EPIDEMIOLOGY OF BLUETONGUE, RIFT VALLEY FEVER AND ORUNGO FEVER VIRUSES IN FOOD ANIMALS OF SOKOTO AND KADUNA STATES OF NIGERIA

BY

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DVM, UNIVERSITY OF IBADAN, IBADAN, 1979

A Thesis submitted to Ahmadu Bello University in partial fulfillment for the degree of MASTER of SCIENCE

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THESIS APPROVAL

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

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DECLARATION

I hereby declare the originality of this piece of work which was carried out by me in the laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria under the supervision of Drs. J. U. Umoh, C. D. Ezeokoli and Professor E. D. Belino.

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

G. O. Ezeifeka

Ahmadu Bello University
June 1983

DEDICATION

Dedicated to my parents, Martin and Virginia, who provided every possible assistance for my academic advancement.

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ABSTRACT

Serum samples were collected from 425 cattle, 210 sheep, 407 goats and 64 camels slaughtered in abattoirs or kept in institutional farms in Zaria, Kaduna, Sokoto, Birnin Kebbi, Gusau, Katsina and Kotorkoshi in Sokoto and Kaduna States of Nigeria. The samples were tested for the presence of antibody to bluetongue virus by the agar gel immunodiffusion test. Bluetongue precipitating antibodies were found in 21.6% of the cattle, 27.6% of the sheep, 21.6% of the goats and 17.2% of the camels tested. At the Livestock Investigation and Breeding Centre Kotorkoshi where the disease was first confirmed in sheep antibodies existed even in six months old goats. The prevalence rates of antibody to bluetongue virus in cattle, sheep and goats but not in camels, were higher in Sokoto State than in Kaduna State.

Sokoto red goats and Ouda sheep, indigenous to northern Nigeria, inoculated by the subcutaneous route with bluetongue type 10 virus did not develop disease. Only mild febrile response was observed which was apparent in more goats than sheep. Viraemia was not detected but both species developed complement fixing and precipitating antibodies. On the 31st day post-infection, gross or microscopic lesions were not observed. Complement fixing antibody developed earlier than precipitating antibody.

The 1106 samples were also tested for the presence of antibody to Rift valley fever by the agar gel immunodiffusion method. The antibody prevalence rates were 2.9% in cattle, 6.7% in sheep 1.0% in goats and 3.1% in camels.

The same serum samples were tested for the presence of antibody to Orungo virus by the complement fixation test. Complement fixing Orungo virus antibody was found in 26.7% of the sheep, 6.8% of the cattle, 30.7% of the goats and 37.5% of the camels tested. The antibody titre ranged from 1:5 to 1:40. Hitherto, Orungo virus infection has not been reported in goats and camels in this country.

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ABBREVIATIONS AND SYMBOLS

B.T	-	Bluetongue
BTV	-	Bluetongue virus
EHDV	-	Epizootic Haemorrhagic Disease Virus
PID	-	Post Inoculation Day
CMM	-	Cubic Millimeter
PCV	-	Packed Cell Volume
OCG	-	Anticoagulant Preservative Solution consisting of potasium oxalate, 5gm; phenol, 5 gm; glycerin 500ml; and distilled water 500ml.
RVFV	-	Rift Valley Fever Virus
RVF	-	Rift Valley Fever
I.C	-	Intracerebral
I.P	-	Intraperitoneal
S.C	-	Subcutaneous
CFT	-	Complement Fixation Test
AGDT	-	Agar Gel Immunodiffusion Test
NT	-	Neutralisation Test
<	-	Less than
No	-	Number

CHAPTER ONE

INTRODUCTION

In Nigeria and other parts of Africa, arboviruses are of great economic and public health significance (Fabiyi, 1980). They serve as potential agents of infection to livestock and could spill over to the human population.

Epidemics of Rift valley fever which occurred in South Africa (Schulz, 1951), Sudan (Eisa and Obeid, 1977), Kenya (Daubney et al,1931) and Egypt (Imam et al, 1979 a and b) were devastating to both livestock and man. In Egypt over 18,000 men and 100,000 animals were affected and about 700 fatal human cases were recorded (Mediterranean Zoonoses Control Center, 1981). A severe outbreak of the disease in imported sheep has been described in Nigeria (Ferguson, 1959).

Bluetongue was reported from Daudawa farm, Kaduna State in 1943 with a mortality rate of 75% (Henderson, 1948). Several outbreaks of bluetongue in imported sheep were also reported from the Livestock Investigation and Breeding Centre, Kotorkoshi, Sokoto State between 1966 and 1973 with high mortality among yearlings and pregnant ewes (Bida and Eid, 1974).

Between 1966 and 1973, 8 strains of Orungo virus were isolated from febrile patients and one strain from

mosquitoes in Nigeria (Tomori, 1978). The virus has been associated with three epidemics of a human illness characterised by myalgia and fever of 3-7 days duration in Nigeria (Fabiyi et al, 1975).

Arboviruses may be involved in many "fevers of unknown origin" affecting man in Africa (Fabiyi, 1980). There is need for a continuous surveillance study on the activity of these viruses in the livestock and human population. This will provide a baseline information for the formulation of control strategies. When clinical evidence of infection may not be apparent, the recognition of the presence of viral activity is usually achieved by antibody studies (Fabiyi, 1980). This study is aimed at determining the present status of bluetongue, Rift valley fever and Orungo viruses infection in the domestic ruminants of Sokoto and Kaduna states of Nigeria.

CHAPTER TWO

LITERATURE REVIEW

Bluetongue

Bluetongue (BT) is an arthropod-borne disease affecting sheep and occasionally goat, but seroconversion occurs in other infected domestic and wild ruminants (Erasmus, 1975).

The first outbreak of bluetongue in Nigeria occurred in Daudawa farm in Kaduna State in 1943 among imported sheep with a mortality rate of 75% (Henderson, 1948). Bida and Eid (1974).described several outbreaks of putative bluetongue with high mortality among yearlings and pregnant ewes of imported sheep at the Livestock Investigation and Breeding Centre (LIBC), Kotorkoshi in Sokoto State between 1966 and 1973. A serological survey for antibodies against BTV in domestic ruminants slaughtered in abattoirs in some eastern and northern parts of Nigeria between 1973 and 1974 showed antibody prevalent rates of 47.7%, 28.4% and 29.4% in cattle, sheep and goats respectively (Taylor and McCausland, 1976). Moore and Kemp (1974) reported antibody prevalent rates of 43.4% in cattle, 37.5% in sheep and 21.0% in goats at Ibadan, whereas rates of 45% in cattle and 33.8% in sheep and goats in the University of Ibadan farm were reported by Durojaiye (1979).

Bluetongue virus belongs to the Orbivirus taxon (Theiler and Downs, 1973). About 20 serotypes of the virus have been recognised in different parts of the world (Nawathe, 1980). In Nigeria, types 6, 10 and 16 were isolated from Culicoides midges, type 7 from Crocidura species and serological evidence was found for types 2 and 12 (Lee et al, 1974; Moore and Kemp, 1974).

Bluetongue is naturally transmitted by Culicoides midges; C. imicola in South Africa, C. imicola and C. milnei in Kenya, C. variipenis and C. pallidipenis in the the United States of America (Du Toit, 1944; Dipeolu, 1977). Dipeolu (1977) identified 33 species of midges trapped in many parts of Nigeria and found a preponderance of C. imicola and C. milnei which are potential vectors of bluetongue. The sheep ked Melophagus ovinus has been found to harbour the virus and transmit the disease and other haematophagus insects like Tabanus and Stomoxys could be considered as potential mechanical vectors (Luedke et al, 1965). Transplacental infection of the virus has been reported in sheep and suggested as an overwintering mechanism (Gibbs et al, 1979). Jochim et al, (1965) reported that sheep could also be infected through the ingestion of infective material. Bluetongue is transmissible through semen (Luedke et al, 1975).

In the United States of America sheep are shown to harbour the virus at high concentrations for about 31 days

goats for longer periods than sheep, and cattle for as long as 300 days (Luedke et al, 1969; Luedke and Anakwenze, 1972). Also, wild ruminants like blesbuck, buffalo, East African greater kudu and antelopes are susceptible to the virus and could act as reservoirs (Erasmus, 1975).

The incubation period in sheep following artificial inoculation varies from 2-15 days whereas that of natural infection is about 7 days (Erasmus, 1975). The signs include pyrexia (41°C - 42°C) lasting 6-7 days, hyperaemia of bucal, nasal and eyelid mucosa; oedema of tongue, lips and intermandibular space; protrusion and dark blue discoloration of the tongue. Later there is necrosis and excoriation of the epithelium of the dental pad, hard palate, gum and lips; occasional haemorrhagic diarrhoea, laminitis, coronitis and lameness (Erasmus, 1975). Goats are less susceptible to bluetongue disease than sheep (Erasmus, 1975). During an extensive outbreak of the disease in Isreal Saanen goats were observed to show clinical signs (Barzilai and Tadmor, 1971). Sapre (1964) reported an outbreak in India in which goats were affected. Inverso et al (1980) isolated 3 strains of BTV from clinically ill goats in the United States of America. Luedke and Anakwenze (1972) on the contrary observed no obvious signs when they infected 18 Saanen goats intradermally and subcutaneously with six American strains. Between 1960 and 1973, BT virus was isolated from 73

cattle herds in the United States of America, most of them from clinical outbreaks of bluetongue - like disease reported by field veterinarians (Hourrigan and Klingsporn, 1975). Luedke et al, (1969) reported viraemia in an experimentally infected cattle, with no overt disease.

Moulton (1961) observed the following pathological changes in the bluetongue - infected sheep. There were oedema of the digestive tract, respiratory tract and body cavities as well as generalised lymphoadenopathy; haemorhages and necrosis of skeletal and heart muscles; erosion and ulceration of the oral and nasal mucosa, inflammation of coronary bands and lamina. He also observed edema of lymph nodes with excessive neutrophyll and macrophage invasion; distortion and hyalination of skeletal and cardiac muscle fibres; pulmonary atelectasis and alveolar emphysema; hyperaemia of the spleen and centrilobular lipidosis of the liver as well as tubular hyaline casts in the kidneys.

When BTV was inoculated intravenously into sheep, the virus had affinity for the endothelial walls, and pericytes of capillaries, caused necrosis and regenerative hyperplasia of these cells which resulted in vascular occlusion and exudation of fluid into the extracellular space (Stair, 1968). The virus concentrated later in

the reticulo-endothelial cells of the lymph node especially those draining the head (Stair, 1968). When the virus was inoculated subcutaneously, it concentrated and replicated first in the lymphoid tissues before the onset of viraemia (Pini, 1974). Concerning the distribution of the virus in the blood Pini et al (1966) reported that the virus was associated more with the buffy coat than the plasma. Spreull (1905) observed that sheep serum was as infective as the whole blood. Other very interesting phenomena were the very early appearance of neutralising antibody and the co-existence of high titres of virus and neutralising antibody (Stair, 1968; Leudke, 1969, and Pini, 1974).

When Saanen goats were inoculated experimentally with BTV Luedke and Anakwenze (1972) observed leucopenia that lasted 2 to 13 days but no overt signs of disease. Lymphocytes and neutrophills decreased earlier while eosinophills decreased later. There was also progressive anaemia and haemoglobinemia. Monocytopenia developed earlier followed by marked monocytosis.

BTV is related to the viruses of epizootic haemorrhagic disease (EHD) of deer and Ibaraki disease of cattle (Frank and Willis, 1975; Inaba, 1975). Both BT and EHD viruses produce disease in deer but EHD virus does not produce disease in sheep. (Frank and Willis 1975).

When ruminant serum samples and mouse brain antigen are used, there is no serological cross-reaction by complement fixation, agar gel precipitation, flourescent antibody and plaque neutralisation tests between BT and EHD viruses but slight cross-reaction exists if immune mouse ascitic fluid is used, (Frank and Willis, 1975). Ibaraki virus of cattle may produce low grade viraemia and neutralising antibody in sheep but no overt disease. Sheep BT antisera does not fix complement with Ibaraki virus antigen (Inaba, 1975).

Rift Valley Fever

Rift valley fever is a haemorrhagic disease characterised by hepatitis, high mortality and abortion in livestock, and a dengue-like disease sometimes accompanied by visual disorders, liver lesions and complications of the central nervous system in man (Mediterranean Zoonoses Control Centre, 1981).

The disease had existed since about 1912 in Kenya under the mistaken identity of malaria in man, baccillary infection in lambs and heart water in ewes (Easterday, 1965). Rift valley fever was first described in 1931 following an epizootic among sheep in a farm near Lake Naivasha in the Rift Valley region of Kenya (Daubney et al, 1931). Between 1950 and 1951 a major outbreak

occurred in South Africa affecting about 20,000 people without mortalities but about 100,000 deaths were recorded of sheep and cattle (Schulz, 1951). In 1953 and 1956 new outbreaks occurred in South Africa (Weiss, 1957). Another outbreak occurred near Entebbe in Uganda in 1960 without concommitant cases in livestock (William et al, 1960). Large epidemics of RVF occurred in the Kosti district of Sudan in 1973; near Khartoun in 1976 (Eisa et al, 1977) and in Egypt in 1977 and 1978 (Imam et al, 1979a, 1979b). The presence of antibody against RVF virus has been reported in animals and man in many other African countries (Easterday, 1965). In Nigeria the disease was first described by Ferguson (1959) among Merino sheep imported to Vom in Plateau State. Lee (1970) isolated the Nigerian strain of the virus from a pool of Culicoides midges and Culex antenatus mosquitoes collected at the University of Ibadan dairy farm.

Rift valley fever virus formerly regarded as an ungrouped virus (Casals, 1957) is now classified in the family of Bunyaviridae, genus phlebovirus (Mediterranean Zoonoses Control Centre, 1981). It is an RNA virus, spherical, enveloped with spikes, sensitive to ether, stable in neutral pH; viable for 4 months at room temperature, 1 year at $4^{\circ}C$ to $-40^{\circ}C$ but destroyed at $56^{\circ}C$ for one hour (Mediterranean Zoonoses Control Centre, 1981). RVF virus isolated from animals and mosquitoes is considered to be

hepatotropic, viscerotropic or pantropic but not neurotropic (Easterday, 1965). The natural hepatotropic virus, however, has neurotropic properties that may be developed experimentally by 30 intracerebral passages in mice to increase the neurotropic effect and decrease the hepatotropic effect (Mackenzie and Findlay, 1936).

Isolation of RVFV could be attempted from blood, placenta, liver and other organs of affected hosts as well as from mosquito vectors (Shope, 1981). Susceptible laboratory animals used for isolation include baby mice, adult mice, hamsters and lambs; baby mice being the animal of choice (Shope, 1981). Alternatively, baby hamster kidney (BHK-21), vero, CER, bovine fetal muscle and primary lamb testis cultures may be used (Davies and Otema, 1978; Coackley, 1965) mice can be inoculated intracerebrally, intraperitoneally, intravascularly and subcutaneously; if extraneural routes are used, younger mice are preferred but if neural routes are used the age of the mice makes no difference (Hims, 1956). The most satisfactory method of egg propagation is the use of 8 day old embryonated egg incubated at 34^{o}C after yolk sac inoculation. Inoculation of mice with neurotropic yellow fever virus, neurotropic or pantropic RVFV inactivated with ultraviolet light, other treatment or killed, or with Rinderpest virus, interfers with subsequent growth of inoculated RVF virus (Findlay and Howard, 1951).

Epizootics of RVF in domestic and wild ruminants including antelopes usually preceed outbreaks in human beings (Mediterranean Zoonoses Control Centre, 1981). A serological survey among wildlife in Kenya showed that 3.2% of blue wildebeests, 6% of grants gazelles and 2% of bubel hartebeests (Davies, 1975); had antibodies against RVFV. In the Egyptian epidemic RVFV was also isolated from rats (Imam et al, 1979a).

The most frequent form of transmission from animal to animal is through mosquito bites and this is why excessive rains increase the number of cases during outbreaks in livestock (Mediterranean Zoonoses Control Centre, 1981). The species of mosquitoes experimentally proven to be able to transmit the disease in Africa include, Aedes caballus, Aedes circumluteolus and Culex theileri in South Africa, Culex antenatus in Nigeria, Culex pipiens fatigans and Taeniorhynachus brevipalpis in Kenya, Mansonia species in Uganda, Eretmopodites species in East Africa and Culex pipiens in Egypt (Mediterranean Zoonoses Control Centre, 1981). Infection of mice with RVFV by the respiratory route has been described (Brown et al, 1981). In some instances, human exposure has occurred through contact with dead animals, aborted foetuses, or viscera meat (Hoogstraal et al, 1979).

During an epidemic, those at highest risk of RVF infection are butchers, slaughterers, herdsmen, veterinarians and laboratory personnel. Respiratory route of infection is considered probable in these individuals (Van Velden et al, 1977). The disease in man is characterised by sudden onset of fever, severe myalgia especially of the lower back, headache, retro-orbital pain and anorexia with a duration of 4 - 7 days (Peters and Meegan, 1981). A small percentage of cases in Egypt developed jaundice and haemorrhagic manifestation followed by death or slow recovery (Shope, 1981). The virus can also though rarely (less than 1% of patients) cause encephalitis resulting in mental confusion, paralysis, salivation, teeth grinding, visual hallucinations, choreal movements, coma and chorioretinitis with transient or permanent blindness (Mediterranean Zoonoses Control Centre, 1981). The 1950 and 1951 RVF epidemic in South Africa affected about 20,000 people and in Egypt in 1977 and 1978 about 18,000 people were affected and about 700 deaths were recorded (Mediterranean Zoonoses Control Centre, 1981). In Nigeria, no human case has been recorded but the presence of RVFV antibody in man has been reported (Tomori, 1980).

RVF in sheep occurs in peracute, acute, subacute, mild or inapparent forms and affected ones have signs like fever, vomiting, mucopurulent nasal discharge and

bloody diarhoea (Easterday, 1965). About 93% mortality occurs in lambs after 24-36 hours of onset, 20% in ewes but almost all pregnant ewes do abort (Bruner and Gillespie, 1973). Mortality rate is 70% in calves and 15% in adult cattle; signs include high rate of abortion, agalactia, laboured respiration, salivation, fetid diarrhoea and excretion of virus in milk (Easterday, 1965). The disease in goats is similar to that of sheep; pigs and cats show viraemia but no overt signs of disease while monkeys produce a mild disease on experimental infection (Easterday, 1965).

RVFV antigens can be detected in infected tissues by agar gel immunodiffusion tests (AGDT), immuno-fluorescence test (IFT), enzyme linked immune assay (ELISA), enzyme linked fluorescent assay (ELFA) and radio-immuno-assay (RIA) (Shope, 1981). RVFV antibody in the serum can be detected by IFT, ELISA, ELFA, haemagglutination inhibition test (HIT), Plaque reduction neutralisation test (PRNT), mouse neutralisation test (NT), complement fixation test (CFT) and AGDT (Mediterranean Zoonoses Control Centre, 1981). Cross-reactions occur between RVFV and other viruses of the phlebotomus fever subgroup depending on the method of antibody detection (Shope et al, 1980). The PRNT, CFT, ELFA and ELISA are more specific while HIT and IFT are least specific and detect antibodies to other members

of the phlebotomus fever group (Shope, 1981). The ACDT has been used satisfactorily in Zimbabwe and its sensitivity can be enhanced by using the counter-immunoelectrophoresis modification of the test (Shope, 1981).

Orungo Virus Fever

Orungo virus was first isolated from a pool of 13 Anopheles fenestus mosquitoes caught at Orungo in the Teso District of Uganda in 1959 during an investigation of an Onyong-nyong fever epidemic (Haddow et al, 1960). The virus was also isolated from the blood of a febrile child at the University College Hospital, Ibadan in 1966 (Tomori, 1978). Before the year 1975 other isolations were made from different parts of Nigeria, 8 from patients with non-descript febrile illness and one from Aedes dentatus mosquitoes (Tomori, 1978). Isolations of Orungo virus have also been reported in Central African Republic and Senegal (Fabiyi, 1980).

The virus belongs to the orbivirus group (Tomori et al, 1976) and has an estimated particle size of between 100nm and 220nm (Tomori, 1976). It is inactivated when heated at 56°C for 8 minutes or 37°C for 12 hours in phosphate buffered saline suspension but stable for 18 months at - 70°C in 5% bovine albumin (Tomori and Fabiyi, 1977a).

It has very low sensitivity to lipid solvents like ether, chloroform and sodium deoxycholate; stable between pH 5.0 and pH 7.0 and keeps at 4°C for at least 18 months if lyophylised (Tomori and Fabiyi, 1977a).

Orungo virus can be propageated in vero cell cultures (Tomori and Fabiyi, 1975); in BHK-21 monolayers (Tomori et al, 1976) but fails to replicate in duck embryo, new-born rabbit kidney (MA-111) and Aedes albopictus cultures (Tomori, 1977). The susceptibility of mice to lethal infection by intracerebral inoculation of Orungo virus is highest in the new-born and decreases with age of the mice but intraperitoneal and subcutaneous inoculation do not kill mice and hamsters of any age (Tomori et al, 1976).

Neutralising antibodies to Orungo virus was detected in 50% of sheep, 24% of monkeys and 14% of cows while none of the goats, horses and camels tested in Nigeria between 1967 and 1971 showed antibodies (Tomori and Fabiyi, 1976). Experimental infection of rabbits, sheep (Tomori, 1976) and Erethrocebus patas monkeys (Tomori, 1978) with orungo virus produced neutralising antibodies without any overt disease. All birds experimentally infected showed neither circulating virus nor neutralising antibody (Tomori, 1976).

Human infection with Orungo virus is prevalent in Nigeria and is associated with both mild and severe human

disease (Tomori, 1976). Of the 10 Nigerian Orungo virus isolates, 4 were recovered from the blood of 1 - 14 years old children with non-descript febrile illness at Ibadan; one from the cerebro-spinal fluid of a seriously ill child with fever, diarrhoea, convulsion and flaccid paralysis of the lower extremities, in a hospital at Ibadan; and three from blood of febrile children at the out-patient clinics in Enugu (Tomori, 1978). During and epidemic of Orungo virus in Jos in 1975, a serological survey conducted showed an antibody prevalence rate of 43% in the exposed human population (Tomori and Fabiyi, 1976). A nationwide serological survey also showed that about 23% of Nigerian had anti-body to the virus; the highest prevalence rate was observed in the Guinea savannah zone and the lowest in the rain forest zone (Tomori and Fabiyi, 1976).

The serological detection of Orungo virus antigen and antibody can be achieved by agar gel immunodiffusion test, complement fixation test and neutralisation test (Tomori, 1976). Haemagglutinating activity was not detected when a sucrose acetone extracted Orungo virus antigen (even after protamine treatment) was tested against goose, goat, sheep, human O, patas and Rhesus monkey red blood cells (Tomori and Fabiyi, 1977a). A study of the serological reaction between Orungo virus

and other arboviruses by complement fixation and neutralisation tests showed that there may be no antigenic relationship between the virus and other viruses (Tomori, 1976).

CHAPTER THREE

A SEROLOGICAL SURVEY FOR BLUETONGUE ANTIBODY IN RUMINANTS OF SOKOTO AND KADUNA STATES OF NIGERIA

Summary

Serum samples collected from 1106 ruminants in seven different locations in Sokoto and Kaduna States of Nigeria were tested for the presence of bluetongue antibody by agar gel immunodiffusion test. These states represent the part of the country where the first clinical outbreaks of the disease in sheep were reported. Bluetongue antibodies were found in 21.6% of the cattle, 27.6% of the sheep, 21.6% of the goats and 17.2% of the camels tested. At the L.I.B.C. Kotorkoshi where the disease was first confirmed in sheep, antibodies existed even in six months old goats. The differences in bluetongue antibody prevalence in the various ruminants species and locations are discussed.

Introduction

Bluetongue is an arthropod-borne disease affecting sheep and occasionally goats, but seroconversion occurs in other infected domestic and wild ruminants. The clinical disease in sheep is characterised by hyperaemia of the muzzle, lip, tongue and palate, ulcerative and necrotic stomatitis, enteritis and lameness, (Bida and Eid, 1974; Nawathe 1980).

About 20 serotypes of the virus have been recognised in different parts of the world (Nawathe, 1980). In Nigeria types 6, 10 and 16 were isolated from Culicoides midges, type 7 from Crocidura Spp. (Lee et al, 1974) while serological evidence was found for type 2 and 12 (Moore and Kemp 1974).

The first clinically diagnosed outbreak of blue-tongue in Nigeria was reported from Daudawa farm in Kaduna State in 1943 with a mortality rate of 75%, (Henderson, 1948). Bida and Eid (1974) described several outbreaks of putative bluetongue in imported sheep at the Livestock Investigation and Breeding Centre (L.I.B.C) Kotorkoshi in Sokoto State between 1966 and 1973 with high mortality among yearlings and pregnant ewes. Serological evidence for bluetongue virus in domestic ruminants from various parts of Nigeria has been reported (Taylor and McCausland, 1976; Moore and Kemp, 1974; Durojaiye, 1979).

This study is designed to determine the present status of bluetongue disease in Sokoto and Kaduna States of Nigeria where a high percentage of the nation's livestock population exists and where the first reported outbreaks of the disease in sheep occured.

Materials and Methods

(i) Collection of Samples

A total of 1106 serum samples from cattle, sheep goats and camels were collected during visits to abattoirs or in institutional farms between February and July 1981. The samples were stored at -20°C until tested. The r ci - species, breeds, sex and ages for animals from institutional farms, were recorded during sampling. The breeds included Bunaji, Rahaji, Gudali and few exotic and crossbred, cattle, Ouda and Balami sheep and the Sokoto red goats.

Three locations were selected each from Sokoto and Kaduna States for sampling and the seventh location was the L.I.B.C. at Kotorkoshi in Sokoto State where the first confirmed outbreak of the disease in Nigeria occurred These locations represent the major livestock markets where most of the animals pooled from the surrounding areas are usually sold for slaughter.

(ii) Virus Antigen and Control Hyperimmune Serum

Bluetongue agar gel immunodiffusion (AGID) antigen and positive control serum were obtained from Dr. G. M. Brown of the United States National Veterinary Services, Ames, Iowa U.S.A.

(iii) The AGID Test

Agar gels for the AGID test were prepared on pre-cleaned 25mm x 75mm microscope slides using 0.9% agarose (Sigma Type 2) in 0.85% sodium chloride and 0.01% sodium azide, pH 7.6. Glass slides were precoated with 2% agarose in 0.85% sodium chloride and air-dried before pouring the agar gel. Two sets of seven well clusters (one central and six peripheral) were punched in each slide as described by the committee on bluetongue of American Association of Veterinary Laboratory Diagnosticians (Joachim and Pearson, 1979). The gels were prepared fresh for each test.

Each serum sample was tested in duplicate and each cluster included two wells of positive control serum. The test were observed from 24 to 72 hours.

Results

In general, 22.5% of all domestic animals were found to be ppsitive for bluetongue antibody (Table 3:1).

About 21.6% of the 425 cattle sera tested were positive. The highest frequencies 29.1% and 27.5%, were in Birnin-Kebbi and Sokoto town respectively while the lowest, 10% and 9.1%, were in Gusau and Katsina respectively (Table 3:2).

Of the 210 sheep sera tested, 27.6% were positive The highest prevalence, 36.5% and 36.4% were in [illegible] Birnin-Kebbi and Sokoto while the lowest, 4.3%, was from Gusau. In Kotorkoshi L.I.B.C. antibodies were detected even in 6 months old kids (Table 3:3).

Seventeen point two percent (17,2%) of the 64 camels tested were positive. Apart from Zaria where the two camels tested were positive the highest prevalence (22.7%) was in Katsina.

Discussion

In this study, positive results were obtained in the four species of domestic ruminants tested. They may be involved in the maintainance of bluetongue virus in Nigeria. Although cattle, camels, sheep and goats are all immunologically responsive to the virus only sheep and goats, especially sheep, show clinical signs (Nawathe, 1980). Sex does not seem to affect the prevalence of bluetongue antibody in those livestock.

Except for camels, the prevalence rate of bluetongue antibody was generally higher in Sokoto State than in Kaduna State. The differences in the species specific prevalence rates obtained in this study may be related to the host preference of the vectors available in these localities. The Milnei group of Culicoides which prefers

cattle to sheep and goats constitutes a lower percentage of Culicides population in these two Northern States but are more common in the Southern States (Dipeolu, 1977).

At the L.I.P.C. Kotorkoshi where the disease was first confirmed in Nigeria in 1974, antibody existed even in 6 months old goats, however, only the oldest ram (9 years old) had bluetongue antibody. This was probably because all the other sheep were recently brought to the farm except that ram which survived the 1974 outbreak.

The agar gel immunodiffusion test was selected for this survey because it is more sensitive for the detection of bluetongue antibodies than complement fixation test, (Coackley et al, 1980). It demonstrates group antibody and has an added advantage of detecting long standing antibodies since precipition antibodies to bluetongue last for up to 400 days, as long as neutralising antibodies persist (Moore and Kemp, 1974).

Bluetongue virus is transmitted by Culicoides midges. The feeding and breeding activities of these vectors in Nigeria are highest in areas with high rainfall, high relative humidity (85-95%), low wind force (2 knots), high population of definitive and reservoir hosts and mean height temperature of 22°C since they feed mainly at night (0100-0500 hours), (Dipeolu, 1977).

These findings suggest that cattle, sheep, goats and camels are involved in the epidemiology of bluetongue virus in Northern states of Nigeria but the mechanism is not clear cut. Furthermore, although the disease has been reported sporadically in sheep, it appears that bluetongue virus does not produce severe clinical disease in this area.

CHAPTER FOUR

RESPONSE OF SOKOTO RED GOATS AND OUDA SHEEP TO EXPERIMENTAL INFECTION WITH BLUETONGUE VIRUS

Summary

Sokoto red goats and Ouda sheep, indigenous to northern Nigeria were inoculated subcutaneously with bluetongue virus type 10. No frank disease was observed in both species following infection. However there was mild pyrexia which affected more goats than sheep. Viraemia was not detected but both species developed complement fixing and precipitating antibodies. On day 31st post-inoculation no gross and microscopic lesions were observed in the lungs, liver, kidney, heart, abomasum, muscle, intestine bladder, spleen and lymph nodes. Complement fixing antibody developed earlier than precipitating antibody and complement fixing antibody titre was higher in goats than in sheep.

Introduction

The degree of susceptibility of sheep to bluetongue disease varies with the breed (Erasmus, 1975). The indigenous South African breeds, the Afrikander and Persian are less susceptible than the Merino whereas most exotic breeds such as the European mutton breeds are more susceptible than the Merino (Spreull, 1905; Neitz, 1948).

Goats are markedly less susceptible than sheep (Erasmus, 1975). Spreull (1905) did not observe any clinical symptom in the experimentally infected Angora goats but goat blood collected on the 5th and 20th days after infection proved infective for sheep. In outbreaks of bluetongue in India (Sapre, 1964) and Israel (Erasmus, 1975) indigenous goats were reported to have shown clinical signs. In Nigeria bluetongue disease has been reported only among imported breeds of sheep in Daudawa farm in Kaduna State (Henderson, 1948) and in the Livestock Investigation and Breeding Centre (LIBC), Kotorkoshi in Sokoto State (Bida and Eid, 1974). Tomori (1980) reported only low level viraemia in the West African dwarf sheep of Southern Nigeria that were experimentally infected sub-cutaneously with an indigenous strain of bluetongue virus. There is very little information about the susceptibility of the northern Nigeria breeds of sheep and goats to bluetongue despite the fact that the disease was first observed in exotic breeds imported into that geographical area. This study is aimed at determining the response of Sokoto red goats and Ouda sheep to bluetongue infection.

Materials and Method

Virus

Bluetongue virus type 10 was obtained from Yale Arbovirus Research Laboratory, United States of America.

The virus was propagated by intracerebral inoculation of 2 day old suckling mice. The stock virus (10% mouse brain suspension of the virus in sterile physiological saline) titre was $10^{4.5}$ mouse LD_{50} per 0.02ml by the method of, Reed and Muench, 1938.

Antigen and Positive Control Antisera

A 10% suspension of the harvested mouse brain in physiological saline, inactivated at 56°C for 30 minutes and stored at -20°C was used as antigen for the complement fixation tests (CFT). Positive control antiserum for the CFT in the form of immune mouse ascitic fluid (IMAF) was prepared using the method of Tikasingh et al (1966).

Bluetongue agar gel immunodiffusion test antigen and positive control antiserum were obtained from Dr. G. M. Brown of United States National Veterinary Services, Ames, Iowa, U.S.A.

Experimental Animals

Six Ouda sheep and six Sokoto red goats were used for the study. Four animals of each species were used as experimental animals while the remaining two were used as controls. The animals were kept for five weeks before virus inoculation. During this period, they were examined for viraemia by intracerebral inoculation

of blood samples into baby mice; precipitating and complement fixing antibodies by agar gel immuno-diffusion and complement fixation tests; pyrexia and clinical signs. All were free from blood parasites when the blood thin smears were examined microscopically, and negative for clinical, virologic and immunologic evidence of bluetongue infection.

Experimental Inoculation

All animals in the experimental group were inoculated subcutaneously at the posterior end of the left shoulder with 2.0ml of bluetongue virus suspension containing $10^{6.5}$ mouse LD_{50}.

Temperature Determination

Rectal temperetures were taken twice daily (morning and evening) throughout the pre-inoculation and post-inoculation periods and the mean daily temperatures recorded.

Collection of Serum and Blood Samples

Heparinised whole blood and clotted blood for serum samples were obtained by jugular venipuncture at weekly intervals during pre-inoculation and daily at post-inoculation until the 12th days then at 4 days interval until the 24th day and daily thereafter until

the 31st day.

Virus Assay

Viraemia was determined by the intracerebral inoculation of a 2 day old suckling mice with the sheep or goat heparinised whole blood mixed with an equal volume of OCG preservative solution and stored at 4°C All blood samples were tested within one week of collection.

Serological Tests

The serum samples were tested for the presence of bluetongue antibodies by the agar gel immunodiffusion method described by Jochim and Pearson, (1979). Complement fixation test was also performed using the microtitre technique of Sever (1962). Positive samples were further tested at serial dilutions to determine the complement fixing antibody titre.

Pathological Changes

The experimental and control sheep and goats were euthanised on the 32nd day post-inoculation and subjected to detailed post-mortem examination. Samples of lungs, liver, kidney, heart, abomasum, intestine, muscle, bladder, spleen and lymph nodes were obtained for histopathological examination.

Results

Clinical Observations

Neither experimental nor control sheep and goats showed obvious clinical signs. All goats and one sheep showed mild febrile response varying from 40.1°C to 40.4°C as against their average normal temperatures of 39.6 - 40°C between the sixth and eleventh day (Table 4.1).

Virus Assay

Viraemia was not detected in any of the sheep and goats throughout the pre-inoculation and post-inoculation observation period.

Pathological Changes

All animals were in good condition and showed no gross or microscopic lesions.

Serological Tests

Complement fixing antibodies to bluetongue developed in both the experimental sheep and goats on the 9th day post-inoculation and precipitating antibodies were detected on the 20th and 25th day in goat and sheep respectively (Table 4.2). The CF antibody titre ranged from 1:5 to 1:10 in sheep but from 1:5 to 1:20 in goats. CF antibody titres of 1:10 and 1:20

persisted up to the 31st day post-inoculation in goats. Two of the experimental sheep had no CF antibody by that day. None of the control sheep and goats had CF or precipitating antibodies.

Discussion

The absence of overt bluetongue disease and the antibody response in sheep observed in this study is partly in agreement with previous reports on Nigerian breeds (Tomori, 1980; Bida and Eid, 1974). Tomori (1980) reported low level viraemia ($10^{1.0}$ to $10^{1.5}$ mouse LD_{50}) in the West African dwarf sheep experimentally inoculated with $10^{9.0}$ MLD_{50} of bluetongue type 7 virus. He also reported the developement of CF and neutralising antibodies but neither febrile response nor overt signs of illness in the same breed. Bida and Eid (1974) reported over 40% mortality due to bluetongue in the imported black-headed Persian and Wiltshire Horn sheep and no overt disease in the incontact indigenous sheep.

The result obtained in Sokoto red goats compares favourably with that obtained in goats in some other parts of the world (Luedke and Anakwenze, 1972. Spreull, 1905). Luedke and Anakwenze (1972) reported a high titre viraemia in Saanen goats inoculated with bluetongue type 8 virus and low titre viraemia in those inoculated with bluetongue type 4 but none of the goats developed

overt illness. However, 13 of the 18 goats developed BT precipitin antibodies 21 days post-inoculation. Spreull (1905) did not observe any clinical symptoms in the experimentally infected Angora goats but goat blood collected on the 5th and 20th days after infection proved infective for sheep.

The higher development of CF antibodies and the relatively more febrile response observed in goats than in sheep indicate that the Sokoto red goats may be immunologically more responsive to BT virus than Ouda sheep. It still remains to be determined whether a similar response will be obtained from the same breeds if inoculated with other strains of BT virus, a higher dose of the same strain, or if other Nigerian breeds of sheep and goats like Balami and Yankasa are inoculated with BT type 10 virus.

The result of this study further suggests the innate resistance of Nigerian sheep and goats to bluetongue disease. Although these indigenous breeds are not clinically affected, they should be considered in control measures especially in importation of more susceptible exotic breeds to improve the local ones.

CHAPTER FIVE

A SEROLOGICAL SURVEY FOR RIFT VALLEY FEVER ANTIBODY IN FOOD ANIMALS OF KADUNA AND SOKOTO STATES OF NIGERIA

Summary

A total of 1106 serum samples from cattle, sheep, goats and camels were collected from Kaduna and Sokoto States of Nigeria. These were tested for the presence of antibody to Rift Valley fever virus by the agar gel immunodiffusion method.

The antibody prevalence rates were 2.85% in cattle, 6.67% in sheep, 0.98% in goats and 3.13% in camels.

Introduction

Rift valley fever (RVF) virus is an ungrouped, arthropod-borne virus which affects man and a wide range of animals especially ruminants (Easterday, 1965).

RVF was first described by Daubney et al in Kenya in 1931 following an epizootic of the disease that resulted in high mortality in lambs and calves and abortion in pregnant farm animals. Subsequent epizootics of the disease have been reported in South Africa (Schulz 1951) Egypt (Imam et al, 1979) and Sudan (Eisa et al, 1977). The presence of antibody to RVF virus has been reported in many African countries (Easterday, 1965).

In Nigeria the disease was first described by Ferguson (1959) among Merino sheep imported to Vom in Plateau State. Lee (1970) isolated the Nigerian strain of the virus from a pool of Culicoides midges and Culex antenatus mosquitoes collected at the University of Ibadan dairy farm.

Although RVF has been experimentally produced in a milder form in the indigenous West African dwarf sheep (Fagbemi et al, 1975) no natural clinical disease has been described in the Nigerian indigenous livestock and human population. However neutralising antibody to RVF virus has been detected in some Nigerian human and animal sera (Fagbemi et al, 1973; Tomori, 1980).

This study is aimed at determining the presence of RVF virus antibody in cattle, sheep, goats and camels in Northern Nigerian where a high proportion of the nation's livestock population exists.

Materials and Methods

Collection of Samples

The samples used are same as those described in the serological survey for bluetongue antibody (Chapter three).

Virus and Reference Antiserum

RVF virus strain IB Ar 55171 and its reference antiserum were obtained from Dr. O. Tomori of the Virus Research Laboratory, University of Ibadan, Ibadan. This virus was originally isolated from pools of Culicoides midges and Culex antenatus mosquitoes collected from the dairy unit of the University of Ibadan farm (Lee, 1970).

Anigen and Control Antiserum

The virus was propagated by intracerebral inoculation of 2 day old suckling mice. A 10% suspension of the harvested mouse brain in physiological saline (pss) inactivated at 56°C for 30 minutes and stored at -20°C was used as crude antigen for the test.

Immune mouse ascitic fluid (IMAF) was prepared using the method of Tikasingh et al, (1966).

Agar Gel Immunodiffusion (AGID) Test

The samples were tested as described in the serological survey for bluetongue antibody (Chapter three).

Results

The results are shown in tables 5:1, 5:2, and 5:3. In Sokoto State, the RVF antibody prevalence rate in the tested sera was highest in sheep (8.11%)

followed by cattle (1.3%), goats (0.5%) and none in camels. But in Kaduna State the prevalence rate was highest in camels followed by cattle and goats and none in sheep. However a combined result of both states depicts a highest antibody prevalence rate in sheep (6.7%) followed by camels (3.1) and cattle (2.8%) but least in goats (1.0%).

Discussion

The reported epidemics of RVF in various parts of Africa have indicated that the disease is of great economic and public health importance. Available evidence support the concept that animals are infected through mosquito bites whereas human beings acquire the infection mainly through direct contact with infected livestock or their tissues (Easterday, 1965). However there is evidence of possible RVF infection in mice through the respiratory route (Brown et al, 1981).

The study reported here shows that RVF virus is present in Nigerian livestock but at relatively low levels of activity. Fagbemi et al (1973), also reported relatively low levels of viral activity in a neutralising antibody survey of Nigerian livestock. Using sera collected between 1964 and 1968 they found the prevalence rates to be 2% in cattle and goats, 9% in sheep and 12% in camels whereas the present study observed prevalence rates of 3% in cattle and camels,

7% in sheep and 1% in goats. Since the population sampled in the two studies are not exactly the same, the possibility of a changing pattern of the disease in the intervening twelve years between the two studies cannot be properly deduced from a comparison of their species specific prevalence rates. However it appears that sheep is gaining in pre-eminence as the most susceptible.

Some of the unanswered questions include the source of the Nigerian strain of the virus, how it is maintained in the country and why the prevalence rate is not higher than observed since RVF virus generally spreads rapidly in a susceptible population as demonistrated in the Egyptian epidemic of 1977 (Imam et al, 1979). Studies by Fagbemi et al (1975) have shown that the West African dwarf sheep may have some innate resistance to the clinical RVF disease when infected. This would not account for the low prevalence of antibody since even such sheep showed sero-conversion. Vector population; life span and seasonal variations in their survival and mechanisms of transmission may contribute to the low antibody prevalence observed in this study.

It may be interesting to conduct similar transmission studies with sheep breeds common in Northern Nigeria to determine if they possess such innate resistance. It is also important to note that such

low level of viral activity being reported in Nigeria was observed in Egyptian sheep prior to the devastating epidemic in 1977. Therefore Nigeria still stands the risk of RVF epidemic despite the present low level of viral activity. This may be possible in the event of introduction of another strain of the virus or foreign breeds of susceptible livestock.

CHAPTER SIX

COMPLEMENT FIXING ANTIBODIES TO ORUNGO VIRUS IN FOOD ANIMALS OF SOKOTO AND KADUNA STATES OF NORTHERN OF NIGERIA

Summary

Serum samples were collected in 1981 from 1106 domestic ruminants in seven different locations in Kaduna and Sokoto States of Northern Nigeria. These states represent the part of the country where there has been no reports of clinical disease or antibody to Orungo virus. Orungo virus antibodies were detected in 26.7% of the sheep, 6.8% of the cattle, 30.7% of the goats and 37.5% of the camels tested by the complement fixation method. The antibody titre in the various positive samples were also determined. Hitherto, Orungo virus infection has not been reported in goats and camels in this country.

Introduction

Orungo virus is a relatively new viral disease agent in Nigeria. It was isolated first in Uganda from mosquitoes (Haddow et al, 1960) and subsequently from man and mosquitoes in Nigeria (Tomori and Fabiyi, 1975). Between August 1966 and June 1973, 9 strains of the virus were isolated in Nigeria, 8 from patients with non-descript febrile illness and one from Aedes dentatus mosquitoes (Tomori, 1978). The virus was considered as a member of the Orbivirus taxon (Tomori, et al, 1976).

The virus has been associated with three epidemics of a human illness characterised by nausea, myalgia and fever of 3-7 day duration in Nigeria (Fabiyi et al, 1975). Following a report of an epidemic of febrile illness associated with the virus in Jos, Plateau State in 1975 a serological survey was conducted which yielded an antibody prevalence rate of 43% in the exposed human population (Tomori and Fabiyi, 1976). Although there has been no report of any clinical Orungo virus infection in animals, there is evidence of the existence of antibodies in some Nigerian monkeys, sheep and cattle (Tomori and Fabiyi, 1976).

Little is known about the role of other domestic animals in the maintenance and transmission cycle of the virus especially in the northern states where there is a much greater percentage of the Nigerian livestock population and a much closer man--animal relationship. This study is therefore aimed at determining the extent of involvement of these food animals in the epidemiology of this viral disease agent.

Materials and Method

Collection of Samples

The samples used are same as those described in the serological survey for bluetongue antibody (Chapter three).

Virus and Reference Antiserum

The indigenous Orungo virus strain IbH13019, isolated from a febrile 4 year old boy at the University College Hospital, Ibadan, Oyo State, in 1973 (Tomori and Fabiyi, 1976) and Orungo virus reference antiserum were obtained from Dr. Oyewale Tomori of the Virus Research Laboratory, University of Ibadan, Ibadan, Nigeria.

Antigen and Control Antiserum

The virus was propagated by intracerebral inoculation of 2 day old mice. A 10% suspension of the harvested mouse brain was made in sterile physiological saline, sonicated, inactivated at 56°C for 30 minutes, stored at -20°C and used as antigen.

Immune mouse ascitic fluid (IMAF) was prepared using the method of Tikasingh et al (1966) and also inactivated at 56°C for 30 minutes.

Complement Fixation Test

The complement fixation tests were performed in plastic plates using the microtitre technique of Weinbrein

(1958). The test sera were inactivated at 56°C for 30 minutes and tested at a dilution of 1:5. Positive serum samples were later tested at 1:5, 1:10, 1:20, 1:40 and 1:80 to determine the antibody titre. All dilutions were made in veronal buffer.

Result

The prevalence rate of Orungo virus antibody in food animals in both Kaduna and Sokoto States as well as the distribution of the virus complement fixing antibody titres in the various animal species studied are shown in tables, 6.1, 6.2, 6.3 and 6.4.

In Kaduna State, when all the animal species tested were considered together with respect to location, the highest antibody prevalence rate (35.6%) was found in Kaduna town while the lowest (12.7%) was found in Zaria. Similarly, in Sokoto State the highest (27.7%) was found in Sokoto town while the lowest (12.6%) was found in Gusau.

In Kaduna State, the highest species specific antibody prevalence rate (37.5%) was found in camels while the lowest (10.4%) was found in cattle. A similar pattern of distribution of specie specific antibody prevalence rate was obtained in Sokoto State where the highest (37.5%) was also found in camels and the lowest

(3.9%) found in cattle. When all the species tested were considered together, chi-square analysis did not show any statistically significant difference between the antibody prevalence rates of 23.0% and 19.8% obtained in Kaduna and Sokoto States respectively ($P>0.05$).

The distribution of complement fixing antibody titres followed a similar pattern in all the species studied. While a greater percentage of the positive samples had antibody titres of 1:5, only a smaller percentage had antibody titres of 1:40, and none of the samples was positive at the dilution of 1:80.

Discussion

There has been reports of epidemics of human illness associated with Orungo virus (Fabiyi et al, 1975) and neutralising antibodies to the virus in both animals and man in parts of Nigeria (Tomori and Fabiyi, 1976). Tomori and Fabiyi, (1976) found neutralising antibodies in 14 out of 99 cattle, 23 out of 44 sheep, 9 out of 38 Cercopithecus species of primates and none out of 48 goats, 12 horses and 10 camels serum samples collected between the years 1967 and 1971. However, none of these positive samples were collected from Sokoto and Kaduna States of Nigeria.

The result presented in this communication shows the high activity and widespread circulation of the virus in cattle, sheep, goats and camels of these two northern states of this country. The antibody prevalence rates of 6.8% and 26.7% found in cattle and sheep respectively for both states were lower than the values of 14.1% and 52.3% respectively obtained by Tomori and Fabiyi (1976) for both species. However, this study shows that in addition to these species, the goats and camels may also play a part in the epidemiology of Orungo virus in Nigeria. Orungo virus is believed to be transmitted by mosquito vectors (Tomori and Aitken, 1978). The high activity and widespread circulation of the virus in the domestic ruminants in these parts of the country may be related to the activity and distribution of the mosquito vectors in these areas. Further studies should be done to determine the extent of human clinical febrile illness caused by this viral agent in these states and the exact species of mosquitoes associated with its transmission cycle.

CONCLUSION

This study shows that cattle, sheep, goats and camels in Sokoto and Kaduna States of Nigeria possess precipitating antibody against bluetongue virus. Some of these animals possess antibody even earlier in life as observed in six months old goats at the Livestock Investigation and Breeding Centre (L.I.B.C), Kotorkoshi. Bluetongue type 10 virus did not produce disease in Sokoto red goats and Ouda sheep. These breeds appear to have an innate resistance to the disease and therefore a low risk of bluetongue epizootic.

Antibody to Rift valley fever virus is also present in these domestic ruminants in the area of study, but at a relatively lower level. The relationship between low antibody prevalence rate and RVF epidemic in Nigeria is still uncertain since such low level of virus activity was reported in Egypt prior to the 1977 epidemic.

This study also shows that in addition to the cattle and sheep that are already known to have antibody to Orungo virus in Nigeria, goats and camels are also infected by this viral disease agent.

Table 3 1 : Summary of Results of Various Animal Species Tested for Bluetongue Virus Antibody By AGDT

SPECIES	TOTAL	RESULT		SOKOTO STATE			KADUNA STATE		
	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive
Cattle	425	92	21.6	233	53	22.7	192	39	20.3
Sheep	210	58	27.6	173	53	30.6	37	5	13.5
Goat	407	88	21.6	185	48	25.9	222	40	18.5
Camel	64	11	17.2	40	4	10	24	7	29.2
TOTAL	1106	249	22.5	631	158	25.0	475	91	19.2

Table 3.2: Summary of Results of Cattle Serum Samples Tested For Bluetongue Virus Antibody B. AGDT

	MALE			FEMALE			TOTAL				STATES RESULTS		
LOCATION	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive		Number Tested	Number Positive	Perc Posi
BIRNIN KEBBI	50	14	28	1	-	0	51	14	27.5	SOKOTO STATE			
GUSAU	36	6	16.7	34	1	2.9	70	7	10		233	53	22
KOTORKOSHI	26	7	26.9	-	-	-	26	7	26				
KADUNA	62	10	16.1	26	8	30.8	88	18	20.5				
KATSINA	9	2	22.2	13	-	0	22	2	9.1	KADUNA STATE	192	39	20
SOKOTO	51	17	33.3	35	8	22.9	86	25	29.1				
ZARIA	51	13	25.5	31	6	19.4	82	19	23.2				
TOTAL	285	69	24.2	140	23	16.4	425	92	21.6				

Table 3.3: Summary of Results of Goat Serum Samples Tested For Bluetongue Antibody By AGDT

LOCATION	MALE Number Tested	MALE Number Positive	MALE Percent Positive	FEMALE Number Tested	FEMALE Number Positive	FEMALE Percent Positive	TOTAL Number Tested	TOTAL Number Positive	TOTAL Percent Positive	STATES RESULTS	Number Tested	Number Positive
BIRNIN KEBBI	1	1	100	21	7	33.3	22	8	36.4	SOKOTO STATE		
GUSAU	27	1	3.7	19	7	5.3	46	2	4.3		185	48
KOTORKOSHI	18	2	11.1	47	17	36.2	65	19	29.2			
SOKOTO	14	1	7.1	38	18	47.4	52	19	36.5			
KADUNA	18	3	16.7	38	4	10.5	56	7	12.5	KADUNA STATE		
KATSINA	62	8	12.9	39	4	10.3	101	12	11.9		222	40
ZARIA	46	14	30.4	19	7	36.8	65	21	32.3			
TOTAL	186	30	16.1	221	58	26.2	407	88	21.6			

Table 3.4: Summary of Results of Sheep Serum Samples Tested For Bluetongue Virus Antibody By AGIT.

	MALE			FEMALE			TOTAL			STATES RESULTS		
LOCATION	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive	Number Tested	Number Positive	Percent Positive		Number Tested	Number Positive	Per Posi
GUSAU	4	-	0	20	2	10	24	2	8.3	SOKOTO STATE	173	53	3
KOTORKOSHI	5	1	20	18	-	0	23	1	4.3				
SOKOTO	6	2	33.3	31	10	32.3	37	12	32.4				
BIRNIN KEBBI	17	9	52.9	72	29	40.3	89	38	42.7				
KAISINA	4	-	0	9	1	11.1	13	1	7.7	KADUNA STATE	37	5	
KADUNA	2	-	0	14	4	28.6	16	4	25				
ZARIA	3	-	0	5	-	0	8	-	0				
TOTAL	41	12	29.3	169	46	27.2	210	58	27.6				

Table 4.1: Pattern of Febrile Response in the Sheep and Goats Experimentally Infected With Bluetongue Virus

Animal Number	Mean 5 Weeks Normal Rectal Temp.	Days 1-5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
Experimental Sheep									
1	40.0	39.9	40.0	39.9	39.9	40.0	40.[illegible]	39.9	39.8
2	39.8	39.9	39.7	*40.2	40.3	40.3	40.3	40.2	40.0
3	39.9	39.8	39.[illegible]	39.9	39.6	39.8	40.0	39.9	40.0
4	39.7	39.8	39.6	39.5	39.7	39.7	39.8	39.[illegible]	39.7
Control Sheep									
1	39.7	39.9	39.8	39.8	39.[illegible]	39.[illegible]	39.8	39.8	39.9
2	39.9	39.9	40.0	39.8	39.[illegible]	39.9	40.0	39.9	39.9
Experimental Goats									
1	40.0	39.9	40.0	40.2	40.3	40.2	40.0	40.0	39.9
2	39.6	39.7	40.0	40.4	40.0	39.9	39.6	39.7	39.7
3	39.9	40.0	40.0	40.3	40.2	40.1	40.[illegible]	40.0	39.8
4	39.7	40.0	40.2	40.3	40.3	40.2	40.[illegible]	39.8	39.8
Control Goats									
1	39.6	39.8	39.8	39.6	39.5	39.5	39.7	39.[illegible]	39.6
2	39.6	39.6	39.8	39.5	39.5	39.6	39.7	39.7	39.7

*Only temperatures above 40.0°C are regarded as febrile response

Table 4.2: Development of Precipitating and Complement Fixing Antibodies in Sheep and Goats Experimentally Infected with Bluetongue Virus

	Days AGDT	0-8 CFT	Day AGDT	9 CFT	Day AGDT	10 CFT	Day AGDT	11 CFT	Day AGDT	12 CFT	Day AGDT	13-24 CFT	Day AGDT	25 CFT	Day AGDT	26-31 CFT
Experimental Sheep																
1	-	-	-	-	-	-	-	+5	-	+10	-	+10	+	+5	+	+5
2	-	-	-	+5*	-	+5	-	+10	-	+10	-	+10	+	+5	+	-
3	-	-	-	+5	-	+5	-	+10	-	+10	-	+10	-	+5	-	+5
4	-	-	-	+5	-	+5	-	+10	-	+5	-	+10	-	+5	-	-
Control Sheep																
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Experimental Goats																
1	-	-	-	+5	-	+5	-	+10	-	+10	+	+10	+	+10	+	+10
2	-	-	-	-	-	+5	-	+10	+	+20	+	+20	+	+10	+	+10
3	-	-	-	+5	-	+5	-	+10	+	+20	+	+20	+	+20	+	+20
4	-	-	-	-	-	-	-	+5	-	+10	+	+10	+	+10	+	+10
Control Goats																
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Figures represent reciprocal of CF antibody titre

Table 5:1: Total Number of Animals Tested and Percent Positive for RVFV Antibody By AGDT . In Kaduna State Of Nigeria

LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive
KADUNA	0/88	0	0/16	0	0/56	0	-	-	0/22	0
ZARIA	8/82	9.76	0/8	0	2/65	3.08	0/2	0	10/157	6.37
KATSINA	1/22	4.55	0/13	0	1/101	0.99	2/22	9.09	4/158	2.53
TOTAL	9/192	4.69	0/37	0	3/222	1.35	2/24	8.33	14/475	2.95

No = Number

Table 5:2: Total Number of Animals Tested and Percent Positive For RVFV Antibody By AGDT In Sokoto State of Nigeria

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LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive
GUSAU	0/96	0	1/47	2.23	1/111	0.90	-	-	2/254	0.79
SOKOTO	1/86	1.16	2/37	5.41	0/52	0	0/27	0	3/202	1.49
BIRNIN KEBBI	2/51	3.92	11/89	12.36	0/22	0	0/13	0	13/175	7.43
	//233	1.29	14@173	8.09	1/185	0.54	0/40	0	18/631	2.85

No = Number

Table 5:3: Total Number of Animals Tested and Percent Positive For RVFV Antibody By AGDT In Both Sokoto And Kaduna States of Nigeria

LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positiv
SOKOTO STATE	3/233	1.29	14/173	8.09	1/185	0.54	0/40	0	18/631	2.85
KADUNA STATE	9/192	4.69	0/37	0	3/222	1.35	2/24	8.33	14/475	2.95
TOTAL	12/425	2.82	14/210	6.67	4/407	0.98	2/64	3.13	32/1106	2.89

No = Number

Table 6.1: Prevalence of Orungo Virus Antibody in Kaduna State

LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	*No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive
KADUNA	16/88	18.2	8/16	50.0	33/56	58.9	-	-	57/160	35.6
ZARIA	2/82	2.4	4/8	50.0	13/65	20.0	1/2	50.0	20/157	12.7
KATSINA	2/22	9.1	1/22	4.6	21/101	20.8	8/22	36.4	32/158	20.3
TOTAL	20/192	10.4	13/37	35.1	67/222	30.2	9/24	37.5	109/475	23.0

* No = Number

Table 6.2: Prevalence of Orungo Virus Antibody in Sokoto State

LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	*No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive
GUSAU	1/96	1.1	10/47	21.3	21/111	18.9	-	-	32/254	12.6
SOKOTO	4/86	4.7	9/37	24.3	30/52	57.7	13/27	48.2	56/202	27.7
BIRNIN KEBBI	4/51	7.8	24/89	26.0	7/22	31.8	2/13	15.4	37/175	21.1
TOTAL	9/233	3.9	43/173	24.9	58/185	31.4	15/40	37.5	125/631	19.8

*No = Number

Table 6.3: Prevalence of Orungo Virus Antibody in Both Sokoto and Kaduna States

LOCATION	CATTLE		SHEEP		GOATS		CAMELS		TOTAL	
	*No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive	No Positive No Tested	Percent Positive
SOKOTO STATE	9/233	3.9	43/173	24.9	58/185	31.4	15/40	37.5	125/631	19.8
KADUNA STATE	20/192	10.4	13/37	35.1	67/222	30.2	9/24	37.5	109/475	23.0
Total	29/425	6.8	56/210	26.7	125/407	30.7	24/64	37.5	234/1106	21.2

*No = Number

Table 6:4: Distribution of Orungo Virus Antibody Titre Within Various Species

Species	*No Tested	No Positive	COMPLEMENT FIXING ANTIBODY TITRES							
			Positives with 1:5 Titre	% with Titre	Positives with 1:10 Titre	% with Titre	Positives with 1:20 Titre	% with Titre	Positives with 1:40 Titre	% with Titre
CATTLE	425	29	13	44.8	4	13.8	6	20.7	6	20.7
SHEEP	210	56	26	46.4	13	23.2	8	14.3	9	16.1
GOATS	407	125	57	45.6	35	28.0	19	15.2	14	11.2
CAMELS	64	24	8	33.3	5	20.8	6	25.0	5	20.8

* No = Number

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