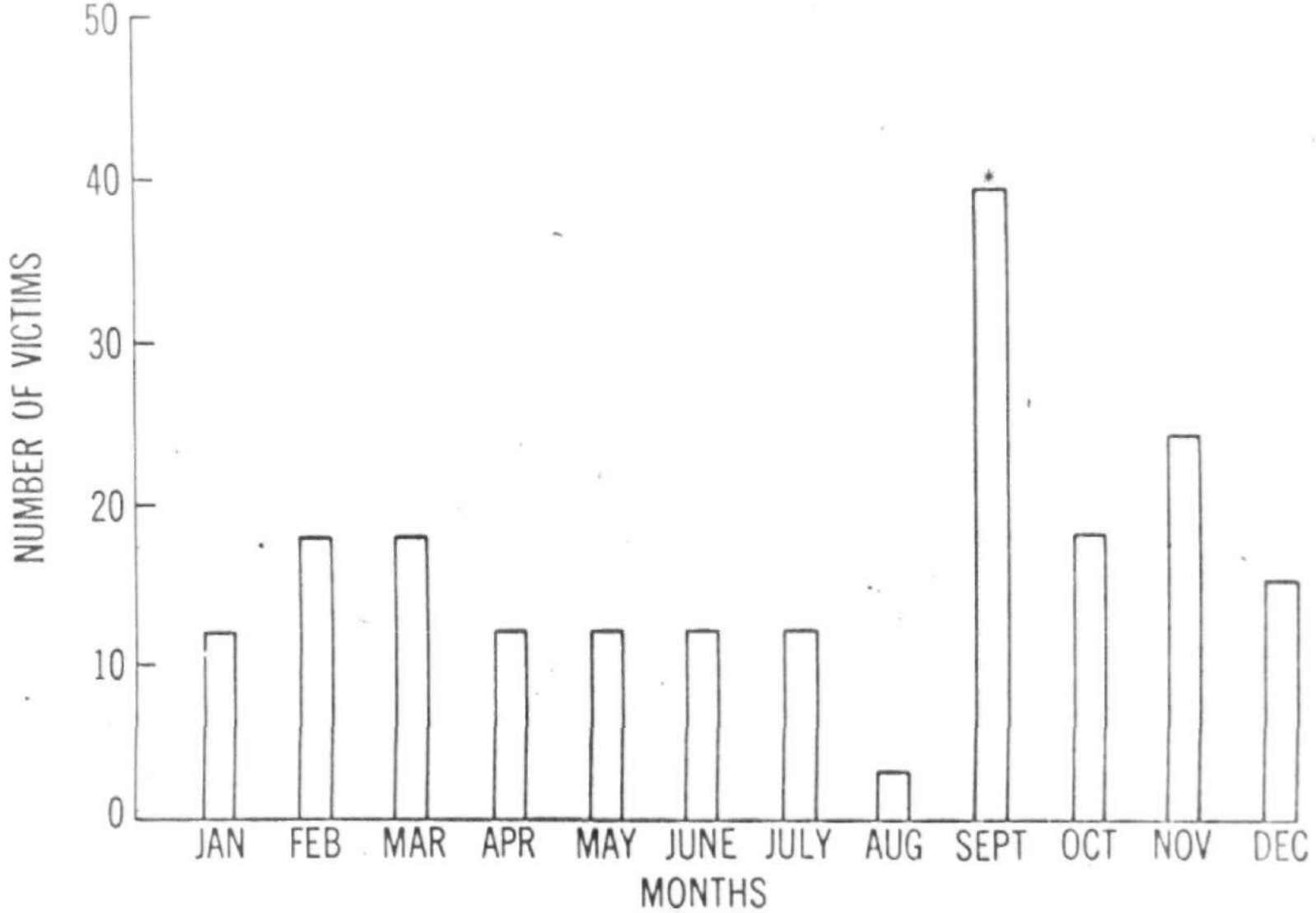


Figure 3. Distribution of Animal Bite Cases in Human Beings by Months, Kaduna States Nigeria, 1971-1978

* 1973 and 1977 epidemics occurred during September

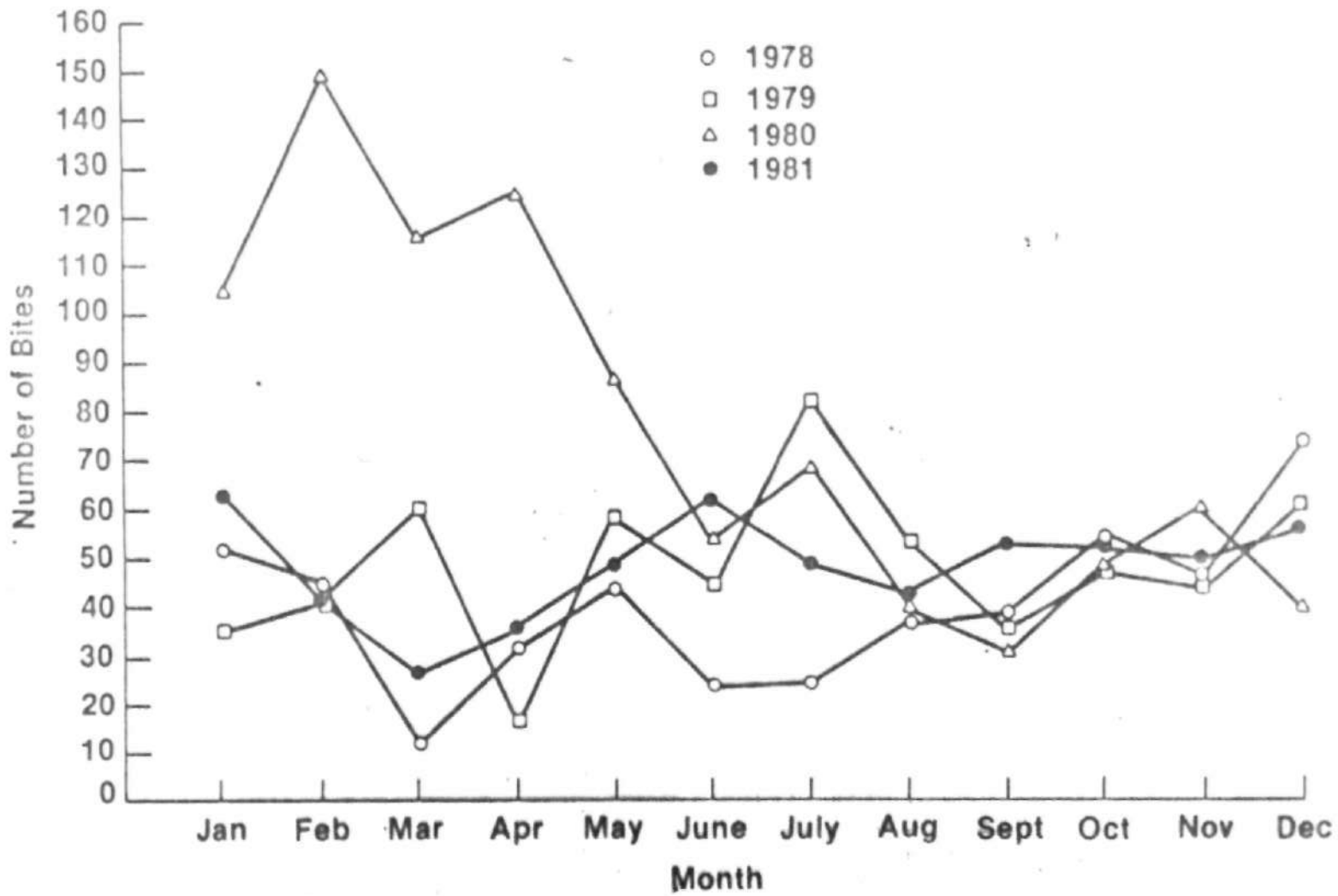


Figure 4. Yearly distribution of Human Beings bitten by animals, Oyo State.

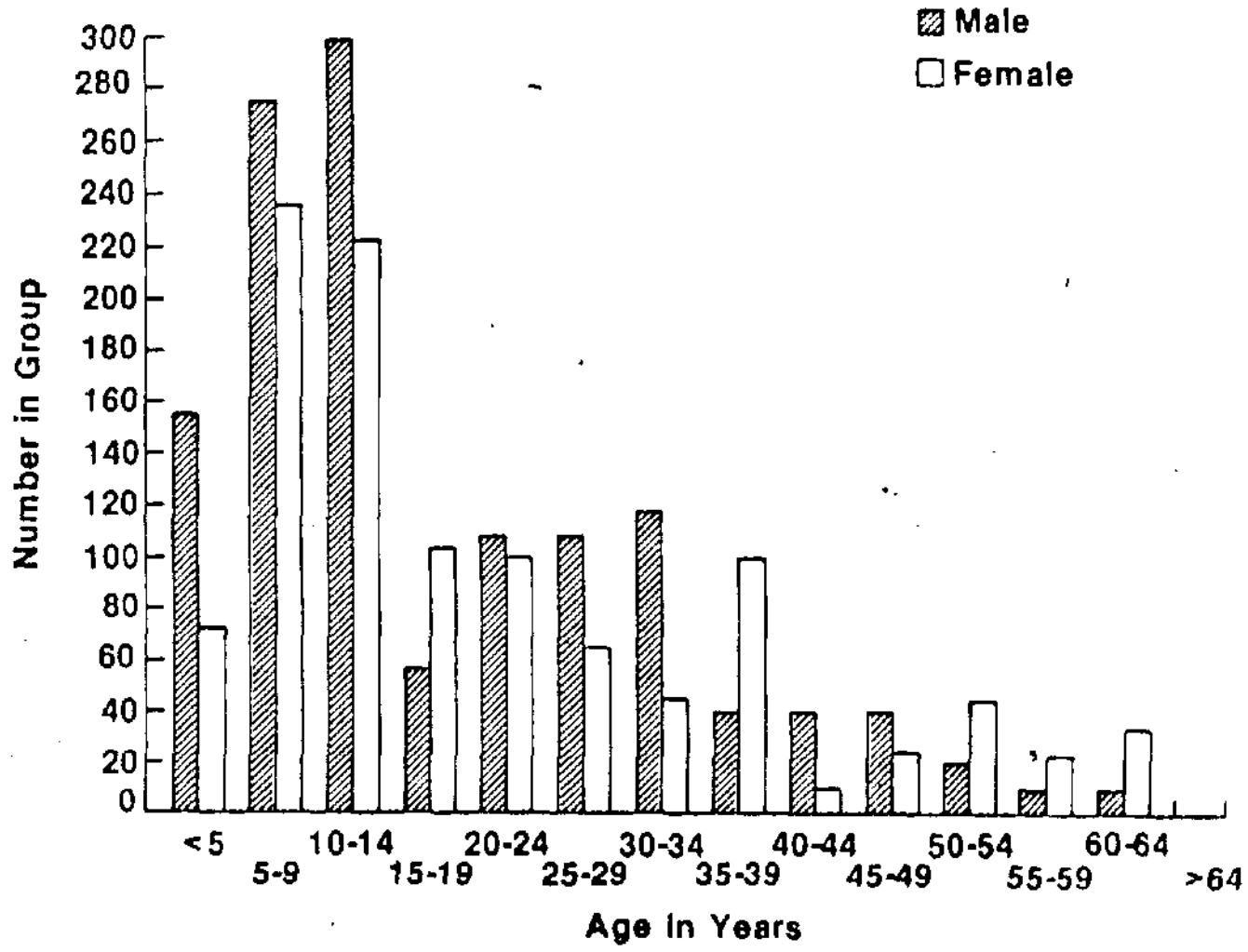


Figure 5. Distribution of Animal Bite Cases in Human Beings by Age and Sex,Oyo State, Nigeria,1978-1981

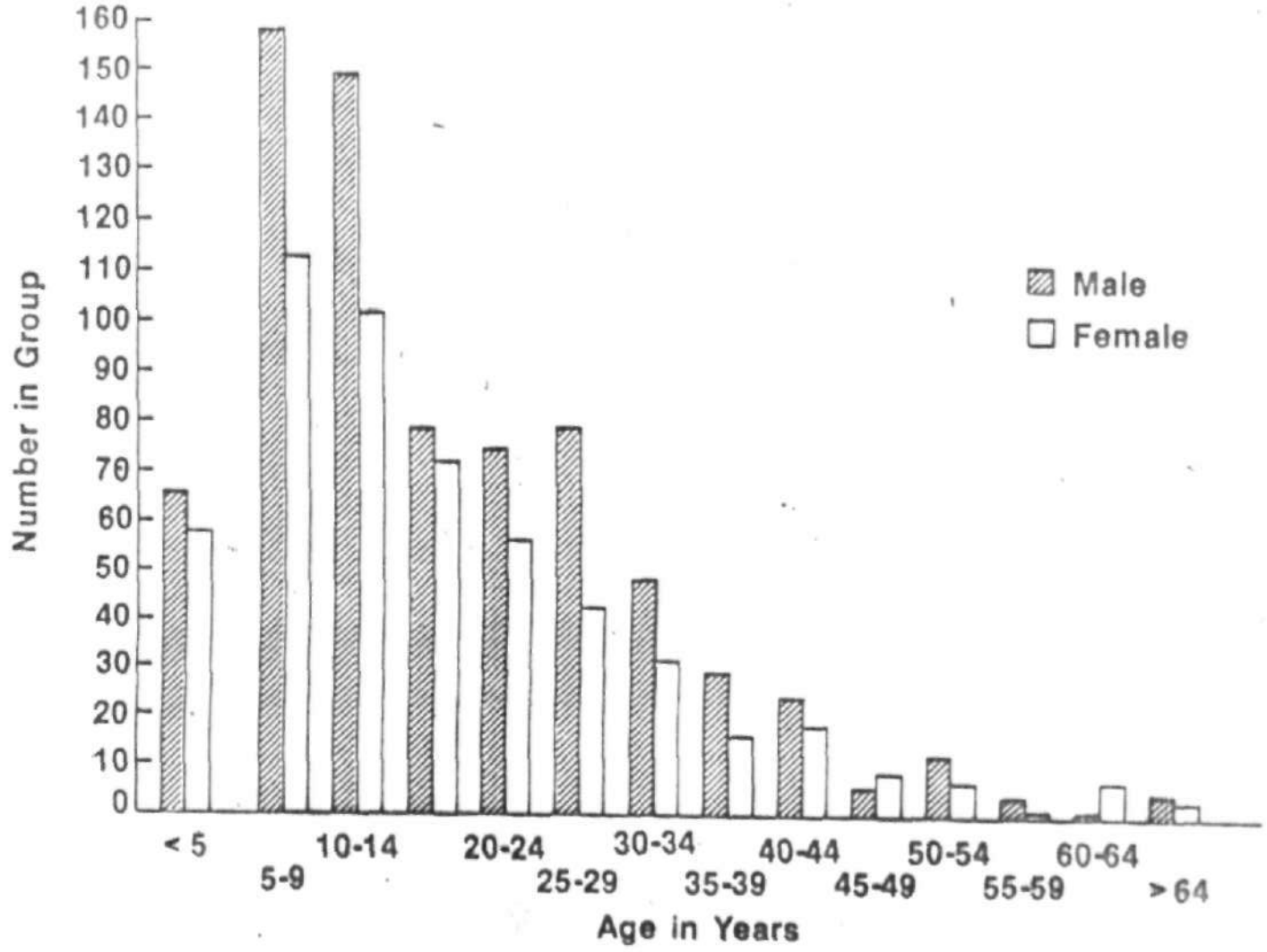


Figure 6. Distribution of Animal Bite Cases in Human Beings by Age and Sex, Lagos State, Nigeria,1979-1982.

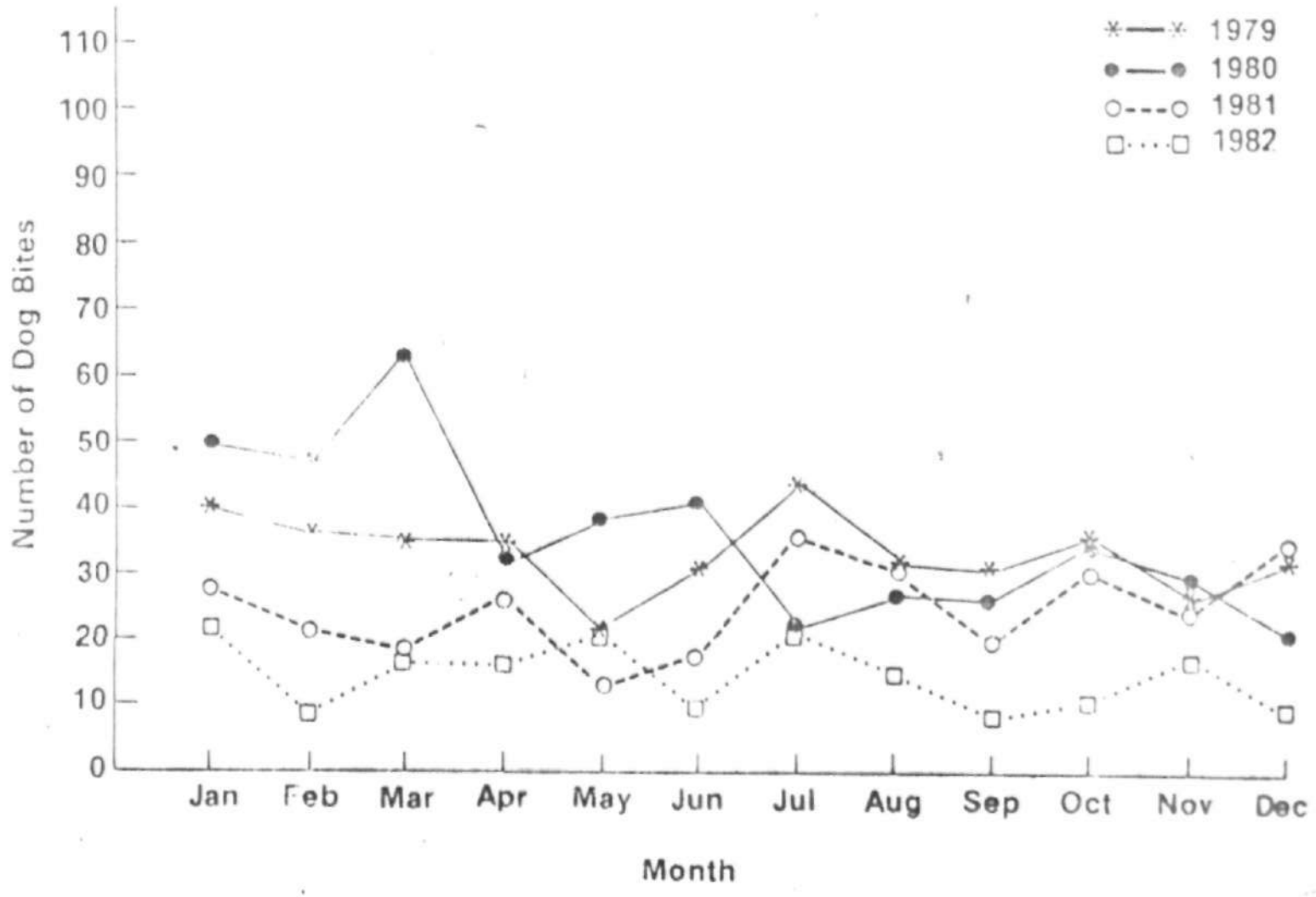


Figure 7. Distribution of **Dog** Bite Cases in Human Beings, Lagos State, Nigeria, 1979-1982

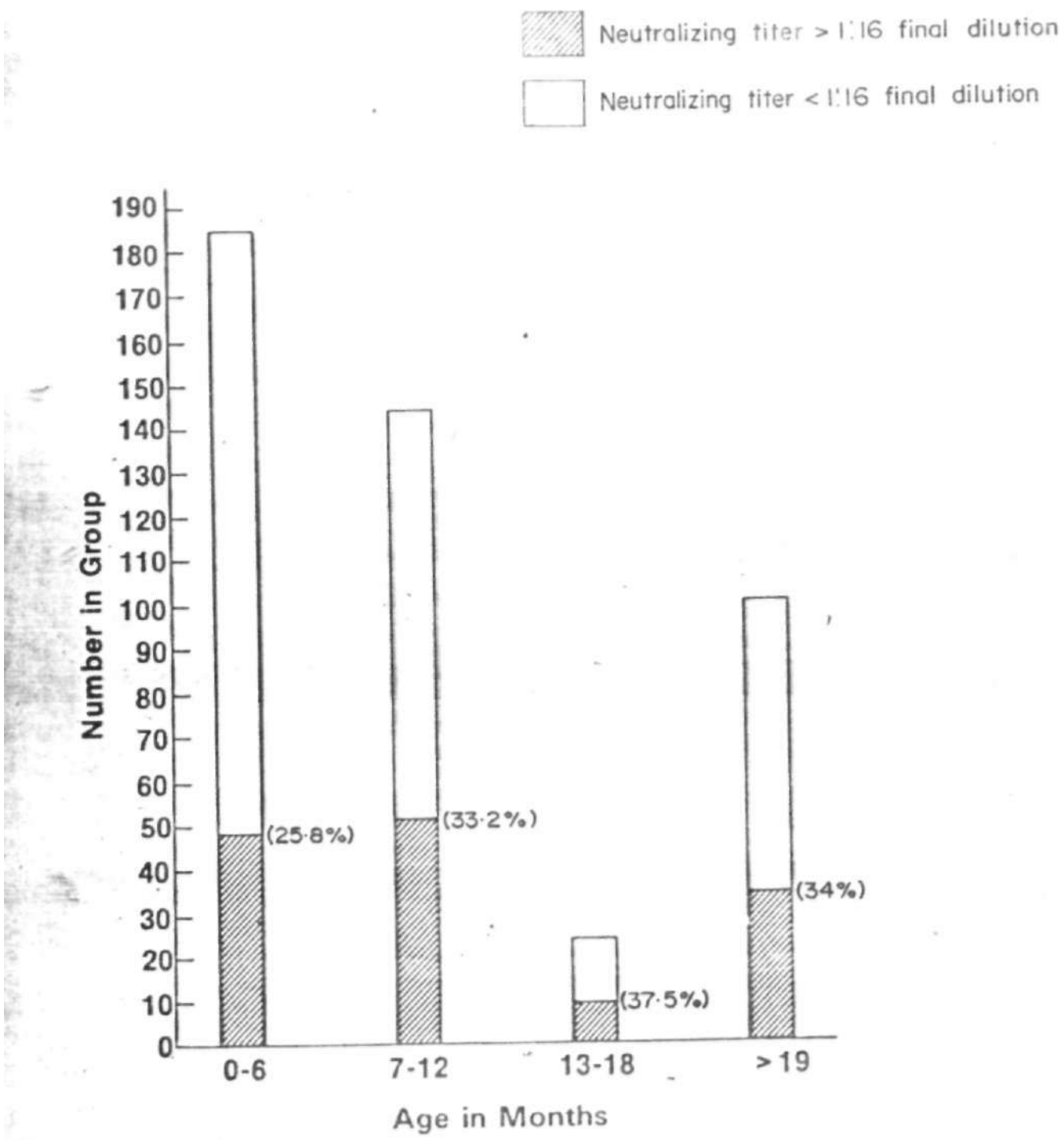


Figure 8. Antirabies Titers in Unvaccinated Owned Dogs in Kaduna, Oyo and Lagos States, Nigeria.

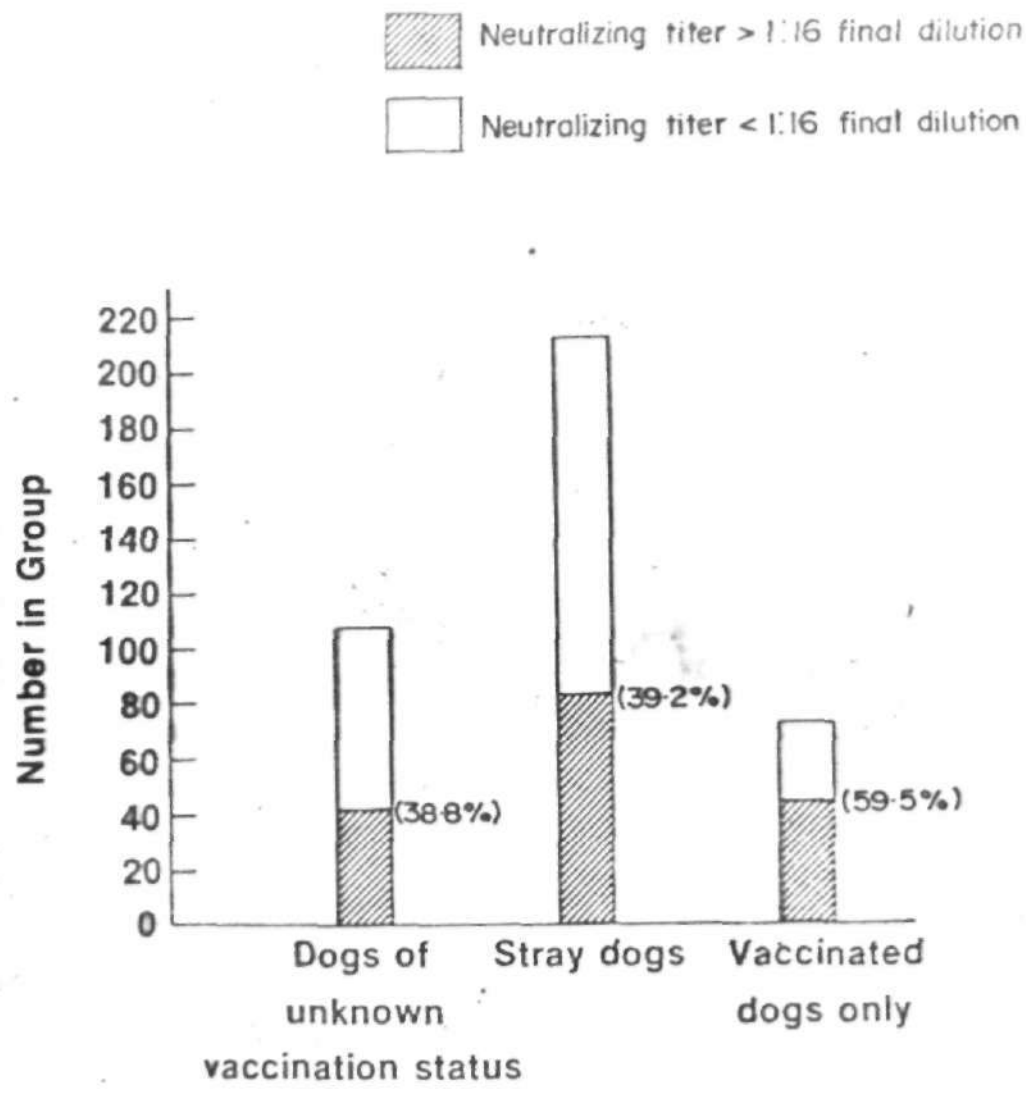


Figure 9. The percentage of Dogs with rabies antibodies in Kaduna, Oyo and Lagos States, Nigeria.

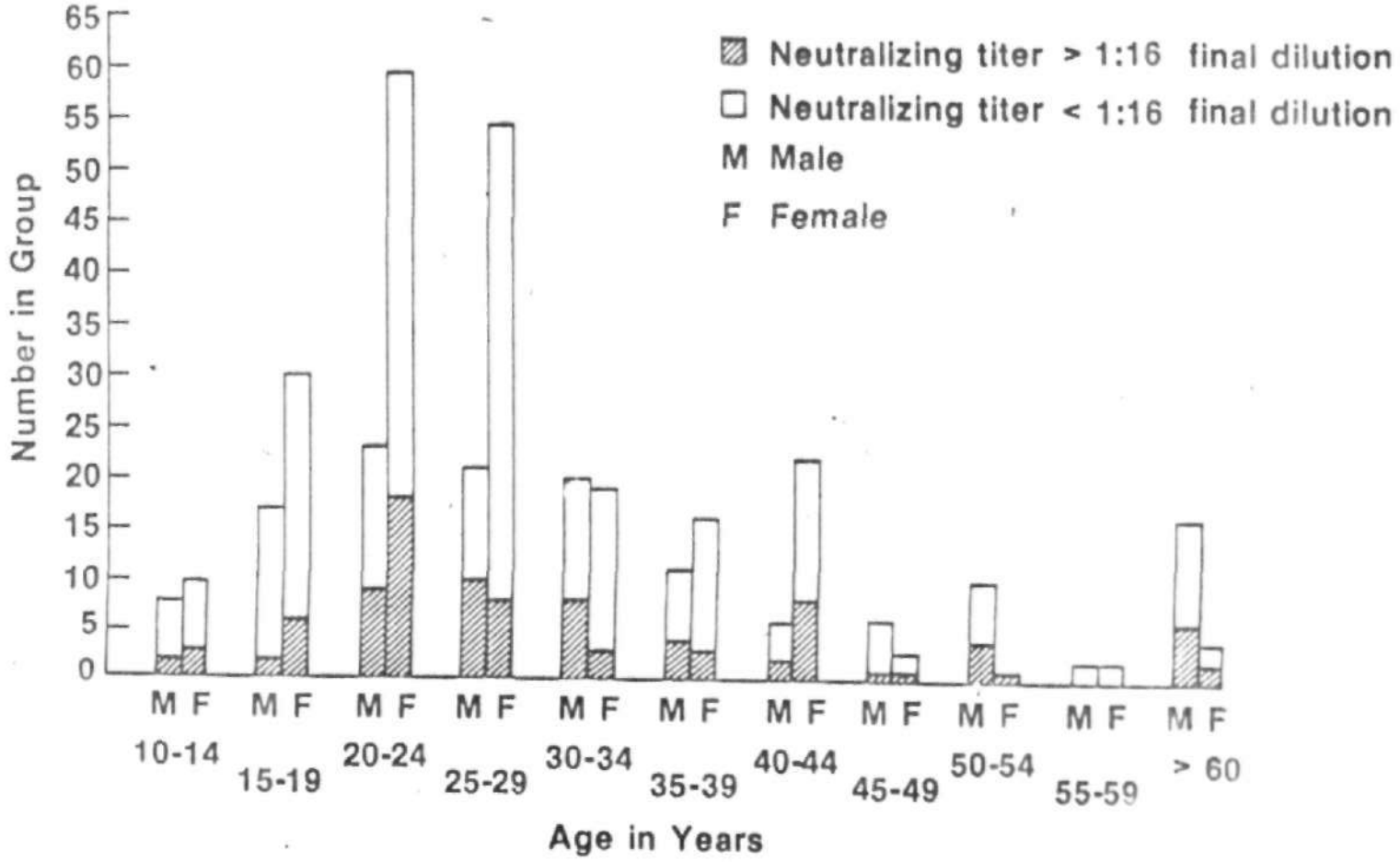


Figure10. **Rabies antibodies** *in Human Beings, Kaduna, Oyo and Lagos States,* **Nigeria, 1983**

DISCUSSION

DISCUSSION

In the 3 states surveyed, the absolute incidence of dog bites and death in both human and animals as a result of rabies could not be determined because complete information on absolute numbers of dog bites, the canine population and even human population was lacking. Reasons given by the Divisional Veterinary Officers and health workers for the inadequate information on dog bites and rabies cases were:
(1) lack of a clear and coordinated channel for reporting cases of either animal bites or rabies, while some people reported to the police, others reported to the hospitals or dispensaries and veterinarians and some did not report at all.
(2) the majority of the local people who kept dogs in these states are used to local treatments which have no recording system.

As observed from the questionaire survey and interviews, it is obvious that both the urban and rural people in the 3 states studied are aware of rabies. Over 60% of all individuals who were interviewed could describe the clinical manifestations of rabies in dogs. The rural people were able to include more details in their descriptions of the

disease than those in the urban areas. It seems the only form of rabies recognized by most people is the furious form of rabies because as the name of the disease depicts in both Hausa and Yoruba "Madness" is the key.

Boulger and Hardy (1960) observed that 68% of 44 cases of rabies in dogs in the Lagos area from 1956 to 1959 were of the dumb type. Of the 10 cases studied by Minor (1977) in Kenya, only 6 (31.6%) displayed furious behavior. If the clinical picture of animal rabies in these states is portrayed by a proportionality similar to these reports, and there is under reporting even of obvious furious rabies, then regretably, the rabies picture so far developed shows only the "tip of the iceberg" and the human toll is probably greater than depicted by the statistics contained in this report.

In Kaduna state, there was an obvious north-south gradient in dog ownership, and occurrence of canine rabies paralleled the dog population density. As noted, more dogs were owned in the south of the state than in the north. The determining factor in dog ownership tended to be cultural and housing. The most important factors in dog ownership appeared

to be utilitarian; hunting, cattle rearing and guarding against prowlers. There were fewer dogs in small rural communities where the close knit societies had less need for crime protection, except in certain parts of the state where dogs were also used for meat as in Kafanchan area. In such areas, large numbers of dogs were reared.

In Oyo and Lagos states, dog ownership cuts across all the segments of the society. Dogs are kept as pets, guard dogs and also for rituals. Dogs are sacrificed to various gods, chiefly "ogun" - the god of iron. The sacrificed dogs are sometimes eaten by the "ogun" worshippers.

Acha and Arambulo (1983) have indicated that in the tropical countries, the problem of dog populations goes beyond their number. It is rather their particular ecology, cultural implication and pattern of dog-ownership which are the sources of the problems. From the results obtained from dog-owner relationship and from general observation, the environment, the prevailing ecological and socio-cultural set-up support the abundance, distribution, predicability of resources (shelter, water, food) for dogs population turnover which is virtually uncontrolled. The implication of this in dog bites,

rabies and rabies control and eradication programme cannot be overemphasized.

From the records in Kaduna state, most of the bite and rabies victims were from the Zaria area, Kaduna and from the Southern part of Kaduna "Southern Zaria". The reasons for this might be that apart from high population of dogs in these areas, better reporting and record keeping exist in these areas as compared with extreme north.

Most of the bite victims were males; the movements of the females are more restricted than of the males. As a result there is a 1:4 female/male ratio of dog bites. This was also reflected in the number of deaths. All the 16 people recorded as victim of fatal rabies infection in the state (1971-1980) were males.

In Kaduna state, the 20-24 years old age group consists of people with enormous amounts of responsibility and ambition. Most people belonging to this group have families and are supposed to care for their families. They go to bushes to fetch firewood and to market to bring food. As a result, they have the greatest exposure potential.

In Oyo and Lagos states, most of the reported cases of bites and human rabies were attributed to either dog or cat bites with only a few of the biting animals being unknown. In general, bites incidents occurred most commonly in younger people (10-14 years) with a slightly higher incidence in males, more than would be expected by the population ratio. The significant differences between sexes seen in Kaduna and Oyo states were not as pronounced in Lagos state. The higher incidence of rabies in the older females in Oyo state was unusual. One possible explanation is the socio-economic role played by these women. Many from this area having raised their children, re-enter society as active workers. The nature of their jobs require their walking to markets and other places, thus exposing them to rabid dogs.

Another factor may be the style of clothing men and women wear. Women wear garments that, on most occasions, do not cover the lower parts of their legs, while men often wear longer "soros" (trousers) that hide their legs. Therefore, attacking dogs may often succeed in inflicting more severe and deeper wounds on women than men. Women are also more frightened than men. Instead of defending

themselves or running from attacking dogs, they tend to get so frightened, that they stand petrified, thus receiving more bites.

The conditions in Lagos and the daily pressures created by prowlers have forced people to keep dogs. There are many companies in Lagos that hire out dogs to other companies on a daily basis to guard and ward-off prowlers. These commercial dogs have increased the number of bites, but the proportion of the increase cannot be evaluated since past records were not available for comparative analysis. Dog bites in the villages and town surrounding Lagos were comparable to that of Oyo state.

The trace-back investigation of the two women bitten by cats which developed rabies in Oyo state illustrated the problems in pursuing rabies investigations and indicated the degree of under-reporting which existed. In one case, it took three days of discussion and intervention by a civic leader to obtain the cooperation of a bite victim's husband. When the cooperation was finally obtained, it required a lot of wisdom to be able to separate the emotional feelings from reality, superstitious beliefs from actual happenings. In one of the investigations, it was found that there had been two

other definite but unrecorded fatal rabies cases in which the victims had been bitten by the same cat. One was the woman who owned the cat and the other was a school boy. In addition, 5 more persons bitten by the same cat were still alive and at least 6 others could not be traced seven months after the death of the index case. In the other cat bite case, the victim could not be traced because of an inadequate address, but at the time of admission, it was noted that 9 other people had been bitten by the same cat. Although cats were seldom involved in cases of rabies in Nigeria, when they were, it appeared they inflicted bites on more people and caused more severe wounds to their victims than dogs.

Since 1972, the use of antiserum has been rare. Of the 3,286 animal bite victims in Oyo State treated at various health centres and hospitals between 1978-1981, none received antiserum.

In urban areas where facilities should have made adequate post-exposure rabies prophylaxis readily available, bite victims often went to nearby chemist (pharmacy) shops rather than to hospitals or clinics because of difficulties in receiving attention at medical care facilities.

In all the states studied, traditional medicine played a greater role in the post-exposure treatment of rabies exposures, especially in rural areas than did modern medical care. The most negative aspects of these practices lay in the fact that some people were believed to possess the power to cause a "mad dog" to attack an opponent. When such an interpretation is given to an animal bite incident, then efforts at post-exposure treatment usually were misdirected.

Similar to the report of Fagbami et al. (1981), it was observed in this study that human rabies deaths were more common than the records indicated. Gaseft and LeBras (1982) in discussing epidemiological problems in Africa stated from their studies that there was usually a gross underestimation of the incidence of disease morbidity and mortality because official statistics were usually unreliable. There was no standardized systems of reporting and recording, and often undertrained health workers were not sensitive to the importance of data and recording of situations. This seemed to be basically the case in Nigeria. If so, it is important, to recognize that in the 3 states studied, the recorded incidence of rabies deaths in the population is low

because of under-reporting and bad record keeping. In contrast to the United States, where there were from 0-4 (average = 1.5) human rabies deaths reported annually in a population of about 200,000,000 inhabitants during 1971-1979 (CDC, 1981) for an average annual rabies mortality rate of 0.008/1,000,000. Oyo state, which had a population of about 7,000,000 during the same period, suffered an average annual recorded human rabies mortality rate of 0.43/1,000,000 population which is already a rate 56 times more than that of the U.S.A.

This study has established a serological evidence of infections with members of lyssavirus group in some anparently healthy dogs and people in the states studied. For the antibodies to be present in these dogs and people, one of the following is likely to have happened. That the dogs and people were exposed, became sick and then recovered or exposed but showed no apparent sickness but developed detectable antibodies or they were all vaccinated against rabies.

It would be most likely that infections initiated at sites of exposure in muscle, subcutaneous tissue or mucous membranes were halted prior to entering nerve pathways. If such may be the case

antibody against the virus would likely be detected in the dogs and the people. Baer and Olson (1972) reported that following apparent recovery from clinical rabies in 4 pigs exposed by a rabid skunk, they developed antibodies within 2 months after exposure. In Grenada, 2 goats and a donkey known to have been bitten by mongooses showed rabies antibodies at low titers between 30 and 54 days after exposure (Everard et al., 1979).

The detection of (SN) antibodies in the human population however, has posed serious question of how these people came in contact with the rabies antigens. If the stimulating antigens were rabies how were the people exposed? Was it by bites of rabid dogs followed by prophylactic vaccination? Of the 350 people examined, only 5 agreed that they had previously been exposed through bites from any dog; 2 of these had SN antibody titers. Four of the 6 dog catchers who worked for several years as catchers had antibodies against rabies and one had antibodies against Mokola virus.

None of these catchers had any history of ever having been bitten by a rabid dog or of receiving prophylactic rabies vaccinations. None of the other tested people had a history of any dog bites or of

antirabies vaccinations.

Considering that only 1.4% of the people tested had a history of dog bites it is difficult to attribute a 28% prevalence of antibody titers to forgotten dog bite exposure. It appears that further explanations are required. It is also possible that the antigens involved could be cross reacting antigens with rabies virus. Such antigen(s) would crossreact strongly with one or more antigens of rabies. It is also possible that such antigen(s) is/are part of an infectious agent. If so, it must be avirulent or of very low virulence, presumably to both dogs and human beings. The evidence would indicate that exposure to this (these) antigen(s) must be prevalent, and that exposure most probably occur early in life and the infectious agent(s) were arthropod borne or that it is a highly invasive agent.

Since the description of the Ouluo Fato strain of rabies virus in Africa (Bouffard 1912) there has always been a suspicion that other variants of rabbies virus may exist. It may be postulated that the infections detected in this study were by one or more strain(s) of rabdoviruses that infect(s) a high percentage of Nigerian people and animals, producing a high level

of viremia readily infecting biting arthropods.

If the antibodies resulted from abortive infections by fully virulent rabies virus, it would appear to defy explanation that the death rates from rabies in people and animals in Nigeria could be so comparatively lower than the prevalence of antibodies in both populations.

Another postulate may be that the infections are caused by a rabies-related virus of close antigenic structure to rabies virus.

Antibodies against Mokola virus have been found in various domestic animals, a bat and a bird (Kemp et al., 1972). In these studies, SN antibodies were found against Mokola and Lagos bat viruses in both dogs and human begins, with 17.4% of dogs and 7.5% of human beings tested having SN antibodies against Mokola virus, while 5.8% of dogs and 2.5% of human beings tested having SN antibodies against Lagos bat virus. Neither of these rabies-related viruses have sufficient antigenic relationship to rabies to be serologically undifferentiable as to infecting virus, but there may be one or more other rabies-related virus(es) which could be more closely antigenically related to rabies virus.

To further elucidate these numerous questions, we propose a longitudinal prospective study of the development and persistence of antibody against rabies in normal unvaccinated dogs and people in Nigeria. Such a study could provide further elucidation on seasonality of exposure, age at exposure, development and decline of antibody, (of re-exposures, associated clinical manifestations and possible mechaniams of exposure). The ultimate objective of such studies will be the isolation, identification and characterization of the etiological agent(s). The prospects of finding a naturally occurring, naturally propagated "vaccine" against rabies is quite overwhelming.

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APPENDICES

Appendix I: Questionnaire on Rabies

To be completed by Medical Doctors,
School of Records and Health Works

Answer the following questions in so far as possible by years, for the period 1970-1980.

1. How many animal bite cases did you see per year?
   1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980

2. Use the attached (I): to complete the age, sex, site of bite distribution and possibl post exposure treatment

3. From which area(s) do you have the highest incidence?

4. How many clinical rabies cases you saw?
   1970.....1980

5. Use attached (II) to complete the age and sex distribution of the victims

6. What treats were given to these victims when they were brought to your health center/hospitals?

7. What were the fate of these victims?
   a. All died ☐
   b. All recovered ☐
   c. Some recovered ☐

8. On what do you base your diagnosis?

9. Which laboratory confirmatory tests were carried out?

10. For laboratory confirmed cases, where were the laboratory examination made if none is situated in your hospital?

11. After an exposure to a rabies suspect, from your experiences, what are the general reactions and understanding of the people to the exposure?

12. When death occurred as a result of rabies, how do people react

13. Please comment on problems of dog bites and rabies in general.

I

Human Exposure, age, sex distribution

and post exposure treatment

Hospital/Clinic:.....

.....

.....

Date:.....

Name and address of the victim	Year of bite	Age	Sex	Bitting Animal	Site of bite	Treat-ment given	Remarks

II

Cases of clinical Rabies, age, sex distribution and clinical presentation

Hospital or Clinic:.....

.....

.....

Date:.....

Case No.	Name and Address of Victim	Age	Sex	Date of bite	Bitting Animal	Date Admitted	Clinical Signs	Date of Death

Appendix 2: Questionnaire on Rabies

To be completed by Veterinary Officer

Answer the following questions in so far as possible by years for the period 1970-1980 for your area of jurisdiction.

1. List number and months of occurrence of outbreaks of rabies. An outbreak is one or more related cases in a specific area.

   1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980

   a. Outbreaks:

   b. Months:

2. List number of animals in which rabies was diagnosed. Indicate number confirmed by laboratory examination.

	1976 Total Confirmed	1977 Total Confirmed	1978 Total Confirmed	1979 Total Confir.	1980 Total Confirmed
a. Dogs:					
b. Cats:					
c. Livestock:					
d. Wild animals:					

3. List number of recorded human exposures by biting animals

	1976		1977		1978		1979		1980	
	Adult	child (0-12yr)	Adult	child (0-12yr)	Adult	child (0-12yr)	Adult	child (0-12yr)	Adult	child (0-12yr)
a. Dogs:										
b. Cats:										
c. Livestock:										
d. Wild animals:										

4. List number of animals vaccinated against rabies by you or under your supervision

	1976	1977	1978	1979	1980
a. Dogs:					
b. Cats:					

5. List recorded number of human deaths from rabies

	1976	1977	1978	1979	1980
a. Adults:					
b. Children:					

6. How many suspect animals did you confine for rabies observation, and of these, how many were diagnosed as rabid?

	1976	1977	1978	1979	1980
a. Cases observed:					
b. Cases diagnosed:					

7. Have you had any vaccine failures in animals? If so, list number of rabies cases recorded in vaccinated animals.

	1976	1977	1978	1979	1980
a. Dogs:					
b. Cats:					

8. For laboratory confirmed cases, where were the laboratory examination made?

9. What is the source and type of vaccine you use for:
   a. Dogs:
   b. Cats:

10. How do you store your vaccine?

11. What is the maximum length of time you store vaccines?

12. Additional comments on rabies:

Appendix 3:

Clinic_____

State_____

Name_____

Address_____

_____

Occupation_____ Age_____ Sex__

Have you been exposed anytime in your life?

Yes_____ No_____

If yes, when?_____

What treatment was given to you?_____

_____ _____

If no, was there any death in your family due to rabies? Yes_____ No_____

If yes, describe the conditions

Have you been vaccinated against rabies?

Yes_____ No_____

If yes, Why?_____

Which vaccine was used?_____

How many doses?_____

Time of Vaccination _____

Place of vaccination_____

Appendix 4: Questionnaire Survey of Dog-owner Relationship

Please mark correct answer to the questions

1. Why do you keep dog(s)?:

   As guard for security ☐

   I love them as pet(s) ☐

   For hunting ☐

   Given as gift(s) ☐

   I don't know ☐

   All of the above ☐

2. What types of food do you give your dog(s):

   Table food (scraps) ☐

   Special pet food ☐

   They feed for themselves ☐

   Table food and occassionally pet food ☐

   I don't know ☐

3.
3. Where are your dogs kept during the day?

   Outside the house ☐

   Within the house compound ☐

   I have a special kennel ☐

   They lie down in my garage ☐

   They rest anywhere they like ☐

   I don't know ☐

Appendix 4 (Continued)

4. Where are the dogs kept during the night?:

They are let loos to keep guard ☐

They are within the compound ☐

They are confined in their kennel ☐

They are kept in the garage ☐

I don't know ☐

5. Do you ever keep dog(s) on a leash?

Never ☐

Keep them chained during the day ☐

Keep them chained during the night ☐

I don't know ☐

6. Have you visited a Veterinary Clinic before?:

Never been before ☐

Yes I have been ☐

I cannot remember ☐

I don't know ☐

7. Who in your household that hates to see the dog(s) around the house most?

My husband ☐ My Wife ☐

Children ☐ The house boy ☐

The house-girl ☐ The visitors ☐

Appendix 5: Distribution of animal bite cases in human beings by age and sex from 1975-1981 in Kaduna State

Age (years)	Male	Percent Total	Percent Male	Female	Percent Total	Percent Female	Bite cases Male/Female
5	21	11.3	14.3	9	4.8	23.1	2.3
5-9	9	4.8	6.1	9	4.8	23.1	1.0
10-14	27	14.5	18.4	3	1.6	7.7	9.0
15-19	6	3.2	4.1	0	0.0	0.0	6.0
20-24	30	16.1	20.4	6	3.2	15.4	5.0
25-29	18	9.7	12.2	0	0.0	0000	18.0
30-34	3	1.6	2.0	3	1.6	7.7	1.0
35-39	12	6.5	8.2	3	1.6	7.7	4.0
40-44	6	3.2	4.1	3	1.6	7.7	2.0
45-49	3	1.6	2.0	0	0.0	0.0	3.0
50-54	0	0.0	0.0	0	0.0	0.0	0.0
55-59	3	1.6	2.0	0	1.6	7.7	1.0
60-64	3	1.6	2.0	3	1.6	7.7	1.0
64	6	3.2	4.1	0	0.0	0.0	6.0
Total	147	79.0	99.9*	39	21.0	100.1*	4.38**

*Totals do not add to unify due to rounding
**The male/female ratio in the Nigerian population was probably about 1.01 (Ilioje, 1973).

Appendix 6: Distribution of Animal Bite Cases in Human Beings by Age and Sex from 1978-1981 in Oyo State

Age (years)	Male	Percent Total	Percent Male	Female	Percent Total	Percent Female	Bite Cases male/female
5	155	7.5	12.1	73	3.1	7.1	2.12
5-9	275	11.6	21.4	236	10.0	21.7	1.12
10-14	299	12.6	23.3	223	9.4	20.5	1.34
15-19	56	2.4	4.4	104	4.4	9.6	0.55
20-24	108	4.5	8.4	100	4.2	9.2	1.08
25-29	108	4.5	8.4	65	2.7	6.0	1.66
30-34	118	5.0	9.2	45	1.9	4.1	2.62
35-39	39	1.6	3.0	99	4.1	9.1	0.39
40-44	49	2.1	3.8	10	0.4	0.9	4.90
45-49	39	1.6	3.0	34	1.4	3.1	1.15
50-54	20	0.8	1.6	44	1.9	4.0	0.45
55-59	10	0.4	0.8	22	0.9	2.0	0.45
60-64	10	0.4	0.8	33	1.4	3.0	0.30
Total	1286	55.0*	100.2*	1088	45.8*	100.3*	1.18**

*Totals do not add to unify due to rounding
**The male/female ratio in the Nigerian population was probably about 1.01 (Ilioje, 1973)

Appendix 7: Distribution of Animal Bite Cases in Human Beings by Age and Sex from 1979-1982 in Lagos State

Age (years)	Male	Percent Total	Percent Male	Female	Percent Total	Percent Female	Bite Cases male/Female
5	66	5.2	9.0	58	4.6	11	1.13
5-9	158	12.4	22.0	112	8.8	21	1.4
10-14	149	11.7	20.0	101	7.9	19	1.5
15-19	78	6.1	11.0	72	5.7	14	1.08
20-24	74	5.8	10.0	56	4.4	10	1.32
25-29	78	6.1	11.0	42	3.3	8	1.9
30-34	48	3.8	7.0	32	2.5	6	1.5
35-39	29	2.3	4.0	16	1.3	3	1.8
40-44	24	1.9	3.0	18	1.4	3.3	1.33
45-49	6	0.4	0.8	9	0.7	1.7	0.7
50-54	12	0.9	1.6	7	0.6	1.3	1.71
55-59	4	0.3	0.5	1	0.08	0.1	4.0
60-64	1	0.08	0.1	7	0.6	1.3	0.14
64	5	0.39	0.7	3	0.2	0.6	1.7
Total	732	57.4*	100.7*	534	42	100.3*	1.63**

*Totals do not add to unity due to rounding
**The male/female ratio in the Nigerian population was probably about 1.01 (Ilioje, 1973).

Appendix 8: Statistical Analysis

The hypothesis tested is that the frequency of dog bite between age group does not depend on sex or location. This was performed using the chi-square test.

where Oij = the observed frequency for the ith sex and jth age group similarly Eij is the expected value for the corresponding ith frequency for ith sex and jth age group.

The significance of the test is determined by comparing the calculated X^2 statistic with the tabulated values.

Appendix 9: Frequency Distribution Of Dog Bites in Different Age Croup By Sex for Kaduns State 1975 - 1981

Age Category/yrs.	Male	Female	Total
5	21(23.7)	9(6.2)	30
5-5-9	9(14.2)	9(3.7)	18
10-14	27(23.7)	3(6.2)	30
15-19	6(4.7)	9(1.2)	6
20-24	30(28.5)	6(7.5)	36
25-29	18(14.2)	0(3.7)	18
30-34	3(4.7)	3(1.2)	6
35-39	112(11.9)	3(3.1)	15
40-44	6(7.1)	3(1.8)	9
45-49	3(2.4)	0(0.6)	3
50-54	0(1.5)	0(4.1)	0
55-59	3(2.3)	0(0.6)	3
60-64	3(4.7)	3(1.2)	6
64	6(4.7)	0(1.2)	6
Total	147	39	186

X^2 = 29.7
Degrees of Freedom = 13
P$\angle$0.01

Appendix 10: Frequency Distribution of Dog Bites in Different Age Group by Sex for Oyo State 1979 - 1981

Age Category (yrs)	Male	Female	Total
5	1 5(125.1)	76(105.9)	231
5-9	275(275.2)	233(232.8)	508
10-14	299(282.8)	223(239.2)	522
15-19	56(86.7)	104(73.3)	160
20-24	108(112.7)	100(95.3)	208
25-29	108(93.7)	65(79.3)	173
30-34	118(88.3)	45(74.7)	163
35-39	39(74.8)	99(63.2)	138
40-44	49(32.0)	10(27.0)	59
45-49	39(39.5)	34(33.5)	73
50-54	20(34.7)	44(29.3)	64
55-59	10(17.3)	22(14.7)	32
60-64	10(23.3)	33(19.7)	43
Total	1286	1088	2374

X^2 = 162.3

Degrees of freedom = 12

$P < 0.001$

Appendix 11: Frequency Distribution of Dog Bites of Different Age Group by Sex for Lagos State 1975 - 1981

Age Category (yrs)	Male	Female	Total
5	66(71.6)	58(52.3)	124
5-9	158(156.1)	112(113.8)	270
10-14	149(144.5)	101(105.4)	250
15-19	78(86.7)	72(63.2)	150
20-24	74(75.1)	56(54.8)	130
25-29	78(69.3)	42(50.6)	120
30-34	48(46.2)	32(33.7)	80
35-39	29(26.0)	16(18.9)	45
40-44	24(24.2)	18(17.7)	42
45-49	6(8.6)	9(6.3)	15
50-54	12(10.9)	7(8.0)	19
55-59	4(2.8)	1(2.1)	5
60-64	1(4.6)	7(3.3)	8
64	5(4.6)	3(3.3)	8
Total	732	543	1266

X^2 = 17.0

Degrees of freedom = 13

P> 0.05

Appendix 12: Frequency Distribution of Dog Bites of Different Age Group by Location in Males

Age Group	Kaduna	Oyo	Lagos	Total
5	21(16.4)	155(143.7)	66(81.8)	242
5-9	9(30.0)	275(262.5)	158(142.4)	442
10-14	27(32.2)	299(282.1)	149(160.6)	475
15-19	6(9.5)	56(83.1)	78(47.3)	140
20-24	30(14.3)	108(125.9)	74(71.6)	212
25-29	18(13.8)	108(121.1)	78(68.9)	186
30-34	3(11.4)	118(100.3)	48(57.1)	169
35-39	12(5.4)	39(47.5)	29(27.0)	80
40-44	6(5.3)	49(46.9)	24(26.7)	79
45-49	3(3.2)	39(28.5)	6(16.2)	48
50-54	0(2.1)	20(19.0)	12(10.8)	32
55-59	3(1.1)	10(10.0)	4(5.7)	17
60-64	3(0.9)	10(8.5)	1(4.7)	14
64	6(0.7)	0(6.5)	5(3.7)	11
Total	147	1286	732	2147

X^2 = 165.7

Degree of freedom = 26

P<0.001

Appendix 13: Frequency Distribution of Dog Bites of Different Age Group by Location in Females

Age Group	Kaduna	Oyo	Lagos	Total
5	9(3.2)	73(91.7)	58(45.0)	140
5-9	9(8.3)	236(233.8)	112(114.7)	357
10-14	3(7.6)	223(214.1)	101(105.1)	327
15-19	0(4.1)	104(115.2)	72(56.5)	176
20-24	6(3.8)	100(106.1)	56(52.0)	162
25-29	0(2.5)	65(70.0)	42(34.3)	107
30-34	3(1.8)	45(52.4)	32(25.7)	80
35-39	3(2.7)	99(77.2)	16(37.9)	118
40-44	3(0.7)	10(20.3)	18(9.9)	31
45-49	0(1.0)	34(28.1)	9(13.8)	43
50-54	0(1.1)	44(33.4)	7(16.3)	51
55-59	0(0.5)	22(15.0)	1(7.3)	23
60-64	3(1.0)	33(28.1)	7(13.8)	43
64	0(7.0)	0(1.9)	3(0.9)	3
Total	39	1088	534	1661

X^2 = 115.3

Degrees of freedom = 26

$P < 0.001$

Appendix 14: Relative Percent Contribution of Each Location to Significant Differences in Males

Age Group	X^2	Kaduna Contribution	X^2	Oyo Contribution	X^2	Lagos Contribution
5		1.27		0.88		3.05
5-9		14.71		0.59		0.48
10-14		0.85		1.00		0.87
15-19		1.29		8.87		19.86
20-24		16.91		2.55		0.075
25-29		1.24		1.43		1.18
30-34		6.25		3.09		1.46
35-39		0.075		1.52		0.14
40-44		0.020		9.16		0.27
45-49		2.06		3.85		6.44
50-54		2.17		0.051		0.12
55-59		2.95		0.0009		0.53
60-64		4.41		0.34		2.94
64		36.94		6.53		0.44
Total		89.09		39.86		37.85

$$\% \text{ Contribution} = \frac{T}{T_1 + T_2 + T_3} \times 100$$

$\therefore$ Kaduna State = 53.93%

Oyo State = 23.63%

Lagos State = 22.42%

Appendix 15: Relative Percent Contribution of Each Location to Significant Difference in Females

Age Group	X^2	Kaduna Contribution	X^2	Oyo Contribution	X^2	Lagos Contribution
5		9.92		3.81		3.74
5-9		0.04		0.01		0.06
10-14		2.85		0.36		0.16
15-19		4.13		1.10		4.20
20-24		1.26		0.35		0.29
25-29		2.51		0.36		1.67
30-34		0.66		1.04		1.53
35-39		0.01		6.09		12.68
40-44		7.09		5.23		6.47
45-49		1.00		1.20		1.68
50-54		1.19		3.35		5.38
55-59		0.54		3.19		5.52
60-64		3.92		0.82		3.36
64		0.07		1.96		4.29
Total		35.306		28.899		51.057

$$\% \text{ Contribution} = \frac{T}{T_1+T_2+T_3} \times 100$$

$\therefore$ Kaduna State = 30.61%

Oyo State = 25.06%

Lagos State = 44.27%

Appendix 16: Solutions Used

I. Glasgow minimum Essential medium (G-MEM)
for the BHK-21 cells properation.

This is a product of GIBCO Laboratories (Grand Island Biological Company, Grand Island, New York). It contains L-glutamine, tryptose phsophate broth, and no sodium bicarbonate in powder form.

Preparation

A measure of 5% deionized distilled water than desired total volume of medium was made, using a mixing container that is as close to the final volume as possible. Powdered medium was added to water 15-30°C while stirring the mixture. A 2.75g of $NaHCo_3$ per liter of medium was added. The solution was then diluted to the desired volume with water and then the pH of the medium adjusted to 0.2-0.3 below desired final working pH using IN NaOH or IN HCl while stirring. The medium was sterilized by membrane filtration and 10% fatal bovine serum and 1% penicillin-streptomycin mixture were added before use.

II. Trypsine-Versene (ATV) Solution:

This was used to remove the cells monolayer from the flask. The solution was prepared by mixing the constituents other than trypsin (NaCl, Kcl, $NaHCl_3$, Glucose or dextrose) gently in 180ml of triple distilled water and heating the mixture to 37°C after which trypsin over approximately 1 hour with continuous stirring to dissolve it completely.

Triple distilled water was then added to make 200ml and the solution was sterilized by filtratio through a Nalgene sterilization filter unit (type 2) with pore size 0.20 microns. This resulted in 10 times the required concentration of trypsin-versine solution and it was diluted 1:10 in triple distilled sterilized water before use in final 1x and 2x solutions. The 2x solution was used for the BHK-21 cells. The pH is tabilized by using NaOH or HCl depending on the initial pH for the required pH of 7.2. The composition of a 10x solution as shown below

Saline A	200ml
NaCl	16.0 gm
KCl	0.8 gm
$NaHCO_3$	1.4 gm
Glucose or dextrose	2.0 gm
Trypsin	1.0 gm
Versene (Na-EDTA)	0.4 gm
Triple distilled water	200.0 ml
Phenol red	0.4 ml

Phosphate buffered saline (PBS) 0.01M-PH-7.4

This was used in several steps in FA preparations. The materials were disolved in triple distilled water, sterilized by autoclaving for 20 minutes at 121°C and adjusted to pH 7.4. The contents are:-

Na_2HPO	4.60 gm
$NaH_2PO_4.H_2O$	1.10 gm
NaCl	34.0 gm
H_2O	4 liters

Appendix 17: Supplies Used

The following supplies were used in this study:

a. 12 x 75 mm test tubes - Falcon (1950 William Drive, Oxnard, California).

b. 75 cm^2 tissue culture flasks - Falcon.

c. 25 cm^2 tissue culture polystyrene flasks HA-26 - Corning Glass Works (New York).

d. 150 cm^2 tissue culture polystyrene flasks - Corning Glass Works.

e. Costar 96-well tissue culture plates with flat bottom wells with 0.32 cm^2 growth area - Costar (205 Broadway, Cambridge, Massachusetts).

f. Micro-test Terasaki cell culture plates, 60 flat bottom wells, fluid capacity 0.010 ml - Falcon (1950 William Drive, Oxnard, California).

g. Cooke Histo-plates, 72 wells - Cooke Laboratory Products (Division of Dynatech Laboratory, M.C., 900 Slaters Lane, Alexandria, Virginia).

h. Lab-Tek tissue culture chamber/slides - Lab-Tek (Division of Miles Laboratory, Inc., Naperville, Illinois).

i. P-20 Model, continuously adjustable digital microliter pipette, 1-20 ml.

Appendix 18: The Rapid Fluorescent Focus Inhibition Technique (RFFIT)

The RFFIT is a rabies-virus neutralization test run in cell culture to determine the rabies antibody level in human or animal sera. Immunofluid rescence staining rather than cytopatic effect is used as an indicator of viral growth. Steps in the RFFIT:

1. Using one of the aliquots from rabies virus pool a ten fold serial dilutions of the virus (0.1ml in 2ml) of volumes of medium without FCS.
2. The BHK-21 cell suspension was prepared. A 0.2ml of the DEAE-Dextran treated cell suspension was added to each chambers with virus dilutions.
3. The slides were incubated in a controlled humidity incubator or airtight chamber with 1-5% CO_2 and $37^{o}C$ for 20 hours.
4. The slides were then fixed in total acetone, stained washed and dried. The 20 hours titer of the virus was deterined by reading 20 low power microscopic fields for each dilution and counting the number of fields containing fluorescing cells. The dilutions that contained 10 fields with rabies infected cells or 50% of the fields counted was selected for the RFFIT (This dilution contained the 50 Fluorescing Foci Dose 50 FFD_{50} 0.1ml).

5. The rabies immunofluoresence conjugate was also titrated to determine the working dilution to be used in the RFFIT.
6. A 3-4 days old monolayer of BHK-21 cells grown in 25ml of medium with 10% FCS in an 8-oz tissue culture flask was trypsinized. The cells were suspended in 10ml of medium with 10% FCS. The cells suspension was adjusted to contain $5x10^5$ cells/ml.
7. The test sera were inactivated for 30 minutes and diluted 1:8 for screening.
8. The control slide was also prepared with a known negative and positive reference serum.
9. 0.2mls of the diluted test serum was mixed with 0.2mls of the rabies virus 50 FFD_{50}/0.1ml in a multi-chambered slide. After allowing the mixture to react in a CO_2 incubator for 90 minutes the already prepared BHK-21 cells in growth medium was then added to each serum-virus mixture.
10. The serum-virus-cell cultures were then incubated for 24 hours in a 5% CO_2 incubator at 37^oC.
11. The cultures were washed, fixed and stained then observed under a fluorescence microscope for fluorescing cells.