PUBLIC HEALTH SIGNIFICANCE OF LEPTOSPIROSIS IN NORTHERN NIGERIA

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THESIS APPROVAL

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DECLARATION

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



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Dedication

This piece of work is dedicated to my children Aisha Qumar and Andrea Yaya and my wife Aman and my parents for their love, understanding and patience.

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ABSTRACT

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INTRODUCTION

Leptospirosis is a disease affecting man and animals and is a major zoonosis. It is primarily regarded as an occupational disease of livestock owners and attendants, veterinarians, slaughtermen, sewage workers and others engaged in related activities. The etiologic agent is a spirochete of the genus Leptospira, tentatively "speciated" into two "complexes": parasitic and saprophytic or biflexa. The International Committee on Nomenclature of Bacteria recognizes more than 140 serotypes divided into 18 serogroups (Anon., 1967).

Although leptospirosis appears to be worldwide, knowledge of its geographical distribution is incomplete (Turner, 1967; Szatalowicz et al., 1969; Sulzer, 1975). Surveys carried out in different areas of the world indicate that it is a water-borne zoonosis and that rodents and wildlife are the important vectors. The presence of these carriers in a moist environment and their numbers,

as well as contact with livestock and man, are important factors in the epidemiology of leptospirosis in any environment. Tropical regions would tend to favor endemic leptospirosis.

In Africa, leptospirosis has been found in Zaire, South Africa, Uganda, Kenya, Sudan, Ethiopia, Egypt, Tunisia, Morocco, Senegal, Mali and Nigeria. The status of leptospirosis in West Africa is largely unknown. In Nigeria, the literature is limited to two small leptospirosis surveys (Collard, 1962; Turner et al., 1975).

Nigeria is primarily an agricultural country with approximately 80 per cent of its population engaged in agriculture and related activities. The population of approximately 70 million live in an area of 923,768 square kilometers, extending from the Gulf of Guinea to the sahelian belt (Appendix 1). Two rivers, the Niger and the Benue, cross the country midway and contribute to a variety of eco-climate zones.

Livestock estimates from the Federal Office of Statistics for 1971 are: 10.8 million cattle, 27.5

million goats, 8.2 million sheep, and 1 million pigs. Over 90 percent of the cattle are located in the savanna and sahelian zones above the Niger and Benue rivers. Associated with cattle is a pattern of transhumance from the northern dry zones to the southern mid-belt area by nomadic Fulbe (Fulani) cattle rearers seeking grazing and water for their herds during the hot dry season (dry and pre-rain).

Goat rearing is important, particularly in the north-western region (Sokoto and Katsina). Sheep are important in the north-eastern states (Borno, Gongola and Kano). Pig rearing is practised mainly in the southern part of Nigeria although large commercial piggeries exist in Kano and Minna.

Sugar cane is an important crop primarily in the irrigated alluvial valleys of Zaria, Kano, Katsina and eastern Sokoto. The Nigerian Government policy is to overcome water shortage in the northern areas by drilling bore holes, building dams, and by establishing irrigation schemes. At the same time, the federal and state governments are providing land to encourage nomadic Fulbe

to settle. While these measures will improve cattle production and the welfare of Fulbe cattle owners, they will create conditions favorable for spreading leptospirosis to cattle, sheep and goats and from animals to man.

The aim of the study was to investigate the public health significance of leptospirosis in northern Nigeria by:

1. A serological survey of livestock and man; and
2. A bacteriological survey of potential reservoirs for man.

PART I.

REVIEW OF LITERATURE

Historical

When Goldschmidt first used the term Weil's disease he was referring to a condition described by Adolf Weil in 1882 (Alston and Broom, 1958). Four patients had been seen by Weil with the following symptoms: hepatomegaly, splenomegaly, jaundice, renal involvement and rapid recovery. Weil differentiated this disease entity (later to be known as leptospirosis) from 1) catarrhal jaundice, 2) primary nephritis, 3) yellow atrophy of the liver, 4) septicemic fever, 5) recurrent fever, and 6) bilious typhoid fever.

Landouzi had earlier associated this particular disease with men working in sewers (Alston and Broom, 1958). He postulated that it came from emanations from sewage and immunity developed after a while. The French called it icterus gravis (infectious jaundice). In 1915 Boggs described the disease as a type of infectious jaundice with fever, enlargement of spleen and liver, nephritis, muscular pain and accompanying icterus. It was not until 1914 that Inada and coworkers characterized the disease by clinical diagnosis (Inada et al., 1916).

They saw spirochetes in hepatic tissue of a guinea-pig infected with blood from a patient suffering from Weil's disease; similar results were obtained from 13 out of 17 patients. Catarrhal jaundice patients failed to yield positive results. They concluded that the spirochete was the causal organism of Weil's disease and named it Spirochaeta icterohemorrhagiae. Inada et al. (1916) proceeded to characterize the disease. In a survey they found 40 per cent of 86 house and ditch rats infected with the spirochete. Clinically, the leptospiruric phase in patients occurred 25 days after onset of illness. They further demonstrated active immunity by persisting titers in infected individuals.

In Germany, Hubner and Reiter (1915) independently discovered the spirochete and named it Spirochaeta nodosa while Uhlenhuth and Fromme (1915) called it Spirochaeta icterogenes. Other pioneer workers in Europe included Stokes and Ryle (1916) in the British Flanders, Dawson and Hume (1916) in Belgium, and Costa and Troisier (1916) in France who described and characterized Weil's disease.

Leptospirosis is not new in the Soviet Union. Bakin in 1880 submitted a theory on the infectious nature of catarrhal jaundice, which was the basis for a later description of a form of icterohemorrhagic leptospirosis. In fact, icterohemorrhagic leptospirosis is referred to in the Soviet Union as "Weil's - Vasilyev" disease (Varfolomeeeva, 1964) in honor of a Russian who pioneered research on the etiology of the disease. The term "marsh-fever" coined by the English is no more than "water-fever" described by Baschenim in 1927 and refers to grippotyphosa fever.

Since then, the bulk of work on leptospirosis has centered around epidemiology and ecology. It has been established in Europe that leptospirosis there is more prevalent in the south than in the northern colder regions.

Present Non-African Status of Leptospirosis

In Finland, seven serogroups have been reported and pomona and tarassovi were not reported in man (Kathe and Mochmann, 1967). In Denmark, it has been found that

leptospirosis is equally distributed in rural and urban areas. Serogroups hebdomadis, icterohemorrhagiae and canicola were the most frequently reported (Turner, 1967).

Eight serogroups are present in the United Kingdom and the disease has occurred sporadically though it remains an occupational hazard.

In France, Gaumont (1966) reported eight serogroups of which icterohemorrhagiae was the most prevalent. Again the disease was primarily an occupational hazard.

In Netherlands, there is a centre in Amsterdam where most of the research on leptospirosis in Europe has been carried out. Leptospirosis is a reportable disease in the Netherlands and L. canicola and L. grippotyphosa are the most important serotypes.

Other countries where leptospirosis has been studied are Hungary (Fuzi et al., 1957), Bulgaria (Matteew and Manew, 1975), Romania (Tropciu and Levin, 1967), Poland and Czechoslovakia (Kmety, 1954).

Fourteen serotypes have been found in the USSR.

Of these, eight (L. icterohemorrhagiae A, B; L. canicola; L. grippotyphosa; L. pomona; L. tarassovi; L. bataviae; and L. hebdomadis) are of pathogenic importance to man and animals (Varfolomeeeva, 1964).

In the Middle East considerable work has been done in Israel.

Serotypes grippotyphosa and ballum have been isolated from hedgehogs and rats (Van der Hoeden, 1958). Serotypes canicola and grippotyphosa are important pathogens for man and animals. More recently, serotypes adamana CHII has been isolated from rats (Shenberg et al., 1975).

In Iran, L. grippotyphosa has been isolated from sheep and cattle (Rafyi and Maghami, 1961).

In the Far East and Australia similar serotypes have been isolated except that L. celledoni has only been reported in Australia (Sundharagiati et al., 1966). It is estimated that 25 - 35 per cent of the population in Thailand have a leptospiral titer.

In the United States of America, 12 main serotypes

have been reported; the most significant being L. canicola, L. icterohemorrhagiae and L. grippotyphosa (Alexander, 1960).

Leptospirosis in Africa

Leptospirosis was recognized in Africa as early as 1933 following an epidemic of jaundice in a colony of Europeans using the same swimming pool (Hadamer and Corti, 1933). The disease had typical signs and symptoms and positive serology was confirmed by Schuffner in Amsterdam and by Petit in Paris. However, it was not until 1946 that systematic study of the disease was started by Van Riel. He isolated 21 strains belonging to eight serogroups around Lake Kivu area of Zaire; 18 from human patients, 2 from rodents (Arvicanthus abyssinicus), and one from water. Later Van Riel and Van Riel (1956) conducted a systematic study of reservoir hosts and prevalence. Serogroups icterohemorrhagiae, grippotyphosa, bataviae, hebdomadis, butembo were found to be most prevalent in the species examined. Some of their isolates are now recognized by the WHO/FAO expert group on leptospirosis as reference strains. These include L. butembo, L. ndambari, L. ndahambukuje, L. kamituga, L. kabura, and L. bafani (Anon., 1967).

A number of surveys for leptospirosis have been carried out in various parts of the African continent. In South Africa, Harris and Saner (1942) started a survey which was followed by Rademan (1964) who isolated seven strains of L. icterohemorrhagiae from rat kidneys. Following an epidemic of canine leptospirosis in Bulawayo in Zimbabwe, Graf (1965) found only one positive out of 505 human sera examined and he was unable to isolate leptospires from rats.

In East Africa, an epidemic of leptospirosis in livestock due to L. grippotyphosa was described by Burdin et al. (1958). This was followed later by an epidemiologic survey of reservoir hosts (Ball, 1966). A prevalence of L. hebdomadis and L. grippotyphosa was found in livestock, while L. grippotyphosa and L. icterohemorrhagiae were isolated from kidneys of rodents caught in Kenya.

The first report of leptospirosis in Ethiopia was by Moch et al., (1975) after testing 758 sera from domestic and wild animals. L. butembo was prevalent in the majority of animals tested and L. grippotyphosa prevailed in dogs and camels.

Surveys in North Africa have been more comprehensive (Mailloux and Kolochine-Erber, 1961; Mailloux, 1963; Durand et al., 1962; Brownlow, 1963). The common serotypes were found except that L. autummalis was found only in Egypt, and L. pomona, L. ballum, L. hyos, and L. tarassovi only in Morocco. Prevalent serotypes are australis, canicola, icterohemorrhagiae, grippotyphosa and hebdomadis. L. grippotyphosa was isolated from Mus musculus in Egypt and from cattle in Tunisia. One isolation reported from Algeria was L. biflexa.

In West Africa, reference to leptospirosis is limited to case reports in West Cameroons, Guinea (Wilbert et al., 1927), (Graf, 1933), and Senegal (Lloverol, 1946). Fry (1967) drew attention to endemic leptospirosis in parts of West Cameroons, by revealing that 25 per cent of 200 patients with F.U.O. (fever of unknown origin) had leptospirosis. Although all the positive patients had pyrexia, only about 35 per cent had icterus and albuminuria.

A serological survey of livestock in Senegal revealed multiple infections and a 20 per cent infection

rate in cattle, 9 per cent in sheep, 4.4 per cent in pigs, and 0.6 per cent in goats. L. icterohemorrhagiae was isolated from a rat (Cricetomys gambianus). A six-year serological study in Dakar of patients with suspected diagnosis of Leptospirosis confirmed only 6.4 per cent (Sankale et al., 1974).

In Ghana, Kinebuchi and Afoakwa (1973) found 21.2 per cent positive seroreactors to leptospirosis in 99 cases of suspected infectious hepatitis. The prevalent serotypes were the same as in Senegal except that no reactors were found for hebdomadis, javanica, pyrogenes, pomona, grippotyphosa, and australis. A greater diversity of serotypes were found in patients from the humid forest areas compared to the drier northern savanna.

There are few records of leptospirosis in Nigeria. As far as can be ascertained from the records of the Ministry of Health and veterinary records, leptospirosis did not even figure in the list of reportable zoonoses. Leach and Cannon (1943) reported a case of leptospirosis in Southern Nigeria from a patient suspected of yellow fever. Collard (1962) demonstrated Leptospira canicola antibodies in 4.5 per cent of the population in Katsina, Kaduna, State.

In 1975, a team of students from the Royal Veterinary College, University of London, tested sera from 955 cattle from Kaduna, Kano, Gongola and Sokoto states and 48.0 per cent were positive. Analysis of the positive reactors revealed that 63.9 per cent came from semi-nomadic herds, 36.8 per cent from permanent farms, and 16.4 per cent from abbatoirs samples (Turner et al., 1975).

Nomenclature

It was recognized early that all leptospira were indistinguishable morphologically (Noguchi, 1917). Noguchi proposed creation of the genus Leptospira to be included in the order Spirochaetales. (Lepto. meaning thin; spira meaning thread).

Babudieri (1949) suggested division of the order Spirochaetales into two families based on optical and ultrastructural studies and presence or absence of septa, axostyles, membranes, flagella and cytoplasmic inclusions (Fig.1).

The eight edition of Bergey's Manual of Determinative

I. Septa present:

Family Saprospiraceae

. undulating membrane (+)	1 Genus Cristispira
. undulating membrane (-)	2 Genus Saprospira

II. Septa absent:

Family Spirochaetaceae

axostyle (+)	flagella (+)		3 Genus Spirella
	flagella (-)	volutin (+)	4 Genus Spirchaeta
		volutin (-)	5 Genus Leptospira
axostyle (-)	crista (+)	flagella (+)	6 Genus Treponema
		flagella (-)	7 Genus Cristispirella
	crista (-)	flagella (-)	8 Genus Trichospira.

Fig. 1 Subdivision of the Order Spirochaetales (Babudieri, 1954)

Bacteriology includes the genus Leptospira in the order Spirochaetales, family Spirochaetaceae with four other genera: Spirochaeta, Cristispira, Treponema and Borellia.

Structure

Leptospira are described as slender, flexuous, helical, single cells of 6 - 20 um length, 0.2 - 0.3 um diameter, and 0.3 - 0.5 um pitch. Both ends may be bent and the hook may disappear on subculturing. Motility is by axial filament by one of three ways:

a) shunting along the axis;

b) rapid rotation along the axis (oscillation); or

c) flexion of the cell.

Leptospira are not readily visible by Giemsa or Gram staining. They are visible in tissue sections stained by Levaditi's silver stain or its modification by Fontana (Drury and Wallington, 1967). In fluid they are best observed by darkfield microscopy and can also be seen by immunoperoxidase staining (Tripathy and Hanson, 1974). Members of the genus are Gram - negative and non-sporing.

Electron microscopic studies of the structure of leptospira have been carried out (Ritchie and Ellinghausen, 1965; Nauman et al., 1969; Chang et al., 1970; Babudieri, 1972; Birch-Anderson et al., 1973) and four main anatomical features have been revealed:

1) a prokaryotic cytoplasmic body delineated by a cytoplasmic membrane containing a less electron dense nuclear region containing delicate stands of DNA material;

2) a rigid mucopeptide containing layer associated with the cytoplasmic membrane which remains intact after treatment of the cells with Teepol;

3) two axial filaments, each inserted in the cytoplasmic body subterminally at opposite ends of the organism. These filaments are structurally analogous to flagella of Gram-negative bacteria in their mode of attachment except in the size and visibility of S and M rings of the basal body (De Pamphilis and Adler, 1971). These structures are actually larger in bacteria than in leptospira; and

4) an outer membrane enveloping the entire membrane thus making it a five-layered envelope: a three-layered organism covered with a two-layered structure (Buckmire, 1970, 1973).

Classification

Leptospira have been classified on the basis of host specificity, disease symptoms, carrier hosts, and geographical distribution (Ido et al., 1918; Schuffner, 1938). Isolation and description of the same serotypes in other parts of the world have limited the usefulness of this basis for classification. Most serotypes described were isolated from field rodents and were also found to infect man.

In his observations of local strains of Weil's disease agent in the neighborhood of Lake Kivu (Zaire), Van Riel (1946) noted that relapsing infectious jaundice (icterus gravis) was only one of the variable types of infection but the most frequent. Both icteric and non-icteric as well as meningitic types occurred. He asserted that symptoms depended on which of the three body parts

were attacked (liver, kidney or meninges). In addition, inapparent cases existed in populations exposed to infection. This refuted the species classification advocated earlier by Schuffner (1938) that the 19 known antigenically autonomous species of Leptospira differed in their geographical range, clinical symptoms, animal hosts, and pathogenicity to laboratory animals. Van Riel further pointed out that the known distribution of leptospira in the world did not follow any obvious rule. The same strains had been isolated in Europe, Africa and Asia. The disease incidence was rather more consistent with an occupational hazard and that the great majority of cases were derived from water.

Since it has been recognized that leptospira are indistinguishable morphologically, culturally and metabolically (Babudieri, 1954), the four most important variable are:

1) Pathogenicity and cross - protection against infection; this varies with age, sex and physiological state of the species of animal concerned. It also depends on the identity and virulence of the

leptospira under test (Chernukha *et al.*, 1975).

2) Clinically, infection varies from a mild febrile illness to a fulminating disease which is fatal. This may depend on the particular stage of disease in the animal, since the animal may be a carrier, convalescent or normal, or it may not be susceptible. Further, not all *Leptospira* sp. produce classical disease symptoms with icterus (Birnbaun *et al.*, 1974).

3) Host selection depends on specificity of the infecting *Leptospira* sp. and on contact possibilities. One animal may act as a carrier for more than one species or conversely one species may infect different hosts (Yu and Wu, 1965).

4) Antigenic composition of the *Leptospira* sp. may or may not be a stable characteristic.

Of these four variables only the fourth seems to be based on intrinsic properties of *Leptospira*, and therefore is the most logical marker for an objective speciation of the genus *Leptospira*.

In 1954, Wolf and Broom (Alston and Broom, 1958) suggested a classification based on cross-absorption agglutination studies. Since most of the above criteria are unstable and the bionomics of leptospira are still poor, they proposed as an interim alternative, the use of the term serotype (serological type) instead of species, to designate strains of the same antigenic constitution. Accordingly, two strains belong to the same serotype if: "after cross absorption with adequate amounts of heterologous antigen, 10 per cent or more of the homologous titer regularly remains in each of the two antisera".

This proposal is elaborate and requires care in performance. It has, in the past led to discrepancies and errors (Babudieri, 1968; Kmety, 1970). It has been accepted, however, and is widely used. It has also taken care of the existence of the so-called "incomplete" and "complete" biotypes (Gispen and Schuffner, 1939).

Antisera to two strains of L. icterohemorrhagiae M20 and RGA were prepared. Absorption of anti M20 with RGA made the anti M20 homologous to M20 only but when anti RGA antiserum is absorbed with M20, no homologous antibodies

remained. Hence M20 strain had antigenic components of RGA in addition to its own. These two strains came to be identified as L. icterohemorrhagiae A. B. for the complete M20 biotype and L. icterohemorrhagiae A for the incomplete RGA biotype (Gispen and Schuffner, 1939).

Similarly biotypes of L. autumnalis, L. canicola A and L. benjamin (Van Riel, 1946) L. grippotyphosa AB, L. wolfii A (Alexander et al., 1955), L. ballum AB, and L. mini A were identified but this nomenclature is no longer used.

Different attempts have been made to find other criteria for classifying leptospira. Enzymatic properties including lipase activity and purine analogue sensitivity have been proposed as factors for classifying leptospira (Bakoss and Chorvath, 1965; Johnson and Harris, 1968). On the basis of sensitivity to 8-Azaguanine and lipase activity, Johnson and Harris (1968) were able to divide the parasitic complex L. interrogans into three groups: Group I species inhibited by 8-Azaguanine at concentrations of 200 mcg/ml or 10 mcg/ml of 2, 6-diaminopurine (DAP) (leptospira in this group possess lipase activity); Group II species, able to

grow in media with 10 mcg/ml 2, 6-DAP and sensitive to 8-Azaguanine; and Group III consists of saprophytic species resistant to the analogues and produce lipase.

Kadis (1974) found urease activity in five parasitic leptospira and none in the saprophytic biflexa complex. He suggested that urease might be another marker for classifying leptospira.

By far the most interesting approach to leptospiral classification has been that of Haapala and coworkers (1969), who demonstrated four genetically distinct groups of leptospira on the basis of G + C content of selected pathogenic and saprophytic (biflexa) serological types. They further found that serological relationship bore no relationship with the relatedness determined by specific duplex formation of DNA.

The Taxonomic subcommittee on leptospira of the International Committee on Nomenclature of Bacteria recognizes more than 140 serotypes of leptospira grouped in 18 serogroups ~~types~~ (Anon., 1967).

Following the basic concept of taxonomy in current use, the Committee accepted serotype as the equivalent of species. In keeping with the thinking of Ainsworth (1955) that "rational and stable basic classification result only by treating pathogens and nonpathogens, parasites and saprophytes, on an equal footing and assessing their overall similarities and dissimilarities" the Committee recommended that the genus Leptospira be considered as monospecific until it was possible to further classify species with confidence. The epithet interrogans was recommended as it was in use earlier than biflexa or icterohemorrhagiae. However, the Committee recognized the existence of the two previously proposed species and suggested their designation as "complexes"; the parasitic complex and the biflexa complex. Thus, serotype as proposed by Alston and Broom (1954) should be represented by a reference strain and the definition of serotype was also modified to read: "Two strains are considered to belong to different serotypes if after cross absorption with adequate amounts of homologous antiserum, 10 per cent or more of the homologous titre regularly remains in at least one of two antisera in repeated tests".

Serotypes of the L. interrogans complex proposed by Turner (1976) are listed in Appendix 2. This is an updated revision of the serotype list by the W.H.O. group of experts on leptospirosis (Anon., 1967).

Epidemiology

Natural hosts of Leptospira sp. include both domestic and wild animals. Man is susceptible but does not serve as reservoir for transmission. Leptospira have occasionally been recovered from non-mammalian species including amphibians (Diesch et al., 1966) and reptiles (Plesko and Hruzik, 1964). These latter findings raise four major epidemiological questions:

(a) What part do lower animals play in the continuity of leptospira in any environment?

(b) What part do predators play in this continuity?

(c) Do pathogenic leptospira survive in water and conserve their virulence or do they "transform" into saprophytic strains?

(d) Do these animals act as vectors for leptospirosis?

Some of these questions were answered by Reilly et.al. (1970) who demonstrated a food chain transmission of leptospira in wild marsupials and carnivores by feeding them leptospira-infected mice in either the leptospiremic or leptospiruric stage of infection.

Pathogenic leptospira have been isolated from surface waters used for recreation (Diesch and McCullough, 1966; Crawford and Diesch, 1969). Moreover, isolation of leptospira from a stream with a flow high enough to maintain a constant dilution indicates that leptospira survive in water (Gillepsie and Ryan, 1963). Environmental factors contributing to survival of leptospira in water are soil pH, temperature (Okazaki and Ringen, 1957; Ryu and Liu, 1966) and bedrock (Kingscote, 1970). Other pertinent observations to the question include the fact that L. icterohemorrhagiae was kept for 22 days in a natural environment (Diesch and McCullough, 1969) and that several species of intestinal bacteria had deleterious effect on leptospiral survival. Moreover, a host of halogen compounds and detergents play a role in leptospiral survival in contaminated

waters (Chang et al., 1948). Shutyaeu (1959) observed that a strain of L. grippotyphosa group remained virulent after 336 days in water and was able to elicit serologic response with high titer in rabbits after 976 days in water. These observations suggest that pathogenicity is not lost in water and that pathogenic species do not transform into "saprophytic" strains.

The relative importance of lower animal species as vectors of leptospirosis probably depends on close association with higher animals, their presence in infected water areas, and their population density.

Two fundamental intrinsic features of leptospira appear in the literature: moisture dependency and extreme environmental sensitivity. Since leptospira leave the host via urine and are transmitted by either direct or indirect contact with urine, seasonal and climatic variations will affect leptospiral survival as well as environmental conditions.

As most mammalian species are susceptible to leptospira infections, the knowledge of specific reservoirs or carriers

is important since they are the main chain of continuity for leptospirosis.

Domestic animals are not only highly susceptible to clinical and subclinical infections, but they are also temporary urinary shedders, hence primary reservoirs. Severity of the initial infection does not determine whether or not affected animals will be shedders.

Wildlife is important for maintaining leptospira. Their population numbers and number of shedder carriers in a moist environment are factors contributing to the enzooticity of leptospirosis, contact between wildlife and domestic animals will also contribute to the risk factor in human leptospirosis.

Some general features of leptospirosis are valid for all domestic hosts and infecting serotypes.

(a) Incidence of infection is much higher than the incidence of clinical disease (Alston and Broom, 1958; Turner, 1967). Both animals with clinical infection and those that recover from the disease continue to excrete leptospira in urine, thus providing a continuous source of infection for

varying lengths of time. Leptospira are shed by cattle for three and half months (Gillepsie et at., 1957) three months by horses, nine months by sheep (Webster and Reynolds, 1955), 11 months by swine (Sleight and Lundberg, 1961), six months to four years in dogs (McIntyre and Seiler, 1953), and up to 11 months in man (Johnson, 1950).

(b) Depending on their number and virulence, leptospira spread from the point of entry at which they produce no lesion to become septicemic (leptospiremia). This phase varies in hosts from mild clinical illness to acute, fatal icteric illness lasting one to seven days.

(c) When generalized infection regresses, it becomes localized in the liver and more particularly, the kidneys. At this point leptospira are shed in the urine.

(d) Transmission of infection results by either direct or indirect contact with infected urine: cutaneous contact (Doherty, 1967), inhalation (Baker and Little, 1948), conjunctival (Van der Hoeden, 1955), and venereal (Roberts, 1958; Sleight and Williams, 1961). Bovine leptospirosis has been experimentally transmitted by transplacental

inoculation (Fennestad and Borg-Petersen, 1962; Hanson and Brodie, 1968). Leptospira have been also successfully isolated from human amniotic fluid (Huei-Lan and Fu-Hsi, 1963). Insect vectors may not be significant but leptospira have been isolated from cattle ticks (Dermacentor marginatus) and dog ticks (Rhipicephalus sanguinem) (Van der Hoeden, 1958; Turner, 1967).

Pathogenesis

Pathogenesis of leptospirosis has been attributed to a number of factors including cytotoxic, hemolysins, toxins and immunologic.

A cytotoxic factor capable of producing a cytopathogenic effect on tissue cell monolayers has been characterized in L. pomona (Yam et al., 1970; Miller et al., 1970). This factor produced reactions in rabbit skin characterized by erythema and edema and histopathological changes in muscle similar to those found in acute leptospirosis. The cytotoxic factor could be responsible for the icterus associated with acute cases. This would suggest a hepatic rather than hemolytic or pre-hepatic cause of leptospiral icterus.

Leptospiral icterus, however, is considered to be due to both hepatic and hemolytic factors. Anemia, characteristic of leptospirosis in lambs has been correlated directly with cold hemagglutinin titers (Bhasin et al., 1971; Smith and Armstrong, 1975).

Pathogenic leptospires have been shown to produce hemolysin in medium in contrast to saprophytic strains (Chorvath, 1975).

Renal failure, a recognized consequence of L. canicola infection in dogs, seldom occurs in other animals except man. Nephritis is a common finding in all species; it has been described in cattle (Fennestad et al., 1967), horse, swine and dogs (Hanson et al., 1969; Taylor et al., 1970; Hanson, 1972) and man (Zach et al., 1973). Ultrastructural changes in renal tubules associated with leptospira infections in sheep include "balloons" in the tubular lumina. Marshall (1974) postulated that these were due mainly to necrosis. He was unable to find active invasion of normal cells even though some severely necrotic cells had leptospira in their cytoplasm. This observation was recorded earlier by Martino et al. (1969). Most significantly, these

observations support the theory that damage is due to toxins elaborated by leptospira (Miller et al., 1970; Harrington and Sleight, 1966). In a later study using mice, Marshall (1975) observed the earliest renal change was interstitial edema, which was present on the second day post infection (PI). Leptospira were only seen in intercellular spaces on the 10th day PI.

Ocular lesions, characteristic signs in equine leptospirosis, result from immune reactions. These lesions can be produced by injection into the ocular sac of a serotype other than the infecting one (Hanson et al., 1969). These signs have also been recognized in man, dogs and cattle (Fennestad, 1963; Hoag and Bell, 1954; Kadamer and Corti, 1933).

Leptospiral infection is abortifacient in cattle, pigs, horses and goats (Roberts, 1971). Infection and or death of the fetus(es) occur. Abortion in cattle depends on the stage of gestation and the infecting serotype; the most critical stage being the last trimester. The common abortive serotypes in cattle are L. pomona, L. grippotyphosa and

L. hardjo. In pigs, full-term stillbirths are more common than abortions. Hemolysin is reported to be the abortifacient factor for cattle (Murphy and Jensen, 1969).

Clinical Manifestations

Clinical signs of meningitis, encephalitis, incoordination and convulsions are not uncommon in both animals and man (Kadamer and Corti, 1933; Alston and Broom, 1958). Leptospira have been recovered from the brain of animals (Langham et al., 1958; Morse and Langham, 1958) and from cerebrospinal fluid of patients with meningitis or encephalitis (Huei-Lan et al., 1958).

Man

The course of leptospirosis in man varies between subclinical and acute manifestations, the seriousness of infection being proportionate to the intensity of icterus and disintegration of kidneys (Van der Hoeden, 1964). Soon after initial symptoms, aseptic meningitis develops. Jaundice follows the febrile period with its associated pathophysiologic changes, bilirubinemia and urobilinuria, and albuminuria as a consequence of interstitial nephritis. In fatal

cases, anuria and uremia precede death. Jaundice does not occur in about 60 per cent of cases. After recovery, renal function usually returns to normal.

Ocular complications, arthritis, iridocyclitis, uveitis are sequelae which appear during convalescence and are well-described in earlier literature (Kadamer and Corti, 1933). The recurrent form peculiar to equine species seldom occurs in man.

Lung manifestations suggestive of tuberculosis; blood-tinged sputum, bronchopneumonia, sore throat are some of the respiratory symptoms observed during the first week of leptospirosis (Poh and Poh, 1970).

Other symptoms contributing to the complexity of differential diagnosis of leptospirosis include a poliomyelitis-like form with weakness of the calf muscles (Christie, 1968). A rash due to subcutaneous and internal hemorrhages develops in 10 per cent of patients. Nausea and vomiting with signs of abdominal tenderness are not uncommon Stoenner, 1971).

The appearance of leptospira in urine is associated with

the convalescent stage when antibodies develop. There is no evidence that a carrier state develops in man even though Johnson (1950) reported leptospira in urine of a man 11 months after recovery from australis B. infection.

Among domestic animals, cattle, dogs and swine constitute the most important hosts of leptospira and the most common source of human leptospirosis. Other animals include sheep, goats, horses, donkeys and cats which are believed to be of lesser importance in the epidemiology of human leptospirosis (Blenden, 1975).

Cattle

The role of cattle in the epidemiology of leptospirosis is obvious from the fact that original cases described by Weil in 1881 were butchers and L. pomona was first isolated from a dairy farmer.

L. grippotyphosa has been isolated in the Soviet Union since 1935 (Varfolomeeva, 1964). L. pomona has been associated with most acute infections in Australia (Sutherland et al., 1949). Heavy losses are also associated with serotype pomona in the U.S.A. (Steele et al., 1957). Outbreaks

of bovine leptospirosis due to L. canicola have been reported in Israel (Van der Hoeden, 1955) and elsewhere (Karash, 1964). Borg-Petersen and Fennestad (1956) report chronic infections associated with L. sejroe and L. saxboebing. Serotype hardjo was associated with infection in cattle in the U. S. A. (Roth and Galton, 1960).

Clinical manifestations of bovine leptospirosis are characterized by pyrexia, icterus, and nephritis. Skin lesions associated with chronic leptospirosis have also been described (Gayot, 1955). In pregnant cows, abortion is an important clinical feature, especially in the last trimester of gestation (Fennestad and Borg-Petersen, 1958; Roberts, 1971). In dairy cattle, the diseases manifested by blood-tinged milk, agalactica and atypical mastitis (Baker and Little, 1948). Young animals are more susceptible than older animals. Redwater of calves caused by L. pomona is an acute form of leptospirosis with high mortality and is characterized by jaundice and hemoglobinuria.

Dog

L. canicola and L. icterohemorrhagiae are the serotypes

recognized to infect dogs although L. pomona has also been isolated from urine of a dog (Murphy et al., 1958). Serotype canicola is more common and is transmitted directly from dog-to-dog while serotype icterohemorrhagiae is transmitted indirectly by contact with rat urine and water contaminated with rat urine. Separation of the two types of infection on the basis of clinical signs alone is almost impossible as they are variable. In all cases of canine leptospirosis, however, whether hemorrhagic icteric or uremic, the kidneys have a variety of lesions of which interstitial nephritis is the most important since the acute stage often becomes chronic with terminal uremia (Bloom, 1954; Taylor et al., 1970). Acute disease terminating with death in two to three days, prevails in the one to three year age group. Shedding of leptospira up to three years after recovery from either a mild or acute clinical disease has been recorded (McIntyre and Seiler, 1953).

Swine

Serotypes pomona, canicola, grippotyphosa, icterohemorrhagiae have been isolated from swine (Hanson, 1972). Other serotypes include autumnalis, hyos (Jhonson, 1942)

australis, bataviae and ballum (Alexander et al., 1964). Apparently healthy pigs excrete large number of leptospira up to six months. Most serotypes produce similar disease patterns in pigs.

Signs most frequently associated with swine leptospirosis include abortion, stillbirths, weak piglets, fever, anemia and hematuria (Fennestad and Borg-Petersen, 1966).

Goats

Most caprine infections remain undetected (Morse and LangHam, 1958; Khanna, 1971). Acute fulminating leptospirosis has been known to wipe out entire herds (Van der Hoeden, 1964). Clinical manifestations include profound jaundice, hemoglobinuria and death within a few days. Abortions occur with pregnant goats (Van der Hoeden, 1953).

L. grippotyphosa and L. pomona have been the serotypes commonly isolated from goats. These isolates support the hypothesis that caprine leptospirosis results from their association with cattle (Morter and Morse, 1956) and swine (Burdin et al., 1958). Other serotypes associated with caprine infections are L. icterohemorrhagiae, L. bataviae,

L. hebdomadis, and L. butembo (Van Riel and Van Riel, 1956).

Sheep

Incidence of leptospirosis in sheep is lower than in goats. Serotypes pomona, grippotyphosa and ballum have been isolated. (Hartley, 1952; Van der Heeden, 1964). The disease tends to follow a course similar to that in goats.

Equine

Equine leptospirosis is associated with periodic ophthalmia and is quite complex. The typical ocular lesions are believed to be an allergic response to localized foreign antigen (Hanson et al., 1969). Acute equine leptospirosis may be manifested by fever, depression,anorexia, and icterus. L. pomona has been reported as a cause of abortion in mares (Roberts et al., 1952; Jackson et al., 1957). The following serotypes have been isolated from horses: L. canicola, L. icterohemorrhagiae, L. grippotyphosa, L. pomona, L. saxkoebing, L. sejroe (Hanson et al., 1969).

Cat

Leptospirosis is expected to occur in cats because of their intimate association with mice and other rodent potential

carriers of leptospira. Leptospiral antibodies have been demonstrated in feline species (Reilly et al., 1968, 1970). In Denmark serological surveys have revealed serotypes icterohemorrhagiae, bataviae, saxboebing and poi (Van der Hoeden, 1964). Experimentally infection was not established in cats (Fessler, 1964). Serotypes L. canicola and L. pomona have been isolated from cats in Peru and in the U.S.S.R.

Camel

In a survey carried out in Egypt, Brownlow et al., (1963) found one reactor to L. bataviae among 18 camels, and in the Sudan, sera from 55 camels were negative (Shigidi, 1974).

Rodents

Rodents are the established reservoirs of leptospirosis for man and domestic animals. Noguchi (1917) examined 21 house mice (Mus musculus), inoculated their pooled kidney suspensions into three guinea pigs; clinical signs identical to those produced by L. icterohemorrhagiae were observed. Alexander et al. (1960) isolated and identified L. icterohemorrhagiae, L. ballum, L. djatzi from 13 mice in Puerto

Rico. The common field mouse (Apodermus sylvaticus) has been found to carry L. ballum and L. grippotyphosa (Fraya de Azevedo and Palmeiro, 1961; Fuzi et al., 1957).

Of 44 house shrews collected in Malaya and cultured, 2 were positive and L. saxboebing and L. autumnalis were identified (Gordon-Smith et al., 1961a).

The common West African brown field rat (Arvicanthus nilcticus) has been found to be a reservoir for Leptospira sp.. L. icterchemorrhagiae was isolated from kidney cultures from 417 rats (A. nilcticus) trapped in the Nile delta (McGuire and Myers, 1957). Brownlow and Dedeaux (1963) tested sera from 131 rats from this area against 14 antigens and found 13 positive with significant titers to serotypes bataviae, australis, zaoni, grippotyphosa, and canicola.

The Norway rat (Rattus norvegicus) and Alexandrian rat (Rattus aegyptius) are known reservoirs for L. icterohemorrhagiae, L. javanica, L. celledoni, and L. canicola (Inada et al., 1916; Turner et al., 1959). The black rat (Rattus rattus) is a known carrier for L. canicola and L. ictero-

hemorrhagiae (Brown and Gorman, 1960; Roth et al., 1961a; Blanc and Mailloux, 1961). In Capetown, Rademan et al. (1964) isolated seven strains of L. icterohemorrhagiae from 256 rats trapped. In the sahelian zone, the predominant rodent species is the white-tailed rat (Cricetomys gambianus). In a survey of 130 white-tailed rats, L. icterohemorrhagiae was isolated from one kidney (Baylet et al., 1971). They found that 17 (13.1 per cent) had significant antibody titers against L. icterohemorrhagiae, L. canicola, and L. butembo.

Other Animals

Other animals susceptible to leptospiral infection and possible carriers include foxes, jackals (Van der Hoeden, 1958), monkeys (Minette and Shaffer, 1968), opossums, skunks and racoons (Reilly, 1968, 1970), and hedgehogs (Van der Hoeden and Shenberg, 1962; Berg-Petersen, 1962).

Diagnosis

Contrary to general opinion, laboratory diagnosis of leptospirosis can be carried out in a general bacteriology

laboratory as it is not difficult. Serological methods, as well as cultural methods, are easily performed. The "problem" was well summarized by Meyer et al. (1939) who stated five main reasons why diagnosis of leptospirosis was often missed in American laboratories.

1) Textbooks always dealt with classic descriptions of symptoms emphasizing chills and splenomegaly which are rarely found.

2) Most laboratories were not equipped to make the diagnosis.

3) Most physicians considered leptospirosis too rare to include it in their differential diagnosis.

4) Few physicians were aware that only 60 - 66 per cent of cases exhibited jaundice.

5) In the few cases where attempts were made to prove the diagnosis, they failed because of lack of familiarity with the precautions needed to establish the diagnosis in each of the four stages of leptospirosis.

It is a misconception that laboratory diagnosis of leptospirosis requires special materials and expertise. Leptospira can be isolated in serum-free culture medium and hence there is no need for rabbits. A darkfield condenser is the most sophisticated equipment required and leptospira are visible at low magnification.

Microscopic, cultural and serologic procedures are employed for the laboratory diagnosis of leptospirosis.

1. Microscopic

(1) Darkfield Microscopy requires a special condenser. This method is successful in cases containing numerous organisms and is used for examining body fluids, blood and urine. Failure to detect leptospira by this technique does not indicate a negative result and it should be followed by culture and/or serology.

(2) Fluorescent antibody (FA). The FA technique was developed recently for leptospirosis (White and Ristic, 1959) and modifed by Torten et al. (1966). Cook et al. (1971) used the FA technique on scrapings from formalized kidney tissues to demonstrate leptospira. Other

workers (Tripathy, 1973) used the indirect technique to demonstrate leptospira. FA findings should be confirmed by cultural and serologic examination.

(3) Histopathologic Staining. A number of techniques are described (Levaditi and Mangnelian, 1906; Bridges and Luna, 1957; Simpson and White, 1961). Most used either metallic salts or negative staining for demonstrating leptospires. Levaditi's original method was modified by Fontana (Drury and Wallington, 1967) by impregnating the whole tissue in stain before imbedding and sectioning. Fontana's modification is far superior to other stains for demonstrating leptospira.

2. Culture

Various media for isolating leptospira have been described and inculde liquid and semi-solid types (Noguchi, 1917; Fletcher, 1928; Korthof, 1932; Stuart, 1946; Roth et al., 1961b; Ellinghausen and McCullough, 1965a). All are complex media incorporating amino acids and supplemented with rabbit or horse serum. Ellinghausen - McCullough's formula is completely

synthetic medium with fraction V bovine albumin and sodium polysorbate instead of serum. Ellinghausen and McCullough (1965b) determined that lack of vitamin B_{12} is a limiting factor for growth and survival of leptospira.

Contamination with mycoplasma is the most serious problem encountered in leptospiral cultures. Yeasts, fungi as well as other Gram-positive bacilli, may occasionally contaminate and overgrow cultures. Fluorouracil and neomycin sulfate can be incorporated in media at concentrations of 100 - 300 mcg/ml (Ellinghausen, 1973) and 5 - 25mg/l (Myer, 1973), respectively. These substances retard bacterial growth without affecting leptospiral growth. Cycloheximide also helps in eliminating yeasts and fungi when present at a concentration of 0.5mg/l (Cousineau and McKiel, 1961). Animal inoculation is the classic and most widely used method for culture purification.

Selection of suitable specimens for culture is

most important since leptospiremia and leptospiruria occur at different stages. Misleading symptoms such as lung manifestations (Poh and Poh, 1970), jaundice and others (Christie, 1968) complicate and frustrate clinicains attempting to diagnose leptospirosis.

Cerebrospiral fluid and blood cultures have the same rate of success and are positive only during the first week of illness prior to leptospiral localization in the kidneys. Anti-coagulants such as heparin, EDTA (Ethylene diamine tetraacetate) or SPS (Sodium polyethylene sulfonate) may be used, but citrate derivatives should be avoided (Turner, 1968).

Urine cultures are successful only after the leptospiremic phase, usually 10 to 14 days after clinical symptoms develop. Repeated urine cultures are more likely to yield positive results than single cultures. Moreover, carriorship varies according to animal species from three months to a lifetime. As with blood cultures, dilution of 1/10 minimizes some of the inhibitory properties of urine.

Tissues for culture obtained at necropsy include liver, spleen, brain and more specifically, kidneys.

3. Identifying Leptospira Isolates

Identifying positive cultures can be done a number of ways (Galton et al., 1962; Turner, 1967; Alston and Broom, 1958). The unknown isolate is grown in liquid culture and tested against a battery of hyperimmune sera prepared against standard leptospiral strains and absorbed. Accordingly, an unknown serotype will be considered as a subserotype within a serotype if after repeated tests, less than 10 per cent of homologous titer remains in one antiserum and 10 per cent or more in the other antisera after cross-absorption with adequate amounts of heterologous antigen (Galton et al., 1962).

Absorption agglutination tests for serological identification of leptospira are fastidious and requires adherence to strict technical precautions. Four factors are known to influence the final results of agglutination absorption tests (Kmety and Galton, 1970):

(a) Time which should be at least 90 minutes

(b) Ratio of antigen to antibody (the titer is inversely proportional to the amount of antigen added i.e. the more antigen present, the lower the titer).

(c) Danysz phenomenon occurs and can be avoided by adding antigen to serum in three equal parts at 10 minute intervals.

(d) Formalin-treated cells yield lower titers than live antigen.

The use of carbon agglutination tests for rapidly identifying leptospira strains was investigated by Pao and Guc, (1974). They used 522 local strains and carbon particles coated with antibodies directed against 14 Chinese national standard strains and found complete agreement with the standard agglutination test. The method was faster since it did not rely on cell lysis. Moreover, only when positive reactions 1+ or more were observed did they resort to the standard agglutination test.

Pyrolysis-gas liquid chromatography (PGLC) has also been found effective in differentiating common strains of leptospira (Reiner et al., 1973). By using 16 coded samples of leptospira the results correlated positively with serological classification by the agglutination absorption test. A further examination of 12 authenticated strains by the authors revealed that each had its own individual profile peak ("finger-print").

The results of identification using PGLC are not complete but are promising. A practical disadvantage is that the procedure would not be available to all laboratories. Schricker et al. (1963a,b) demonstrated that leptospira serotypes contain two types of surface precipitinogens: fraction A and S. Fraction A is heat-stable, alcohol-soluble, and contains 20 per cent carbohydrate and little protein and amino acids. This fraction produces lines common to different serotypes indicating perhaps a genus specific antigen. The second antigen (S fraction) contains serotype specific precipitinogen and is heat-labile. Sinagawa and

Yanagawa (1972) isolated and characterized this specific antigen for L. kremastos and called it type specific main antigen (TM antigen). Results of their analyses indicated that it consists of 68 per cent carbohydrate, 23 per cent lipid, 5 per cent protein and 2.8 per cent phosphorus. In addition, 13 amino acids were identified. It proved to be similar to Gram-negative endotoxin except that during the phenol extraction procedure it remained in the phenol phase whereas endotoxin is in the water phase. Neutral sugars contained in this antigen were identified as galactose, xylose, arabinose, rhamnose and glucosamine. A comparison of the general pattern of infrared absorption of the TM antigen also demonstrated similarity with Gram-negative endotoxin.

Kasai and Yanagawa (1974) found that the glucosamine content in TM hydrolysate correlated with the degree of inhibition of the complement fixation test using L. canicola. They also found that glucosamine alone did not inhibit the test. This suggested that proteins may be important as antigenic determinants

in the TM antigen of leptospira (Adachi and Yanagawa, 1975).

Analysis of antigenic determinants will in future prove more useful for identifying and classifying leptospira. The general trend of investigations have indicated that serotype specific antigen of leptospira is essentially a lipoplysaccharide (Auran et al., 1972; Chang et al., 1974; Faine et al., 1974a; Schricker et al., 1963a,b; Shinagawa and Yanagawa, 1972). It is known that the sheath is the agglutinating element (Yanagawa and Faine, 1966).

The findings of identification studies using immunodiffusion have clearly indicated type specific reactions and weak cross reactions, which occur more frequently in immunodiffusion than agglutination tests. Genus specific reactions have also been observed (Yanagawa et al., 1974).

The data available to date confirms what is known by using classical serological methods such as the complement fixation test, erythrocyte lysis test, or

agglutination tests.

4. Serology

Serological diagnosis relies on different procedures which are either genus specific or serotype specific and uses four kinds of genus specific tests: sensitized erythrocyte lysis test (SEL), complement fixation test (CFT), indirect immunofluorescent test, and agglutination tests (MSA and MA). The first three tests are positive earlier in the course of leptospirosis and also revert to being negative earlier.

The SEL test uses erythrocyte sensitizing extracts of leptospira which are genus specific (Chang and McComb, 1954; Cox et al., 1958). Antibodies detected by this substance are actually different from agglutinins and complement fixing antibodies. Even though it is a good screening test, it has the disadvantage that all sera must be absorbed free of heterophile antibodies.

The CF test is also broad and not routinely used.

Serotypes of the Semaranga group are used (Sturdza et al., 1960). Seroreactors of this group will usually react at high titers with pathogenic serotypes. Complement fixing antibodies usually indicate clinical disease or recent infection.

The indirect hemagglutination technique (Sulzer et al., 1975) uses soluble alcohol extracts of Adamana strain absorbed to group O erythrocytes. It is more sensitive and more specific than the standard slide agglutination test (SAT). It correlates well with the SAT during clinical illness and convalescence up to 12 months.

The microscopic agglutination technique and modifications are the standard tests in common use (Sulzer and Jones, 1973; Sulzer et al., 1973; Galton et al., 1962; Galton and Health, 1965). The test is performed by diluting serum and adding fixed amounts of actively growing culture and reading 50 per cent agglutination as the endpoint of titration.

Agglutination tests can be described as being

more serotype specific as the reaction is specific at higher serum dilutions when heterologous non-specific agglutinins have been diluted out. Agglutination tests may be either macroscopic or microscopic, the difference being one of sensitivity. The microscopic agglutination (MA) is more sensitive and will detect more cross reactions, whereas the macroscopic slide agglutination (MSA) test is useful only with active cases of leptospirosis.

Immunofluorescence tests have been described (Shandu and White, 1972). They are generally called genus specific but the problem is that each serotype would have to have its own antiserum. Indirect immunofluorescence tests are used most often for diagnosis leptospira in tissues.

Interpretation of serological data for diagnosing leptospirosis is straight forward in cases of repeated negative (or absence of) titer and in positive sera with a rise in titer. In the former, the diagnosis of leptospirosis is ruled out whereas in the latter,

the presence of the disease is confirmed.

Positive reactors can result from one of three ways:

(a) Passive immunity (administration of immune serum to an individual);

(b) Past infection (resulting in active immunity); and

(c) Presence of clinical disease.

Genus specific tests are known to fail to detect leptospirosis in recently infected cattle and rabbits (Ris, 1975).

Alston and Broom (1958) recommended a titer of 1/300 as diagnostic for man. These authors observed that no significant titer was detectable before the 6th day of illness. Out of 169 patients, titers of 1/300 were observed in seven per cent after one week and 1/30,000 in only four per cent after the same period. After recovery, titers generally fluctuate between 1/100 and 1/300 for many years. Marius (1964) argued that meaningful results are

obtained only when there was a four-fold rise in antibody titer in paired sera.

After demonstrating infection by the CFT in field investigations, Stramkova et al. (1972) followed the dynamics of antibody formation in 82 patients with leptospirosis. They found a rise in titer during the first three weeks and a maximum antibody titer between the 2nd and 4th month. Titers greater than 1/100 remained for two to three years after recovery.

Other workers including Turner (1968) have observed that titers of 1/30 in which 75 - 100 per cent lysis occurred in suspensions using serotypes endemic to particular regions. They suggested that any titer be regarded as positive and that the onus is on those who regard such reactions as "non-specific" to demonstrate alternative causes. There is general agreement, however, that repeated examination of serum for leptospiral titer is most definite.

Reaction of sera with two or more serotypes is frequently observed in MA tests. This could indicate multiple infection or crossreacting antigens in different serotypes (Wiesmann and Suter, 1952). Fuhner (1950) noted in the early stages of leptospirosis that the titer of non-specific antibodies may be higher than specific ones and referred to this phenomenon as the "paradoxical reaction" in Weil's disease. This observation emphasizes that the particular stage of the disease must be considered when interpreting serological results and the need for paired serum samples (or repeated serological examination) for a correct diagnosis of leptospirosis.

Immunity

It has long been known that laboratory animals recovered from leptospirosis were immune to challenge with homologous strains and simultaneously to certain heterologous strains (Inada, 1925; Fletcher, 1928; Ido et al., 1916; Larson, 1945).

Active immunity against leptospirosis involves

formation of antibodies against both structural components of the cell and serological factors elaborated by Leptospira sp..

On the basis of known antigenic structure, two studies are worth noting. Auran et al. (1972) induced spherical forms of L. canicola, treated these salt-altered cells with 0.02 per cent sodium lauryl sulfate to solubilize the sheath, and then injected hamsters with the different fractions. All fractions, as well as formalized cells, protected the hamsters against subsequent infection with the homologous serotype. Takashima and Yanagawa, (1975) used the outer envelope, cell wall, type specific TM antigen (Shinagawa et al., 1972), protein constituents of the outer envelope, lyophilized live cells as well as lyophilized heat-killed cells to immunize guinea pigs. These workers found that the outer envelope was protective and that one out of four animals receiving TM antigen was protected. The protein constituent of the sheath failed to immunize. The protective dose of heat-killed lyophilized cells injected was much higher than that of lyophilized live cells.

The inference that can be drawn from these two experiments is that the outer envelope of the cell plays an important role in immunity against leptospirosis. Since two out of four animals (50%) receiving TH antigen survived, a higher dose with adjuvant would probably provide better protection. The fact that heat-killed lyophilized cells gave less protection than live lyophilized cells is significant.

Leptospiral lipolysaccharide IgM antibodies produce peak titers in three weeks in guinea pigs, rabbits, and mice. These antibodies were not only leptospirocidal *in vitro*, but they conferred resistance to development of a carrier state in mice (Faine et al., 1974b).

Vaccine prophylaxis trials in man and experimental animals have shown that they can be rendered immune from infection with virulent strains of *L. icterohemorrhagiae* (Smith, 1937). Vaccination did not prevent development of carriers or shedders (Feigin et al., 1973); Shenberg and Torten, 1973). These observations suggest the possibility that local immunity may operate in the carrier state. Passive immunity is not operative via maternal antibodies.

Fennestad and Borg-Petersen (1962) followed antibody formation in bovine fetuses and found that 132 and 168 days-old fetuses had antibodies and plasma cells, were leptospiremic, and had evidence of nephritis.

Kemenes (1964) found that cross immunity existed among L. icterohemorrhagiae, L. canicola, L. pomona and to a lesser extent, L. grippotyphosa. None of these serotypes exhibited cross immunity with L. hyos and L. sejroe. Solid type cross immunity could only be established by infection with virulent strains. These observations prompted speculation that leptospiral immunity is directed against the biological activities of leptospira rather than speicific antigens. The cross immunity findings are consistent with classifications using genetic techniques (i.e. strains with the same G+C content demonstrated cross immunity). This postulate is not new in the sense that Alexander et al. (1956) found cross protection with a hematoxin active against mammalian erythrocytes in serotypes pomona, canicola, australis and grippotyphosa. Note the presence of hematoxin does not necessarily imply virulence even though it contributes to pathogenicity.

Natural immunity against leptospirosis occurs. It may be due to diference in host species. This could explain the predilection of L. icterohemorrhagiae for the rat and L. canicola for dogs. Also leptospiral antibodies against L. biflexa occur naturally in mammals (Johnson and Gary, 1963). In lower vertebrates such antibodies have been found to be bactericidal to serotypes canicola, arborea, ballum, pomona and grippotyphosa (Charon et al., 1975).

Cell-mediated immunity may be important in the development or prevention of the renal carrier state and other persistent forms of infection (Blenden et al., 1961; Alexander et al., 1971).

Treatment

Experimental work has shown broad spectrum antibiotics to be effective for treating leptospirosis (Kemp, 1964; Kocen, 1962; Burner and Meyer, 1950; Larson, 1945).

In canine species, a combination of penicillin and streptomycin is accepted as best. Supportive therapy of high quality protein diet and supplementation with B-complex

vitamins is essential.

In food animals treatment with tetracycline and streptomycin is effective. Inclusion of these drugs in the feed has helped eliminate carriers in infected herds (Blood and Henderson, 1968).

Recovered carrier dogs are primarily responsible for maintaining serotype canicola in the population. Similarly, cattle play a major role in maintaining serotypes hardjo and pomona. Serotype pomona, however, is found in wildlife as well as pigs hence its mechanism of continuity is more complex.

In man, treatment with penicillin, streptomycin, tetracycline, chloramphenicol, erythromycin and oleandomycin are all effective even though data on their efficacy is conflicting (Wintrobe et al., 1974). There is general agreement that antibiotics must be administered within four and preferably two days of onset of illness to be effective; later periods correspond to more advanced stages of the disease with renal tubular degeneration and other associated changes.

Prophylaxis

Vaccination of livestock and all potentially exposed individuals has become accepted as a better long term prophylactic measure against leptospiral infections. Elimination of rodents and other potential carrier species is impracticable in view of the complexities of the ecological relationships and cost involved. Accordingly, vaccination against prevalent virulent serotypes is already practised in most countries. Most vaccines contain killed organisms and have an immunizing potency acceptable for one year.

Trivalent bacterins of L. pomona, L. hardjo, and L. grippotyphosa are available and have provided good relative protection against renal leptospirosis, in the United States (Hanson, 1972, 1973; Hanson et al., 1972).

York and Bruckner (1953) developed an egg-propagated vaccine attenuated by freezing and thawing. This bacterin induced protection in calves and in guinea pigs without stimulating appreciable levels of antibodies. A vaccine inactivated by thimerozal and by freezing and thawing forti-

fied with adjuvant also failed to induce sufficient titers in calves (Brown et al., 1955).

Similarly, a heat-extracted bacterin which prevented leptospiremia, leptospiruria and kidney lesions in challenged animals, induced only limited antibody response (Hoag and Bell, 1955). Many other reports indicate that relative protection and antibody response do not necessarily correlate (Babudieri, 1959; Gillepsie and Kenzy, 1958; Killinger et al., 1970). Moreover, it has bee found that relatively low titers of agglutinins, 1/10 to 1/100, indicated a good level of protection against both experimental and natural infections (Plesko et al., 1967).

We now know that difficulties other than those associated with immunogenicity exist. The preferred route of administration of vaccines has been found to be intramuscular (Slinko, 1974). Differences in temperature of inactivation and of the growth medium do not markedly affect immunogenicity (Painter and Ellinghausen, 1976). The development of leptospiruria appears to be the hardest to control.

The lack of easily measurable clinical signs in cattle

and the fact that certain serotypes such as hardjo do not consistently kill hamsters has been emphasized by Hansen (1973). The hamster protection test is regarded as the most reliable procedure for measuring immune response of cattle. Kemenes (1964) emphasized previously that serotype icterohemorrhagiae was highly virulent to guinea pigs and it presented a problem when evaluating vaccines. The in vitro growth inhibition test (Tripathy et al., 1971) and indirect hemagglutination test (Sulzer et al., 1975) provide alternative techniques to measure the immune response of vaccinated animals.

A vaccine for use in man has been developed (Shenberg and Torten, 1973). It is grown in a chemically-defined medium and has proved to be highly immunogenic. It could induce full protection in animals challenged with 10,000 LD_{50} of virulent Leptospira. A trial of this vaccine in Israel found a low level of antibody response, which parallels the response obtained in animals.

PART II

SEROLOGIC SURVEY OF NORTHERN NIGERIA FOR LEPTOSPIROSIS: 1. LIVESTOCK

INTRODUCTION

Leptospirosis is a worldwide disease of man and animals and is a major bacterial zoonosis. The principal animals involved are rodents, dogs, cattle and swine and they all act as reservoirs of infection (Alston and Broom, 1958; Turner, 1967; Schwabe, 1969). Sheep, goats, camels, horses, cats and wild animals are also infected. In animals, the incidence of subclinical infection is higher than clinical disease (Alston and Broom, 1958; Turner, 1967). After recovery animals become shedders of leptospira for variable periods: cattle three and half months (Gillespie et al., 1957), horses three months and sheep nine months (Webster and Reynolds, 1955), pigs eleven months (Sleight and Lundberg, 1961) and dogs up to four years (McIntyre et al., 1953).

Livestock play an important role in the epidemiology of human leptospirosis. Infection is transmitted by direct or indirect contact with infected urine. Cutaneous, inhalation, conjunctival, oral, venereal and transplacental transmission have been reported (Baker and Little, 1948; Van der Hoeden,

1955; Roberts, 1958; Sleight and Williams, 1961; Fennestad and Borg-Petersen, 1962; Sadler, 1963; Doherty, 1967; Hanson and Brodie, 1968). Milk-borne infection in man has been suspected (Schwabe, 1969).

Knowledge of the geographical distribution of leptospirosis is incomplete (Turner, 1967; Szatalowicz et al., 1969; Sulzer, 1975). Reports of leptospiral surveys in livestock in Africa are limited: Morocco (Blanc et al., 1961), Egypt (Brownlow and Dedeaux, 1963), Ethiopia (Moch et al., 1975), Sudan (Shigidi, 1974), Tunisia (Durand and Lequerie, 1962), Zaire (Van Riel and Van Riel, 1954), and Senegal (Van Riel et al., 1971). Serotypes deteced were Leptospira canicola, L. icterohemorrhagiae, L. ballum, L. tarassovi, L. hyos, L. bataviae, L. hebdomadis, L. pomona, L. grippotyphosa, L. australis and L. pyrogenes.

In Nigeria, one livestock survey for leptospirosis was carried out in 1975 by a student team from the Royal Veterinary College, London. The team tested 955 cattle from Kaduna, Kano, Gongola and Sokoto states and found a 48 per cent prevalence rate. Of the cattle tested from semi-nomadic

and permanent herds and abattoirs, 63.9 per cent, 36.8 per cent, and 16.4 per cent, respectively, were positive for leptospirosis (Turner et al., 1975).

Leptospirosis in cattle is characterized by pyrexia, hemoglobinuria, icterus, mastitis, nephritis and abortion (Blood and Henderson, 1968). The important seretypes are L. pomona, L. hardjo and L. sejroe (Sutherland et al., 1949; Steele et al., 1957; Alexander and Evans, 1962; Sulzer, 1967). L. icterohemorrhagiae and L. grippotyphosa have been isolated from cattle (Field and Sellers, 1950; Varfoloomoeva, 1964; Ellis et al., 1976). An outbreak of leptospirosis in cattle in Israel due to L. canicola was traced to dogs (Van der Hoeden, 1955).

L. pomona, L. grippotyphosa and L. hardjo have been reported in sheep and goats (Van der Hoeden, 1964; Ris, 1975) and also L. ballum in goats (Van Riel and Van Riel, 1956).

The study reported here was undertaken to determine the presence and incidence of leptospirosis in livestock in eight northern states of Nigeria.

MATERIALS AND METHODS

Survey Area

The area surveyed was limited to the northern part of Nigeria above the Benue and Niger rivers (Appendix 1) consisting of the states of Bauchi, Borno, Gongola, Kaduna, Kano, Niger, Plateau, and Sokoto. Collection centers were selected from maps of cattle trade routes available at the Ministry of Agriculture and Natural Resources, Kaduna. Major points on the trade routes were selected to insure a greater number of cattle from many areas grazing in the vicinity at the time of sampling.

Survey Organisation

Letters were sent to the Ministries of Agriculture and Natural Resources in each state informing them of the proposed survey and requesting their cooperation. Later, a second letter was sent giving specific details of the number of herds and sera to be collected and the date and time of testing. Because of mail delays, ample time was allowed between notification and testing. As a safeguard to insure local cooperation, copies of all pertinent correspondence was

carried to each collection center.

A Toyota van with a freezer, generator, bench centrifuge,[1] sterile disposable syringes and needles, and sterile MacCartney and bijoux bottles, was used for the survey.

Animal Species

Blood was collected from cattle, sheep, goats, donkeys, horses, camels, and pigs. The predominant cattle breeds in the states surveyed were: Niger and Sokoto -- Sokoto Gudale and Bunaji (White Fulani); Bauchi, Borno, Gongola and Plateau -- Bunaji, Adamawa Gudale and Rahaji; Kaduna -- Bunaji; and Kano -- Rahaji and Bunaji, and Kadawa farm -- Berkshire, South Devon and Chianina (Bida, 1974). The predominant sheep and goat breeds were Uda and Yankasa, and Kano brown and Sokoto red, respectively.

Serum Samples

Cattle: As it was not practical to collect blood samples from the cattle during the rainy season (June to October),

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1 Gallenkamp Ltd., Chadwell Heath, Essex, England.

samples were taken during the dry and pre-rain seasons (January to May, 1975 and December, 1975 to June, 1976). The collection procedure was to proceed from north to south to increase the numbers of semi-nomadic and settled Fulbe herds sampled. Blood was taken only from semi-nomadic to nomadic herds or settled herds with traditional management practices except for Kadawa farm in Kano and Talata mafara in Sokoto.

A minimum of five herds were sampled whenever possible. The number of blood samples per herd depended upon herd size and owner cooperation; the minimum number collected being: 10 - 4; 10 to 20 -- 5; 21 to 30 -- 6; 31 to 40 -- 8; 40 - 10. Where there was no cooperation, blood was obtained postmortem at the local slaughter establishment or by antemortem bleeding of slaughter cattle.

Sheep and Goats: Blood was collected from sheep and goats in cattle herds and from villages, and from slaughter establishments.

Horse and Donkey: Blood samples were taken from all available horses and donkeys in the collection centers.

Camel: Blood samples from camels were collected at Kano and Sokoto following slaughter.

Handling and Storage of Sera: Blood (5 - 10 ml) was collected with a disposable syringe[2] and a 21 gauge needle via the jugular vein, transferred to a sterile, labelled MacCartney bottle, and allowed to clot. After clotting, serum was separated by centrifugation at 1,500 rpm for 10 minutes. Each serum was decanted into a sterile, labelled bijoux bottle, 0.3 ml glycerol added as a bacteriostat, transported to the microbiology laboratory at Ahmadu Bello University, and stored at -20°C until tested.

Leptospira Strains

The following nine leptospiral serotypes[3] were used in the survey.

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2 Terumo Corporation, Tokoyo, Japan.

3 Kindly supplied by Dr. L. G. Turner WHO/FAO Leptospirosis Reference Laboratory, Colindale, London and Dr. H. Dikken, WHO Leptospirosis Laboratory, Royal Tropical Institute, Amsterdam.

Serotype	Strain	Passage	Source
L. grippotyphosa	Adamans CH 31	P.1227	Colindale
L. hebdomadis	Hebdomadis	P.1147	"
L. canicola	Hond Utrecht IV	P.1475	"
L. biflexa	Patoc 1		"
L. pomona	Pomona	P.1169	"
L. icterohemorrhagiae	Ictero. I.	P.209	Amsterdam
L. ~~hardjo~~ pyrogenes	Salinem	P.1262	"
L. tarassovi	Perdipelicin	~~P.1262~~ 161	"
L. hardjo	Hardjoprajitno	P.700	

The serotypes were maintained in semi-solid Ellinghausen-McCullough medium and transferred every three months. After initial incubation and growth, the cultures were stored in the dark in a cupboard at room temperature (25 to 27°C).

E.M. Medium

The sodium polysorbate 80 bovine albumen medium of Ellinghausen and McCullough (EM) was prepared according to Turner (1970). To bovine albumen (fraction V)[4], 5-~~flucuracil~~ fluorouracil (5Fu)[5] was added, pH adjusted to 7.2 ± 0.1, filtered through

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4 Sigma Chemical Company, St. Louis, Missouri, USA.

5 Ibid

a 0.22 micron filter, and stored until added to the filter-sterilized basal medium. The final concentration of 5Fu in the medium was 400 mcg/ml.

Leptospiral Antigens

Five to seven day live cultures of Leptospira serotypes in EM media were centrifuged at 1,500 rpm for 10 minutes in a bench top centrifuge[6] to remove extraneous debris. The supernatant was diluted with phosphate buffered sterile saline (PBS) and standardized to 150 - 200 leptospira per high dry field with a darkfield microscope[7] (Galton et al., 1962).

Microscopic Agglutination Test

All sera were screened at 1:20 dilution and discarded if negative. Positive sera were then titered at doubling dilutions from 1 : 100 to 1 : 1600.

The microscopic agglutination test (MA) of Galton et al.

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6 Baird and Tatlock (London) Ltd., Chadwell Health, Essex, England.

7 Olympus Optical Co., Tokoyo, Japan.

(1967)[2] was carried out with Cole et al. (1973) modification to handle lesser volumes using live antigens. The tests were carried in plastic hemagglutination plates[8] with ten vertical and eight horizontal rows. Each hole had a capacity of 0.5 ml. Eight samples were screened against nine leptospiral antigens per plate.

One-tenth ml of diluted serum was transferred to the vertical rows and 0.1 ml of antigen added to each of the eight horizontal rows by means of a 2 ml pipette[9] calibrated to deliver 0.1 ml in 2 drops. Each plate was carefully shaken to insure thorough mixing, covered with aluminum foil and stacked. The stack was covered with a moist towel and incubated at 28 - 30°C for three hours before examination.

The tests were read by transferring a drop of the mixture to a slide and examining for agglutination or "clumping" with a darkfield microscope at low power; 50 per cent or more clumping with definite "translucent mothballs" was considered

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8 Baird and Tatlock (London) Ltd., Chadwell Heath, Essex, England.

9 Mescipharm Supply Company, Zaria, Nigeria.

positive.

Controls of 0.1 ml PBS and 0.1 ml antigen were set up with each batch of tests.

After examination, the plates were immersed in 2.5 per cent phenol for 30 minutes, rinsed overnight in running water, thoroughly washed in hot water and soap, rinsed with distilled water and allowed to dry before re-use.

Terms

The following criteria of Schwabe (1969) for incidence and prevalence were used in this study.

Incidence rate - Dynamic measure of the number of new cases of leptospirosis occurring in a given population in a stated period of time.

Prevalence rate - Static measure of the total number of affected individuals in a population at a given time.

RESULTS

A total of 2,852 sera was tested: 1,153 cattle, 568

sheep, 448 goats, 395 donkeys, 146 horses, and 142 camels (Table 1). Cattle sera represented 40.4 per cent of the total livestock samples tested.

Incidence rates of leptospirosis in livestock in the eight states are given in Table 2 and Fig. 1; the overall incidence was 7.4 per cent. State incidence in decreasing order was: Niger, Kaduna, Kano, Borno, Plateau, Gongola, Sokoto, and Bauchi. Leptospiral serotypes varied between states, the nine serotypes used for testing were found only in Kaduna state (Table 3).

Bauchi state had the lowest incidence rate (3%) and the predominant serotypes were L. icterohemorrhagiae, L. hebdomadis, and L. pomona. Cattle, sheep, horses and donkeys were involved and 50 per cent of the reactors had titers of 1/200 or more (Appendix 3, Tables, 1, 6, 8).

In Borno state, only cattle and horses had leptospiral antibodies. L. hebdomadis, L. pomona, L. hardjo and L. icterohemorrhagiae were the main serotypes and 60 per cent of the reactors had titers of 1/100 or more (Appendix 3, Tables 1, 8). Five of the positive sera had coagglutination and the other

five reacted specifically with only one serotype.

In Gongola state, only cattle and horses had leptospiral titers (Appendix 3; Tables 1, 8). Cattle seroreactors had titers of 1/100 or more to L. pomona and two L. tarassovi.

Kaduna state was the only state with leptospiral reactors in all livestock species except camels (Table 2). Although horses had a 32 per cent incidence, it was confined to L. pyrogenes, L. canicola and L. icterohemorrhagiae (Appendix 3, Table 8). Donkeys, however, reacted to L. grippotyphosa, L. icterohemorrhagiae, L. hardjo, L. hebdomadis, L. pyrogenes and L. australis. Incidence rates in sheep and goats were low and L. pyrogenes predominated (Appendix 3, Tables 6 - 7).

Kano state had the highest incidence of leptospirosis in cattle (Table 2). L. hebdomadis, L. hardjo and L. pomona predominated and titers of 1/100 were recorded (Appendix 3, Table 5). Horse sera reacted against L. icterohemorrhagiae, L. canicola and L. pyrogenes and donkey sera mainly against L. pomona and L. pyrogenes (Appendix 3, Tables 9 - 10).

Niger state had the highest incidence rate (13.0%) with all serotypes employed being detected except L. australis (Table 5; Appendix 3, Tables 4, 6, 9). The main equine serotypes were L. pyrogenes and L. hardjo.

All serotypes except L. australis were detected in cattle, sheep and horses in Plateau state (Tables 2, 5, 11). The main serotypes were L. pomona, L. tarassovi, L. hebdomadis, L. canicola, and L. pyrogenes (Appendix 3, Tables 3, 6, 9).

In Sokoto state, seroreactors were confined to cattle, horses and donkeys (Table 2). The predominant bovine serotype was L. pomona with titers up to 1/1600 (Table 5; Appendix 3, Table 2). The main equine serotypes were L. hardjo and L. pyrogenes (Appendix 3, Table 9).

Analysis of the results of animal species revealed the following:

1. Cattle

Eleven hundred and fifty-three sera were collected from 131 herds containing a total of 6,288 cattle (Table 4). More herds were tested in Kaduna and Kano states and both had more

positive herds. In Borno state with a 27 per cent, only one herd was sampled. Fifty-six per cent of the sera collected at abattoirs had leptospiral antibodies. The incidence rate for the eight states varied from 2.3 to 27.0 per cent with an overall incidence of 11.9 per cent for bovine leptospirosis (Fig. 2). Distribution and mean titer of the leptospiral serotypes in the eight states are summarized in Table 6 and Fig. 3.

All nine leptospiral serotypes were found in cattle in Kaduna state (Table 5). Serotypes hebdomadis, pomona, hardjo, and icterohemorrhagiae were prevalent in all eight northern states except there were no seroreactors to L. icterohemorrhagiae in Gongola or to L. hardjo in Plateau (Table 5). Reactors to L. canicola were found in Bauchi, Kaduna, Plateau and Niger states. L. australis was found in Kaduna state where L. tarassovi was also prevalent (Tables 5-6).

2. Sheep

Only 1.8 per cent of 568 sera collected were positive for leptospirosis (Table 7; Fig. 4). The seroreactors were found in Bauchi, Kaduna, Niger and Plateau states. The

predominant serotypes were L. pyrogenes and L. grippotyphosa (Table 8; Fig. 3).

3. Goats

Only 1.1 per cent of 448 goat sera were positive for leptospirosis and the reactors were detected in Kaduna and Kano states (Table 9; Fig. 4). The seroreactions were limited to L. pyrogenes, L. icterohemorrhagiae and L. canicola (Table 10; Fig. 3).

4. Donkeys

The incidence in the five states positive for leptospirosis varied from 2.8 to 11.1 per cent with an overall incidence of 6.1 per cent (Table 11; Fig. 5). The incidence rate was highest in Niger and Kaduna states.

All nine serotypes were detected in donkeys (Table 12; Fig. 6). High titers were found against L. hebdomadis, L. pomona, L. pyrogenes, and L. australis. The predominant serotypes were L. icterohemorrhagiae and L. pyrogenes.

5. Horses

Horses had the highest incidence rate (24.7%) for leptospirosis for the species surveyed in the eight states (Table 11). All nine serotypes were found in horses (Table 13; Fig. 6); the predominant being L. icterohemorrhagiae and L. pyrogenes.

6. Camels

There were no leptospiral reactors in the 142 samples taken from camels slaughtered in Kano and Sokoto states.

7. Age and Sex

The majority of animals were mature to aged and female (Appendix 2, Tables 1 - 11).

DISCUSSION

This study established the existence of leptospirosis in livestock in Bauchi, Borno, Niger and Plateau states of Nigeria, and confirmed it in Kaduna, Kano, Gongola and Sokoto states.

In interpreting results the 50 per cent agglutination of

leptospira organisms at 1/20 dilution was considered significant. There has been controversy over what is significant in leptospiral serology. Turner (1967) considered 75 - 100 per cent agglutination at 1/10 dilution significant since serological reactions at that level could be regarded as being indicative of serogroups occurring in the particular regions where the test was carried out. He further challenged those who regard such reactions as non-specific to demonstrate alternative causes.

Observations of sex and age difference were of little value as female animals were more numerous and more readily available for sampling than males, and as almost all were adult to aged.

Cattle were the largest group in the survey. This was because of the importance of the cattle industry in the northern states and the fact that the survey was organized around main centers of the cattle trade routes so that cattle under traditional methods of husbandry could be sampled. Fulbe cattle owners were reluctant to have their cattle bled and this kept the number sampled from each herd to a minimum.

An interesting observation on the incidence of bovine leptospirosis is that Borno and Kano had the highest rates. They also happen to have the most cattle of the eight states surveyed. Plateau and Niger were the next highest in incidence, yet they have the least number of cattle. It can be inferred that two factors are operating here: population density and the geoclimatic factor (Gordon-Smith et al., 1961). Niger and Plateau states are located in the southern Guinea savanna whereas states like Kano and Sokoto are in the dry Sudan savanna area (Appendix 1 maps 2,3). The leptospirosis incidence in cattle in these four states and Kaduna are above the total incidence rate (7.4%).

Kinebuchi and Afoakwa (1973) observed in Ghana that there was more variety of serotypes and more infection in man with leptospira in the forest area than in the savanna. In this survey the incidence rate for livestock recorded for Kaduna state seem to follow this pattern even though it is a different savanna subregion. Kaduna state extends from the Sudan savanna in the north to the souther Guinea savanna zone. There were more seroreactions in more species in the moist Southern area of Kafanchan than in Katsina in the far dry

North (Appendix 1).

In the state distribution of serotypes in cattle, all nine leptospira serotypes occurred in Kaduna and eight in Niger. These findings support observations by Twigg et al, (1974) that leptospira infection rates of dairy cattle in Britain were highest in marshy rat-infected land. Also connected with this was the practice of transferring cattle from one area to another.

In early cattle surveys in the U.S.A., reactions to L. autumnalis, L. ballum, L. grippotyphosa, L. hardjo and L. icterohemorrhagiae were found (Galton et al., 1956). As there were no isolation of these serotypes in cattle, it was concluded that the presence of these agglutinins was probably due to cross reactions (Alexander and Evans, 1962). The isolation of L. pomona from cattle with serologic reactions to other serotypes later helped reinforce this concept (Hanson, 1973).

In this survey serotypes pomona and hebdomadis predominated with 3.9 and 3.3 per cent incidence, respectively. These were actually 24 and 22.5 per cent of all positive sero-

reactions in cattle. Interestingly enough neither L. pomona nor L. hebdomadis have been isolated but L. pyrogenes has been from kidneys of slaughtered cattle (Part VI). Three unidentified isolates reacting to L. australis, L. ballum, L. canicola and L. icterohemorrhagiae have been found. On the basis of titers, it is unlikely that either pomona or hebdomadis seroreactions are due to cross reactions. Further studies should lead to isolation of these two serotypes from Nigerian cattle. As in other African surveys of the same latitude, serotypes grippotyphosa, hebdomadis, icterohemorrhagiae, pomona and tarassovi were found (Van Riel et al., 1971; Shigidi, 1974; Moch et al., 1975).

The incidence of leptospirosis in sheep did not follow the same pattern as in cattle except for one general observation. In Kaduna state all positive seroreactors were found in the Kafanchan area in the moist southern Guinea savanna. There was evidence for the presence of six serotypes (grippotyphosa, hebdomadis, pomona, pyrogenes, tarassovi and australis). In the survey of livestock in Senegal, Van Riel et al. (1971) also found six serotypes. Shigidi (1974) in Sudan found only three serotypes but he used less serotypes in his screening

battery. In addition, he used commercial antigen which we found unsuitable for this purpose. The serotypes found in northern Nigeria are found also in Sudan.

There is no doubt that goats can transmit leptospirosis. In view of the extremely low percentages obtained in most surveys involving goats, its role in the epidemiology of leptospriosis is questionable. In northern Nigeria goat rearing is more important only in the more arid states in the sudan and sahelian savanna zones (Borno, Sokoto, Kano, and Northern part of Kaduna). It is interesting to observe the positive reactor in Kafanchan (Kaduna state) reacted at 1/1600 to L. pyrogenes, which is the prevalent serotype occurring in siwne in the area (Part V). L. pyrogenes has also been isolated from a bovine kidney from Kaduna state. The reactions to icterohemorrhagiae and canicola serotypes indicates that infection was contacted from dogs or rats in view of the browsing habits of goats.

Horses appeared to be more affected than donkeys. Serotypes icterohemorrhagiae, and pyrogenes were most prevalent although serological reactions to other serotypes were detected. The relative high incidence is no indication of specific predisposition to infection. Among positive reactors in both

donkeys and horses there were approximately the same percentages for the different serotypes. The smaller sample of horses tended to inflate the incidence rate.

The lack of positive camel sera indicates that there is little likelihood of camels playing a role in the epidemiology of human leptospirosis in northern Nigeria. Camels are found in the northern Sudan savanna. They are brought from the Republic of Niger and slaughtered for meat or used for transporting goods back.

In conclusion, the data presented indicates that cattle and horses have a high incidence of leptospirosis and that they may be more important carriers in the epidemiology of human leptospirosis in northern Nigeria. The high incidence of L. pomona and L. hebdomadis serogroup antibodies suggests that a systematic study will lead to isolation of these serotypes.

SUMMARY

A total of 2,852 sera was tested for leptospiral antibodies in eight northern states of Nigeria by the microscopic

agglutination test: 1,153 cattle, 568 sheep, 448 goats, 395 donkeys, 146 horses, and 142 camels. Nine serotypes were used: grippotyphosa, icterohemorrhagiae, canicola, hebdomadis, pomona, hardjo, pyrogenes, tarassovi, and australis. The overall incidence for leptospirosis was 7.4 per cent. The incidence rates for the eight states in decreasing order were Niger (13.0%), Kaduna (8.7%), Kano (8.0%), Borno (7.3%), Plateau (6.4%), Gongola (5.7%), Sokoto (4.4%), and Bauchi (3.0%). Leptospiral serotypes detected varied between the states from three to nine, Kaduna state only had all nine.

Livestock species found positive for leptospirosis in each state were: Bauchi - cattle, sheep, horses and donkeys; Borno and Gongola - cattle and horses; Kaduna - all except camels; Kano - cattle, goats, horses and donkeys; Niger - cattle, horses, donkeys, sheep and goats; Plateau - cattle, sheep and horses; and Sokoto - cattle, horses and donkeys.

The overall species incidence rates were: horses (24.7%), cattle (11.9%), donkeys (6.1%), sheep (1.8%), and goats (1.1%). All nine serotypes were found in cattle, horses and donkeys,

and in sheep (*gripptyphosa*, *hebdomadis*, *pomona*, *pyrogenes*, and *tarassovi*), and goats (*pyrogenes*, *icterohemorrhagiae*, and *canicola*).

TABLE 1: Livestock Sera From Eight Northern States of Nigeria Tested for Presence of Leptospira Antibodies in 1975 - 76.

Species	State								
	Bauchi	Borno	Gongola	Kaduna	Kano	Plateau	Niger	Sokoto	Total
Cattle	172	37	120	374	137	87	105	121	1,153
Sheep	21	67	20	158	81	70	65	86	568
Goats	22	41	33	157	81	61	16	32	448
Donkey	13	10	14	110	118	-	57	73	395
Horse	5	9	5	25	40	17	26	19	146
Camel	-	-	-	-	42	-	-	100	142
Total	233	164	192	824	499	235	269	436	2,852

TABLE 2: Percentage Incidence of Leptospirosis in Livestock From Eight Northern States of Nigeria during 1975 - 76.

Species	State								
	Bauchi	Borno	Gongola	Kaduna	Kano	Plateau	Niger	Sokoto	Total
Cattle	2.3	27.0	8.3	12.3	20.4	13.8	14.3	10.7	11.9
Sheep	4.8	0	0	2.5	0	1.4	6.1	0	1.8
Goat		0	0	1.3	3.7	0	0	0	0.9
Donkey	7.7	0	0	10.9	3.4	-	8.8	2.7	6.1
Horse	20.0	22.0	20.0	32.0	15.0	17.6	42.3	21.0	24.7
Camel	-	-	-	-	0	-	-	-	0
Total	3.0	7.3	5.7	8.7	8.0	6.4	13.0	4.4	7.4

TABLE 3: Leptospira Serotype in Livestock from Eight Northern States of Nigeria from 1975 - 76.

Serotype	State								
	Bauchi	Borno	Gongola	Kaduna	Kano	Plateau	Niger	Sokoto	Total
L. grippotyphosa	0	0	0	11	1	1	7	2	22
L. icterohemorrhagiae	3	2	0	15	12	2	6	4	44
L. hardjo	1	3	2	8	4	1	5	6	30
L. hebdomadis	2	7	2	11	13	4	5	7	51
L. pomona	2	3	9	6	13	3	7	10	53
L. canicola	1	0	0	5	1	2	2	0	11
L. pyrogenes	2	1	1	16	5	6	9	1	41
L. tarassovi	0	1	0	16	1	5	3	2	28
L. australis	0	0	2	17	1	1	0	0	21
Total	11	17	16	105	51	25	44	32	301

TABLE 4: Summary of Cattle Samples Per Herd and State.

State	Herds		Cattle				No. abattoir samples positive
	No. tested	No. positive	No. involved	No. sampled	Positive	%	
Bauchi	6	2	528	172	4	2.3	1 (2.5%)
Borno	1	1	200	37	10	27.0	9 (56%)
Gongola	15	7	797	120	10	8.3	0
Kaduna	32	11	976	374	46	12.3	15 (55.6%)
Kano	27	12	1,376	137	28	20.4	0
Plateau	10	6	560	87	12	13.8	0
Niger	19	7	652	105	15	14.3	0
Sokoto	21	7	1,199	121	13	10.7	0
Total	131	53	6,288	1,153	138	11.9	25

TABLE 5: Number of Leptospira Seroreactors* In Cattle Tested From Eight Northern States of Nigeria in 1975 - 76.

Leptospira Serotype	State								
	Bauchi	Borno	Gongola	Kaduna	Kano	Plateau	Niger	Sokoto	Total
L. grippotyphosa	-	-	-	8	-	3	1	-	12
L. icterohemorrhagiae	2	2	-	4	4	4	1	1	18
L. hardjo	1	3	2	8	4	3	-	6	27
L. hebdomadis	2	7	2	8	13	3	3	7	45
L. pomona	2	3	9	6	12	4	2	10	48
L. canicola	1	-	-	2	-	1	2	-	6
L. pyrogenes	-	-	-	5	-	2	1	-	8
L. tarassovi	-	-	2	15	1	1	4	1	23
L. australis	-	-	-	13	-	-	-	-	13
Total	8	15	15	69	34	21	14	24	200

*Includes multiple reactors

TABLE 6: Summary of Titers of Leptospiral Reactors in Cattle Tested From Eight Northern States of Nigeria in 1975 - 76.

Leptospira Serotype	Titer						Positives		% Incidence
	20	100	200	400	800	1600/+	Total	%	
L. grippotyphosa	5	4	2	1	-	-	12	8.7	1.0
L. icterohemorrhagiae	13	4	1	-	-	-	18	13.0	1.6
L. hardjo	18	3	5	2	1	-	9	21.0	2.3
L. hebdomadis	24	13	7	-	-	1	45	32.6	3.3
L. pomona	13	18	5	7	2	3	48	34.8	3.9
L. canicola	4	-	-	2	-	-	6	4.3	0.5
L. pyrogenes	2	4	-	2	-	-	8	5.8	0.6
L. tarassovi	6	6	2	3	5	1	23	16.7	1.9
L. australis	3	4	3	3	-	-	13	9.4	1.1
Total	88	56	25	20	8	5	202		

TABLE 7: Sheep Sera Collected From Eight Northern States of Nigeria and Tested for Leptospiral Antibodies in 1975 - 76.

State	No. Tested	No. Positive	% Incidence
Bauchi	21	1	4.8
Borno	67	0	0
Gongola	20	0	0
Kaduna	158	4	2.5
Kano	81	0	0
Niger	65	4	6.1
Plateau	70	1	1.4
Sokoto	86	0	0
Total	568	10	1.8

TABLE 8: Distribution of Leptospira Titers in Sheep Seroreactors From Eight Northern States of Nigeria in 1975 - 76.

Leptospira Serotype	Titer						Positives		% Incidence
	20	100	200	400	800	1600/+	Total	%	
L. grippotyphosa	2	1	-	-	-	-	3	30.0	0.5
L. hebdomadis	-	-	1	-	-	-	1	10.0	0.2
L. pomona	-	-	-	-	-	1	1	10.0	0.2
L. pyrogenes	4	1	-	-	-	-	5	50.0	0.9
L. tarassovi	1	-	-	-	-	-	1	10.0	0.2
L. australis	-	1	-	-	-	-	1	10.0	0.2
Total	7	3	1	-	-	1	12		

TABLE 9: Goat Sera Collected From Eight Northern States of Nigeria and Tested for Leptospiral Antibodies in 1975 - 76.

State	No. Tested	No. Positive	% Incidence
Bauchi	22	0	0
Borno	41	0	0
Gongola	33	0	0
Kaduna	157	2	1.3
Kano	81	3	3.7
Niger	16	0	0
Plateau	61	0	0
Sokoto	37	0	0
Total	448	5	1.1

TABLE 10: Distribution of Leptospira Titers In Goat Seroreactors From Eight Northern States of Nigeria in 1975 - 76.

Leptospira Serotype	Titer						Positives		% Incidence
	20	100	200	400	800	1600/+	Total	%	
L. icterohemorrhagiae	-	1	1	-	-	-	2	50.0	0.4
L. canicola	-	-	-	-	-	-	1	25.0	0.2
L. pyrogenes	2	-	-	-	-	1	3	75.0	0.7

TABLE 11: Equine Sera Collected From Eight Northern States of Nigeria and Tested for Leptospiral Antibodies in 1975 - 76.

State	Donkey			Horse		
	No. Tested	No. Positive	% Incidence	No. Tested	No. Positive	% Incidence
Bauchi	13	1	7.7	5	1	20.0
Borno	10	0	0	9	2	22.2
Gongola	14	0	0	5	1	20.0
Kaduna	110	12	10.9	25	8	32.0
Kano	118	4	3.4	40	6	15.0
Niger	57	5	8.8	26	11	42.3
Plateau	-	-	-	17	3	17.6
Sokoto	73	2	2.8	19	4	21.0
Total	395	24	6.1	146	36	24.7

TABLE 12: Distribution of Leptospira Titers in Donkey Seroreactors From Eight Northern States of Nigeria in 1975 - 76.

Leptospira Serotype	Titer						Positives		% Incidence
	20	100	200	400	800	1600/+	Total	%	
L. grippotyphosa	1	2	-	-	-	-	3	12.5	0.7
L. icterohemorrhagiae	8	2	-	-	-	-	10	41.7	2.5
L. hardjo	-	1	-	-	-	-	1	4.2	0.2
L. hebdomadis	1	1	-	1	-	-	3	12.5	0.7
L. pomona	-	1	-	1	-	-	2	8.3	0.5
L. canicola	1	-	-	-	-	-	1	4.2	0.2
L. pyrogenes	5	2	-	1	-	-	8	33.3	2.0
L. tarassovi	1	-	-	-	-	-	1	4.2	0.2
L. australis	1	1	-	-	-	1	3	12.5	0.7
Total	18	10	-	3	-	1	32		

TABLE 13: Distribution of Leptospira Titers in Horse Serореactors From Eight Northern States of Nigeria im 1975 - 76.

Leptospira Serotype	Titer						Positives		% Incidence
	20	100	200	400	800	1600/+	Total	%	
L. grippotyphosa	2	2	-	-	-	-	4	11.1	2.7
L. icterohemorrhagiae	8	2	3	-	-	1	14	38.9	9.6
L. hardjo	1	2	-	-	-	-	3	8.3	2.0
L. hebdomadis	3	-	-	-	-	-	3	8.3	2.0
L. pomona	3	-	-	-	-	-	3	8.3	2.0
L. canicola	1	2	-	-	-	-	3	8.3	2.0
L. pyrogenes	12	2	-	-	-	-	14	38.9	9.6
L. tarassovi	2	1	1	-	-	-	4	11.1	2.7
Total	32	11	4	-	-	1	48		

Fig. 1 Incidence of Leptospirosis in livestock from eight states of Nigeria.

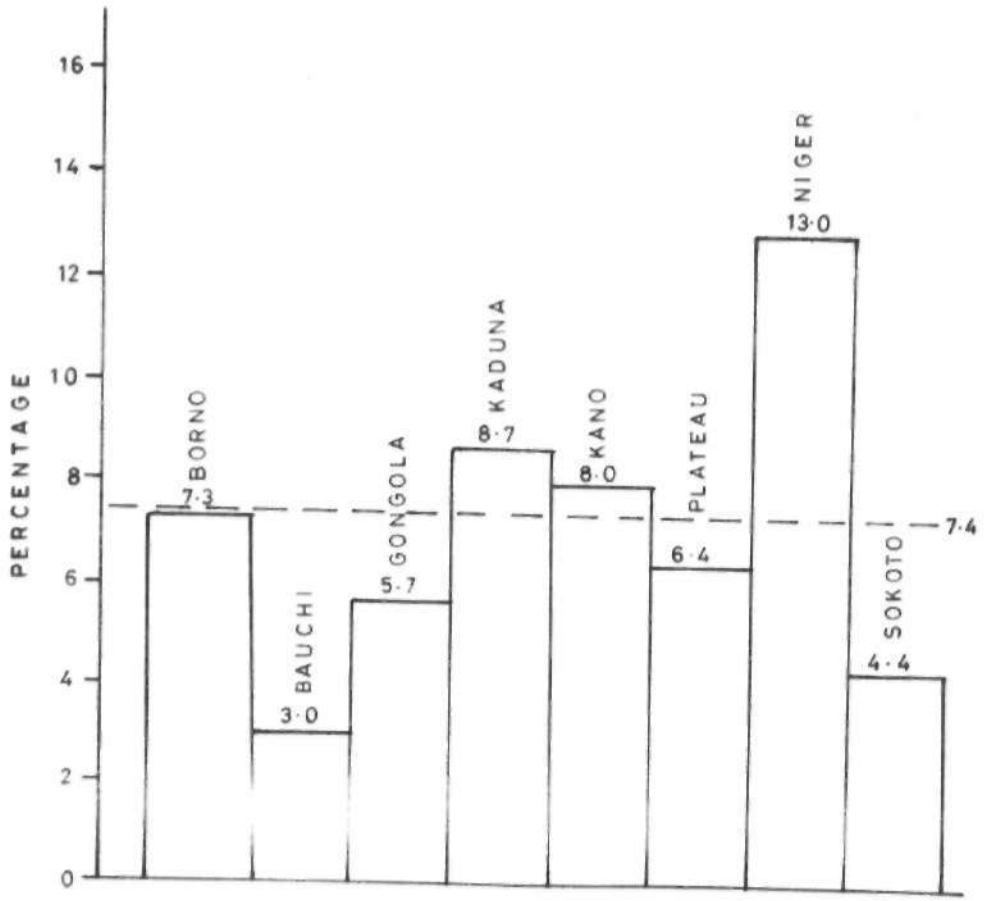

PERCENTAGE
16
14
12
10
8
6
4
2
0
BORNO
7·3
BAUCHI
3·0
GONGOLA
5·7
KADUNA
8·7
KANO
8·0
PLATEAU
6·4
NIGER
13·0
SOKOTO
4·4
7·4

Fig. 2 Incidence of Leptospirosis in cattle from eight states of Nigeria.

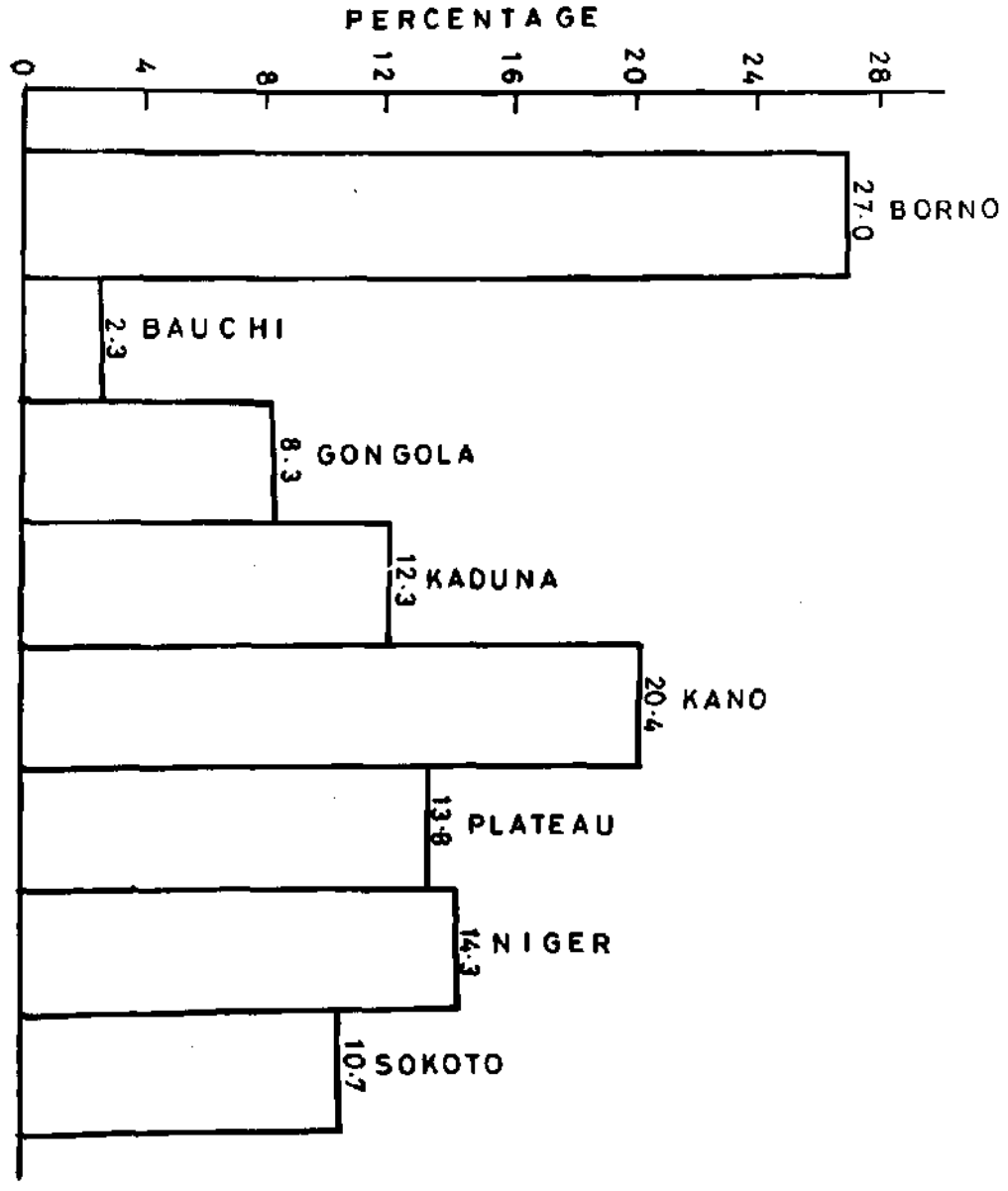

PERCENTAGE
0
4
8
12
16
20
24
28
27·0
BORNO
2·3
BAUCHI
8·3
GONGOLA
12·3
KADUNA
20·4
KANO
13·9
PLATEAU
14·3
NIGER
10·7
SOKOTO

Fig. 3 Distribution of Leptospira serotypes in cattle, sheep and goats from eight northern states of Nigeria.

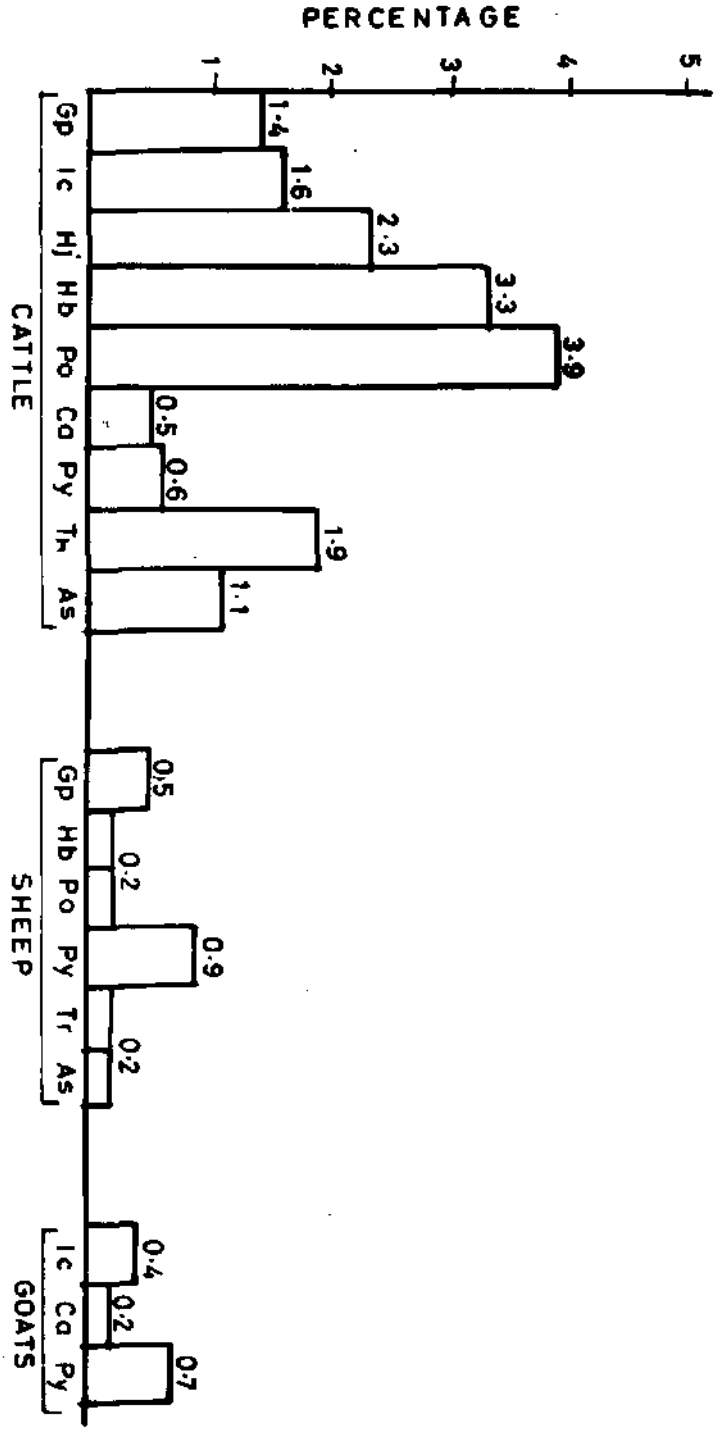

PERCENTAGE
1
2
3
4
5
CATTLE
Gp
Ic
Hj
Hb
Po
Co
Py
Th
As
1·4
1·6
2·3
3·3
3·9
0·5
0·6
1·9
1·1
SHEEP
Gp
Hb
Po
Py
Tr
As
0·5
0·2
0·9
0·2
GOATS
Ic
Co
Py
0·4
0·2
0·7

Fig. 4 Incidence of Leptospirosis in Man and Animals in eith northern states of Nigeria.

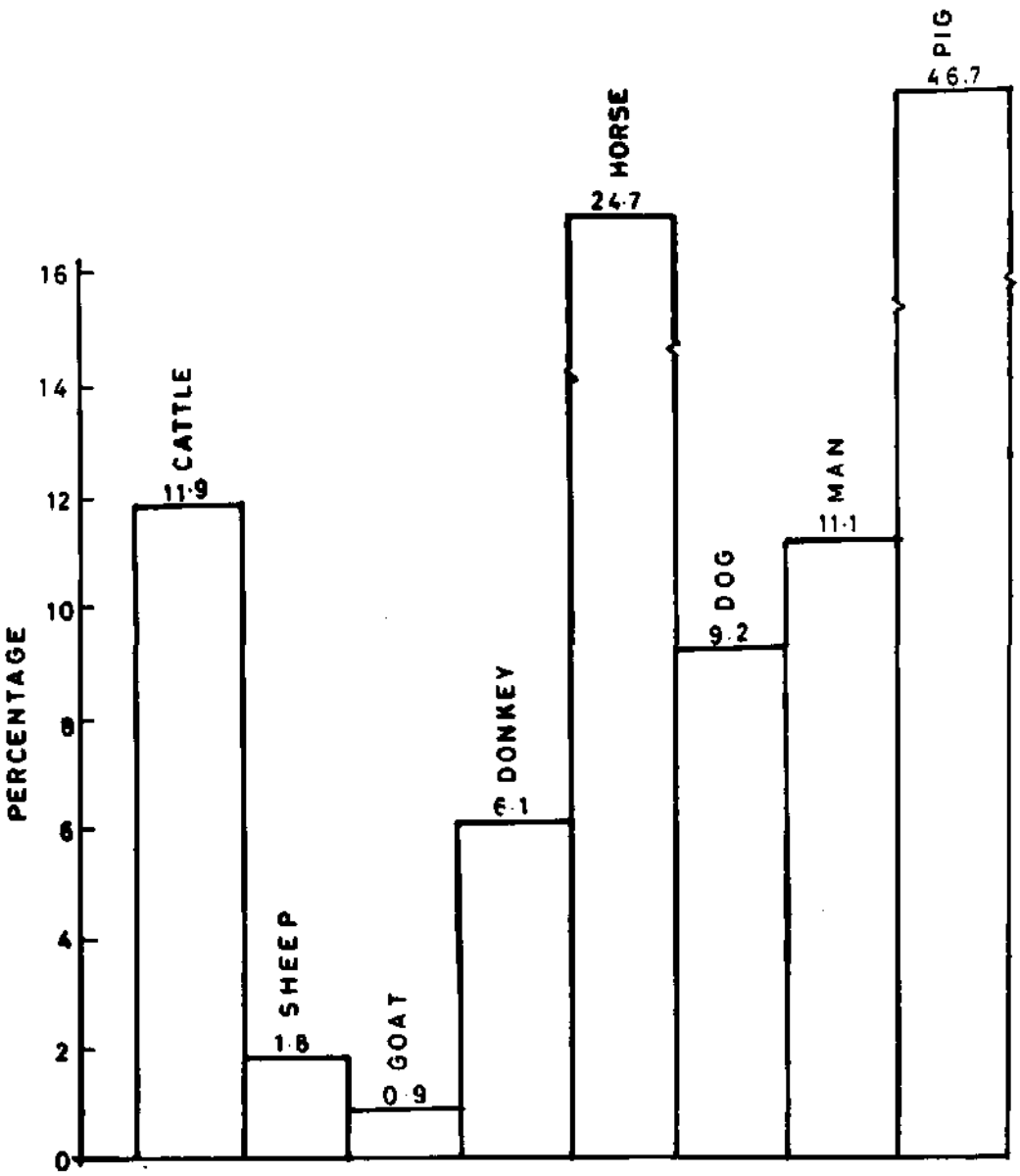

PERCENTAGE
0
2
4
6
8
10
12
14
16
CATTLE
11·9
SHEEP
1·8
GOAT
0·9
DONKEY
6·1
HORSE
24·7
DOG
9·2
MAN
11·1
PIG
46·7

Fig. 5 Incidence of Leptospirosis in equine species from eight states of Nigeria.

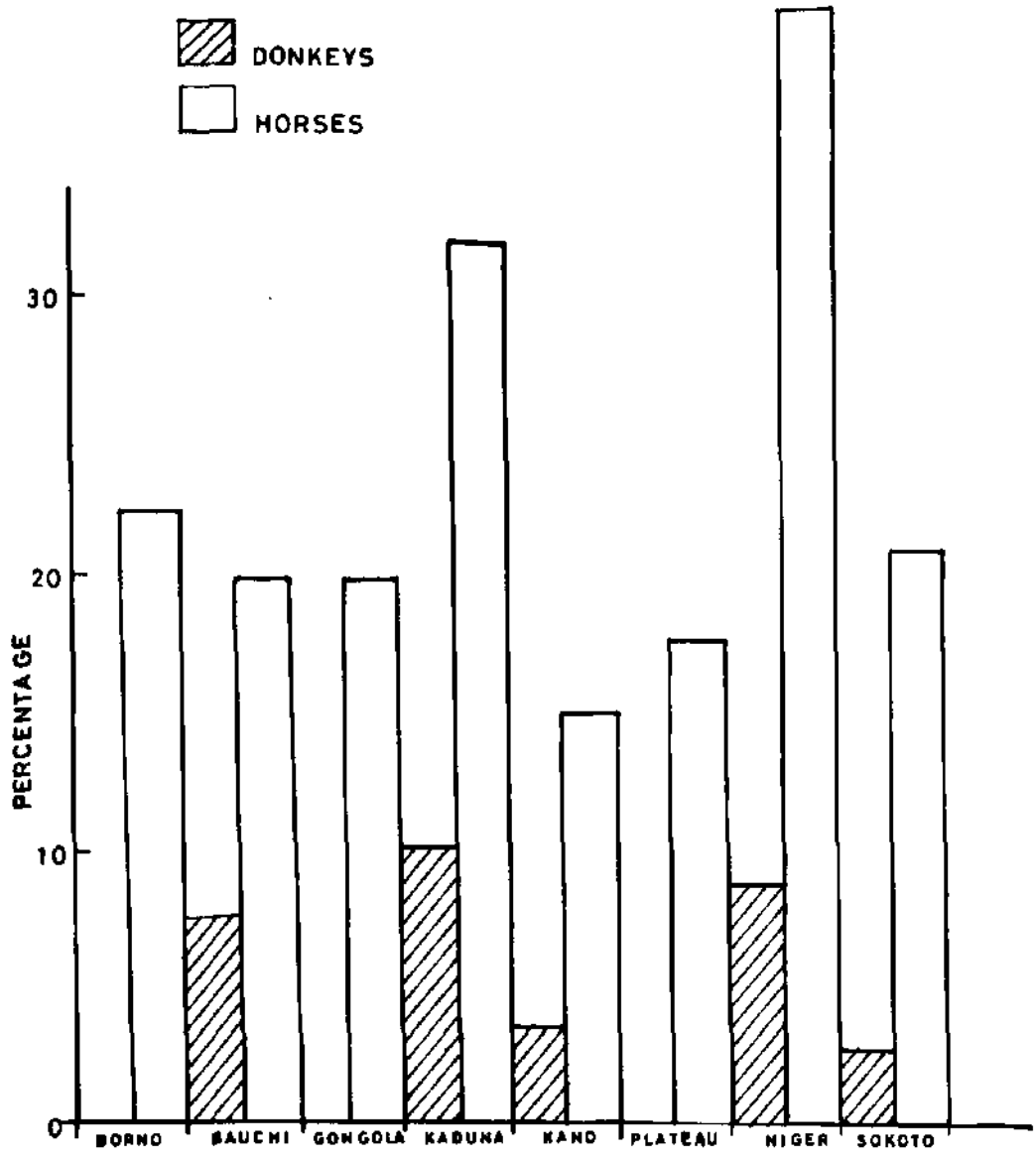
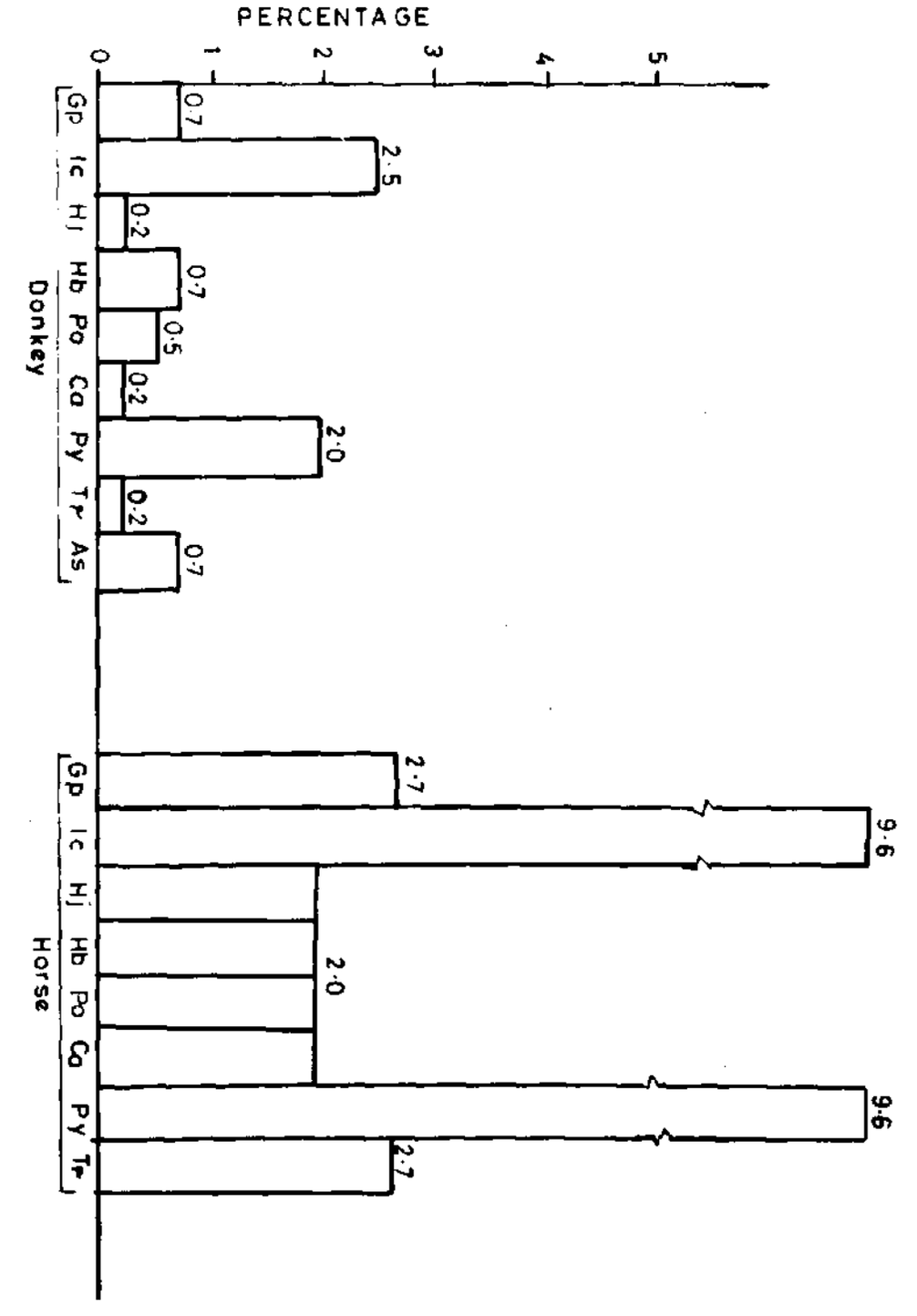

DONKEYS
HORSES
PERCENTAGE
30
20
10
0
BORNO
BAUCHI
GONGOLA
KADUNA
KANO
PLATEAU
NIGER
SOKOTO

Fig. 6 Distribution of Leptospira serotypes in equine species from eight northern states of Nigeria.


PERCENTAGE
0
1
2
3
4
5
Donkey
Gp
Ic
Hj
Hb
Po
Ca
Py
Tr
As
0·7
2·5
0·2
0·7
0·5
0·2
2·0
0·2
0·7
Horse
Gp
Ic
Hj
Hb
Po
Ca
Py
Tr
2·7
9·6
2·0
9·6
2·7

PART III

SEROLOGIC SURVEY OF NORTHERN NIGERIA FOR LEPTOSPIROSIS: 2. MAN

INTRODUCTION

Leptospirosis is an ubiquitous water-borne zoonosis recognized throughout the world. It is caused by a spirocheto Leptospira interrogans, which is classified into 18 serogroups and has over 140 morphologically identical serotypes (Anon., 1967). Environmental factors such as those existing in tropical regions tend to favor endemicity of leptospirosis. In man the disease varies from a mild clinical illness caused by the majority of serotypes to a fulminating type caused by L. icterohemorrhagiae and L. canicola.

Much still remains to be clarified about the epidemiology of leptospirosis (Szatolowicz et al., 1969; Turner, 1967). Reports of leptospirosis in Africa date from 1933 when Kadamer and Corti described an epidemic of leptospirosis in a colony of Europeans living in Zaire. Since then leptospirosis has been reported in Algeria, Senegal, Guinea and Mali (Mailloux and Kolochine-Ebert, 1961; Mailloux et al., 1974) and in Cameroon where Fry (1967) demonstrated its endemicity by

showing that 25 per cent of patients with F.U.O. (fever of unknown origin) in the area of Ndian had leptospira antibodies and that 35 per cent of patients with leptospirosis had signs of icterus. In Nigeria, Leach and Canon (1943) reported a case of leptospirosis which was mistaken for yellow fever in the then Western State.

In other parts of Africa surveys in man have been carried out. In Zimbabwe, Graf (1965) found one positive out of 484 sera tested for leptospirosis. In a six year survey of patients suspected to have leptospirosis in Dakar, Sankale et al. (1974) found 6.4 per cent positive. These investigators concluded that leptospirosis was not a problem in Dakar. Kinebuchi and Afoakwa (1973) examined 99 cases diagnosed as infectious hepatitis or jaundice in Ghana. Eleven of 38 cases providing paired sera and 10 out of the remaining 61 sera (16.4%) were positive for leptospirosis. Collard (1962) found 4.5 per cent prevalence of leptospira antibodies in a survey conducted in the Katsina area of Kaduna State in Nigeria.

The aim of this study was to investigate the incidence of leptospirosis in eight northern states of Nigeria by a

serological survey using serotypes reported in Africa.

MATERIALS AND METHODS

Survey area and organization, leptospiral serotypes, and microscopic agglutination testing were described in Part II. Serotype biflexa was added to the screening battery.

The Chief Medical Officers-in-Charge of the area hospitals in all the survey centers were notified and their cooperation requested. All sera were collected from the pathology laboratories from routine blood samples taken from people reporting sick.

RESULTS

Results of the leptospirosis survey in man are summarized in Tables 1 - 4 and Appendix 3 (Tables 11 - 14). A total of 1,374 people were tested and 152 were positive, a 11.1 per cent incidence rate. Seventy-four of the positives were males and 78 were females.

The highest incidence rate per state was recorded in Plateau state (31.8%), and Bauchi, Gongola and Kaduna were

next with 16.7, 10.5 and 9.4 per cent, respectively (Fig. 1). In Borneo there was no seropositives among the 30 samples taken.

Age distribution is shown in Table 2. The adult group seemed more predisposed to leptospirosis with 77.6 per cent incidence; the older age group and children had 4.6 and 0.7 per cent incidence rates, respectively.

All serotypes tested were present (Table 4) 40.8 per cent of the positive sera reacted at titers of 1/100 or more. L. grippotyphosa, L. hebdomadis, L. pyrogenes, L. tarassovi and serotype biflexa patoc 1 had titers up to 1/400. Serotypes icterohemorrhagiae, hardjo, pomona, australis and canicola exhibited titers ranging from 1/20 to 1/100. There were relatively fewer reactors to L. australis and L. canicola than any other serotype.

DISCUSSION

Collection of human blood was more difficult and delicate as it was not possible under the conditions of the survey to mobilize the manpower necessary and to obtain the

authority and cooperation to bleed healthy people. The sampling method of obtaining sera from blood already collected from people reporting sick served to determine the status of leptospirosis in the human population of the eight states. Others may consider the results more meaningful to clinicians than to epidemiologists interested in incidence of leptospirosis in the population as a whole. The sampling is not regarded as being biased towards leptospirosis as the patients were reporting ill for various reasons and as the survey covered a two-year period, it could be regarded as being reasonably representative of the population in each of the eight states.

The incidence of leptospirosis would be expected to be relatively high in a tropical agricultural country like Nigeria. The choice of serotypes was based on earlier reports of prevalent serotypes in the sahelian zone. However, it was not possible or practical to include all the serotypes reported.

Incidence reported was not as high as that found in Ghana by Kinebuchi and Afoakwa (1973). It was nevertheless

more significant than that reported by Sankale et al. (1974) who concluded that leptospirosis was not a human problem in Dakar. They acknowledged the high incidence of leptospirosis in rodents and livestock in Senegal (Van Riel et al., 1969; Van Riel et al., 1971). Their argument was that, since only seven cases of leptospirosis were found in six years, it might not be important clinically even though it was a recognized public health problem. Collard's (1962) results in Nigeria seemed to be higher. He used the CF test and serotype canicola only. The CF test is known to be more sensitive than the MA test but it is also less specific.

A high frequency of all serotypes tested was found at low dilutions (1/20 to 1/100). L. grippotyphosa, L. hebdomadis, L. pyrogenes and L. tarassovi had high titers, which were indicative of definite infections. It has been suggested by many authors that seroreaction at low dilutions are non-specific. This is possible since in Weil's disease the "paradoxical reaction" is known to occur. A high percentage of L. biflexa reactors and at high dilutions is indicative of current infection as well as the possibility of infection with serotypes not included in the screening battery (Hergt, 1976).

L. grippotyphosa, L. hebdomadis, L. pyrogenes and L. tarassovi are known to cause fever in man (Alston and Broom, 1958; Varfoloomeeva, 1964). The same serotypes are also important causes of leptospirosis in livestock.

The incidence pattern in Bauchi, Plateau and Gongola states suggests that there may be a relationship between altitude and a higher incidence of leptospirosis. It is also known that overgrazing is a livestock problem for the Plateau geographical region which includes these three states. The distribution of serotypes in these states gives a better picture on incidence. In Gongola state there were two positives out of 19 sera sampled. Compared to the sample size in Kaduna, Kano and Plateau states, the results in the latter are more meaningful.

There was an age but no sex difference among the sero-reactors to leptospirosis. The adult group was more predisposed to infection than either the aged or children groups.

It is known that about 65 per cent of human cases leptospirosis are anicteric. Berman et al. (1973) found that leptospiral infections were a common cause of acute fever in

U. S. military personnel in South Vietnam in 1973. Muscle tenderness and conjunctivitis were more common than typical jaundice and central nervous system signs. Fry (1967) already showed that 25 per cent of 200 cases of F.U.C. investigated in Cameroon were of leptospiral origin and that icterus was present in only 35 per cent.

The incidence in the states, especially Plateau, Bauchi and Kaduna, clearly indicates the presence of leptospirosis in the human population of northern Nigeria. A record of occupation was not kept and this could be useful in future studies (Schwabe, 1969). The results obtained will form the basis for a more systematic epidemiologic study of human leptospirosis in Nigeria including sampling higher risk occupational groups such as slaughtermen, veterinarians and Fulbe cattlemen.

SUMMARY

A total of 1,374 sera collected from hospitals in eight northern states of Nigeria were tested for leptospiral antibodies to serotypes grippotyphosa, icterohemorrhagiae, hardjo,

hebdomadis, pomona, canicola, pyrogenes, biflexa, tarassovi, and australis by the microscopic agglutination test during 1975 - 76. The incidence rates for the eight states in decreasing order were: Plateau (31.8%), Bauchi (16.7%), Gongola (10.5%), Kaduna (9.4%), Sokoto (5.1%), Kano (4.5%), Niger (3.0%) and Borno(nil). Antibodies to all ten serotypes were detected in Plateau and Kaduna states only. Serotypes were detected in the following decreasing frequency: biflexa 52, icterohemorrhagiae 33, hebdomadis 28, pyrogenes 27, pomona 24, hardjo 20, tarassovi 19, grippotyphosa 18, canicola 8, and australis 3. There was an age but no sex difference among the seroreactors; adults 77.6 per cent incidence rate, aged 4.6 per cent, and children (< 10 years) 0.7 per cent.

TABLE 1: Sex Distribution of Human Seroreactors to Leptospirosis From Eight Northern States of Nigeria.

State	Total Sample	Positive			% Incidence
		Total	Male	Female	
Bauchi	66	11	9	2	16.7
Borno	30	0	-	-	0
Gongola	19	2	2	0	10.5
Kaduna	424	40	18	22	9.4
Kano	201	9	4	5	4.5
Niger	100	3	3	0	3.0
Plateau	223	71	36	35	31.8
Sokoto	311	16	2	14	5.1
Total	1,374	152	74	78	11.1

TABLE 2: Age Distribution of Human Seroreactors to Leptospirosis From Eight Northern States of Nigeria.

State	Age group in years				Total	Percentage
	0 - 10	11 - 20	20 - 45	46+		
Bauchi	0	3	5	3	11	7.2
Borno	0	0	0	0	0	
Gongola	0	2	0	0	2	1.3
Kaduna	0	7	32	1	40	26.3
Kano	0	0	9	-	9	5.9
Niger	0	3	0	0	3	1.9
Plateau	0	3	65	3	71	46.7
Sokoto	1	6	9	0	16	10.5
Total	1	25	118	7	152	
Percentage	0.7	16.4	77.6	4.6		

TABLE 3: Distribution of Human Seroreactors to Leptospirosis in the Eight Northern States of Nigeria.

Leptospira Serotype	State								Total
	Bauchi	Borno	Gongola	Kaduna	Kano	Niger	Plateau	Sokoto	
L. grippotyphosa	3	-	-	3	-	1	11	-	18
L. icterohemorrhagiae	-	-	-	8	6	1	17	1	33
L. hardjo	1	-	-	5	-	-	14	-	20
L. hebdomadis	2	-	-	11	-	-	11	4	28
L. pomona	4	-	-	7	-	-	12	1	24
L. canicola	1	-	-	3	-	2	2	-	8
L. pyrogenes	1	-	1	14	-	1	19	1	27
L. biflexa	-	-	-	15	3	-	30	4	52
L. tarassovi	2	-	1	4	1	-	7	4	19
L. australis	-	-	-	3	-	-	-	-	3

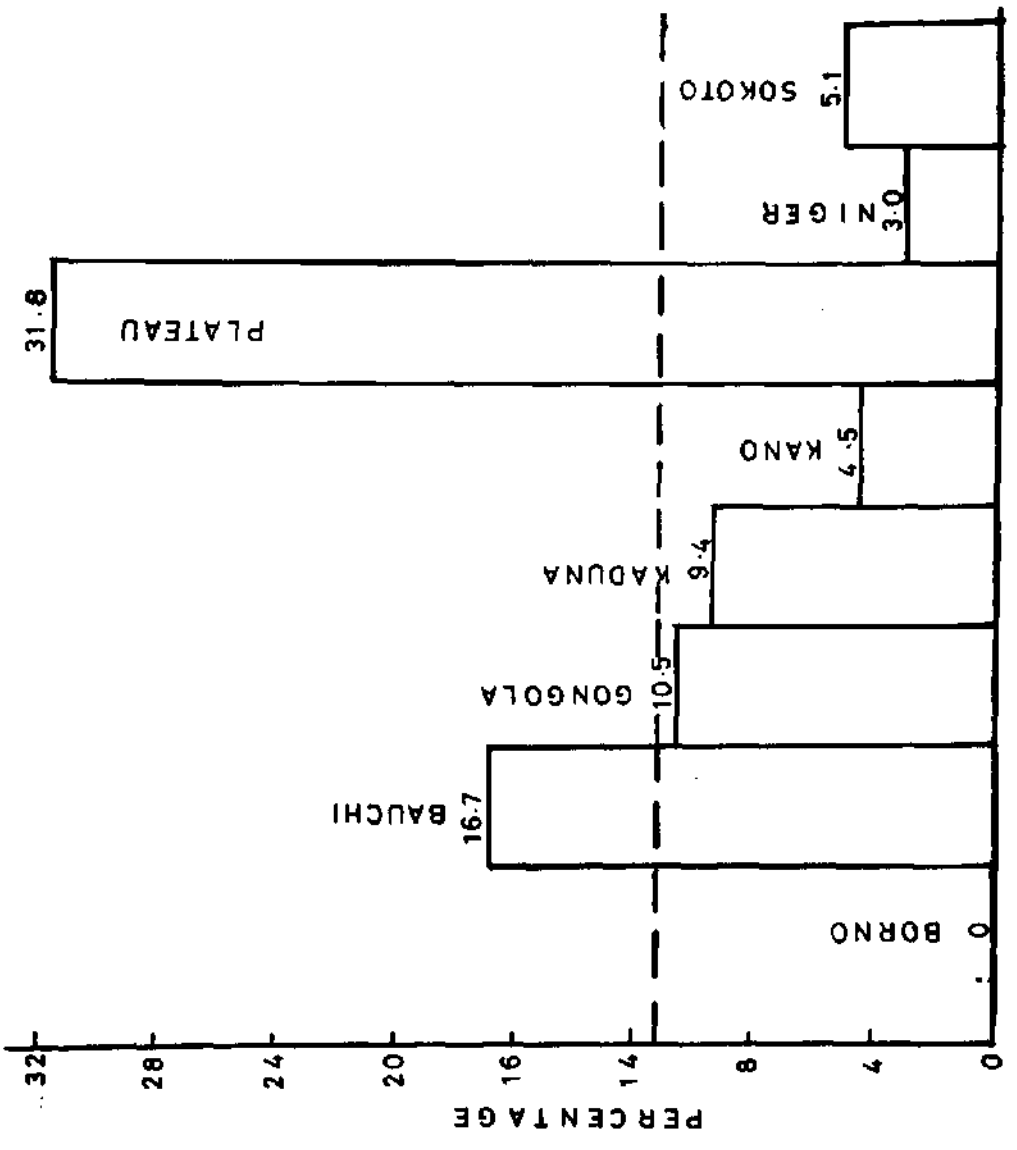
TABLE 4: Frequency and Distribution of Leptospira Serotypes in Human Seroreactors From Eight Northern States of Nigeria.

Leptospira Serotype	Titer						Total	%	% Incidence
	20	100	200	400	800	1600/+			
L. grippotyphosa	8	7	1	-	1	1	18	11.8	1.3
L. icterohemorrhagiae	24	9	1	-	-	-	34	22.4	2.5
L. hardjo	13	6	-	-	-	1	20	13.2	1.5
L. hebdomadis	26	10	2	1	3	-	42	27.6	9.1
L. pomona	12	8	-	-	-	-	20	13.2	1.5
L. canicola	6	2	-	-	-	-	8	5.3	0.6
L. pyrogenes	17	4	3	1	2	-	27	17.8	1.9
L. biflexa	39	11	-	1	1	-	52	34.2	3.8
L. tarassovi	10	2	2	2	-	-	16	10.5	1.2
L. australis	-	3	-	-	-	-	3	1.9	0.2

* Including multiple reactors



Fig. 1 Incidence of Human leptospirosis in eight states of Nigeria.


SOKOTO
5.1
NIGER
3.0
PLATEAU
31.8
KANO
4.5
KADUNA
9.4
GONGOLA
10.5
BAUCHI
16.7
BORNO
0
32
28
24
20
16
14
8
4
0
PERCENTAGE

PART IV

CANINE LEPTOSPIROSIS IN ZARIA AND JOS, NIGERIA

INTRODUCTION

The dog is a recognized carrier host for leptospira organisms. It has been suggested that people rarely contact leptospirosis at home except for "canicola fever" caused by L. canicola, and "icterohemorrhigica fever" caused by L. icterohemorrhagiae when the premises are heavily infested with rats (Meyer, 1964; Geistfield, 1975).

Acute leptospirosis in dogs is caused by two serotypes, Leptospira icterohemorrhagiae and L. canicola, which cause disease entities with distinct signs. The former is characterized by jaundice and severe nephritis with uremia and the latter rarely causes jaundice. Both diseases, however, are characterized by nephritis (Alston and Broom, 1958). Infection with L. icterohemorrhagiae is by contact with rat urine or water contaminated with rat urine, while infection with L. canicola is by dog-to-dog contact. Younger dogs, one to three years old, are more susceptible to infection than older dogs. Subclinically

infected or convalescent canine carriers are of great importance in the epidemiology of leptospirosis, because they excrete leptospira in their urine for long periods.

In a 13 year study of 483 cases of human leptospirosis (Galton and Heath, 1965), 12 serotypes were isolated including L. canicola and L. icterohemorrhagiae. Infection with these two serotypes was most commonly associated with direct or indirect contact with rats and dogs.

Other leptospira species have been known to affect dogs. Prevalence of agglutinins other than L. canicola and L. icterohemorrhagiae, as well as isolation of L. pomona from a dog, have been reported (Murphy et al., 1958; Ryu et al., 1975; Sebek and Wurst, 1975; Ryu, 1975).

In Africa, reports of canine leptospirosis are few. Darasse and Tidori (1956) reported isolating L. icterohemorrhagiae from a dog in Dakar. In a survey involving 50 dogs in Morocco, both L. icterohemorrhagiae and L. canicola were isolated (Mailloux and Kolochine-Erbert, 1961). Following a survey for leptospira carriers in Capetwon, Beyers (1965) isolated L. canicola from dogs.

Ball (1966) could not find any seroreactor in five dogs from Kenya and Uganda. In Ethiopia, Mech et al. (1975) found one seropositive among 12 dogs examined for leptospirosis. In Nigeria, there have been clinical reports of canine leptospirosis at Zaria (Adeyanju, 1976).

Reported here are the results of a survey for leptospira agglutinins in the dog population of Zaria and Jos, Nigeria.

MATERIALS AND METHODS

The procedures for leptospiral testing were described in detail in Part II.

Sera were collected from dogs in the veterinary hospital, Ahmadu Bello University, Zaria, Kaduna State, and from the veterinary clinic at Jos, Plateau State, from January, 1976 to December, 1976.

RESULTS

Two hundred and eighty-two dogs were sampled and 26

(9.2%) reacted to one or more leptospira serotypes (Tables 1 - 2). The agglutinin titers of the positive sera are given in Table 3; 61.5 per cent had titers of 1/100 or more. The monthly distribution of leptospira reactors and prevalence are given in Table 4 and Fig. 1.

L. canicola and L. icterohemorrhagiae were the predominant serotypes with a 5.7 and 4.3 per cent incidence, respectively. Reactors to both serotypes were found in Zaria in contrast to Jos where L. canicola was prevalent. There was no difference in the leptospirosis incidence in the canine population of the two areas (Table 1).

Two dogs reacted with L. pyrogenes and one with L. australis.

DISCUSSION

The results of the survey confirmed findings elsewhere that leptospirosis is relatively common in the canine population and that L. icterohemorrhagiae and L. canicola are the primary serotypes. Initially, it was intended to collect sera from dogs in the vicinity of the collection

centers for the leptospirosis survey in livestock in the eight northern states (Part II) but this was found to be impractical because of lack of adequate help and local cooperation. The canine survey was limited to the veterinary hospital at Ahmadu Bello University, Zaria and the veterinary clinic at Jos. The Ahmadu Bello University veterinary hospital treats dogs from Zaria, Kaduna, Kano, Sokoto, other parts of northern Nigeria, southern Nigeria including Lagos, and overseas. A number of expatriate university faculty bring their dogs with them. In contrast, the canine population at Jos is less cosmopolitan and more local. This would explain why the Zaria sera reacted to four leptospira serotypes and the Jos area primarily to L. canciola.

The important public health question is whether these dogs are carriers and shed leptospira in their urine thereby endangering man and other domestic animals. There is no doubt that clinical cases of leptospirosis occur since 61.5 per cent of the seropositive dogs had titers above 1/100. Upon recovery, these dogs can shed leptospira in their urine from 6 months to four years (McIntyre et al., 1953). Of

additional importance, is the finding of sero-reactors to L. pyrogenes and L. australis and the isolation of L. pyrogenes from the kidney of slaughtered cattle and L. australis from rats (Arvicanthus niloticus) (Part VI). This suggests that dogs contact these infections from other animal species.

It is not possible to draw any valid conclusions regarding the monthly distribution and prevalence of reactors. This would require a longer observation period and a larger number of sera from a wider dog population.

One of the problems of public health surveys in a developing country like Nigeria is the ability to obtain a statistically meaningful sample against a background of different social attitudes. The dog is considered man's best friend and a pet, but it does not receive the same treatment in different communities. An urban Nigerian will keep a dog more as a companion and watchdog than the cattle herdsman. The former will also pay more attention to the health of his dog. This is important as he and his family will have closer contact with their dog and hence a greater danger of contacting zoonotic diseases like leptospirosis.

In Zaria, increased urbanisation has resulted in an increased number of households having pet dogs. This has been an increasing trend over the past five years.

The serological survey indicates that dogs in Zaria and Jos may be carriers of leptospirosis. Whether they constitute an important public health hazard will have to be determined by actual attempts to isolate leptospira from their urine and correlating results with surveys of man.

SUMMARY

Two hundred and eighty-two dogs were tested for leptospiral agglutinins by the microscopic agglutination test and 26 (9.2%) were positive for one to three serotypes; 61.5 per cent had titers of 1/100 or more. L. canicola and L. icterohemorrhagiae were the predominant serotypes with a 5.7 and 4.3 per cent incidence, respectively. Reactors to both serotypes were found in Zaria in contrast to Jos where L. canicola was prevalent. Two dogs reacted with L. pyrogenes and one with L. australis. There was no difference in the leptospirosis incidence in the canine population of Zaria and Jos, Nigeria.

TABLE 1: Dog sera collected from two Northern States of Nigeria in 1976 and tested for leptospiral antibodies.

State	No. Tested	No. Positive	% Incidence
Kaduna	160	15	9.4
Plateau	122	11	9.0
Total	282	26*	9.2

*Includes multiple reactors

TABLE 2: Summary of Titers of Leptospiral Reactors in Dogs tested at Zaria and Jos, Nigeria in 1976.

Leptospira Serotype	Titer						Positive		
	20	100	200	400	800	1600/+	Total	%	% Incidence
L. icterohemorrhagiae	8	2	-	-	-	1	12	46.1	4.3
L. canicola	5	6	2	2	1	-	16	61.5	5.7
L. pyrogenes	2	-	-	-	-	-	2	7.7	0.7
L. australis	-	-	1	-	-	-	1	3.8	0.3
Total	15	8	3	2	1	1	31		

TABLE 3: Agglutinin Titers of Leptospira-positive Sera collected from Dogs in Zaria and Jos, Nigeria during 1976.

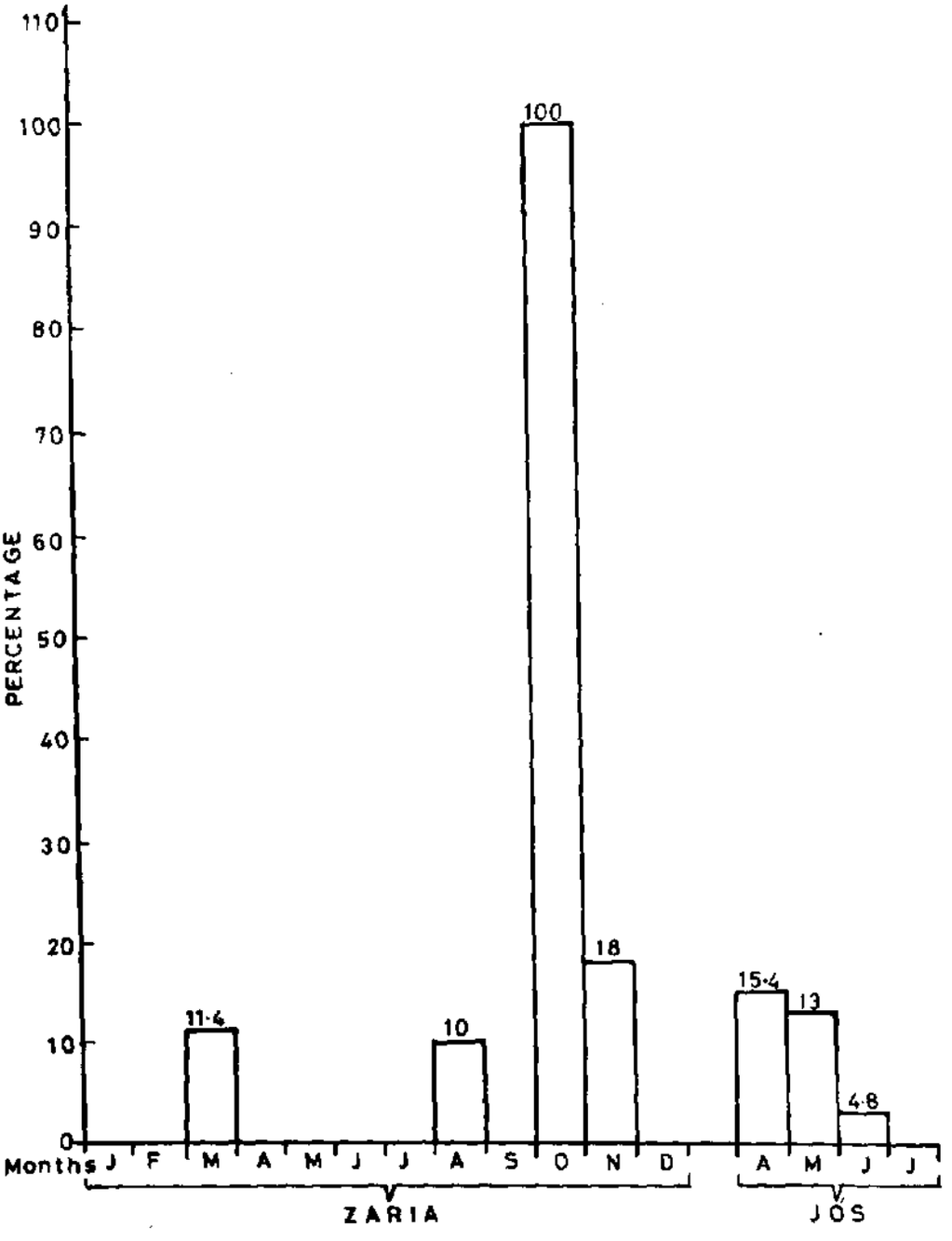
City	Serum No.	Titer icterо.*	canicola	pyrogenes	australis
Zaria, Kaduna State	3	20	100	20	-
	36	-	20	-	-
	51	20	100	-	-
	54	-	800	-	-
	67	20	-	-	-
	72	20	20	-	-
	81	400	-	-	-
	84	20	200	-	-
	85	100	-	-	-
	97	100	-	-	-
	125	20	-	-	-
	133	1600+	-	-	-
	137	-	20	-	-
	143	-	-	-	200
	145	-	100	-	-
Jos, Plateau State	5	-	20	-	-
	8	20	-	-	-
	10	-	400	-	-
	15	-	200	-	-
	35	-	-	20	-
	52	-	400	-	-
	58	-	100	-	-
	67	-	100	-	-
	69	-	20	-	-
	70	-	100	-	-
	106	20	-	-	-
	Total	12	16	2	1

*icterohemorrhagiae

TABLE 4: Prevalence of Leptospirosis in Dogs from Zaria and Jos.

Zaria				Jos			
Month	Total	Positive	%	Month	Total	Positive	%
March	34	4	11.7	April	26	4	15.4
August	20	2	10.0	May	46	6	13.0
October	1	1	100.0	June	35	1	2.8
November	44	8	18.1				

Fig. 1 Monthly distribution and prevalence of canine leptospirosis in Zaria and Jos.


110
100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE
100
18
15·4
13
11·4
10
4·8
Months J F M A M J J A S O N D A M J J
ZARIA
JOS

PART V

SWINE LEPTOSPIROSIS IN KADUNA AND KANO STATES OF NIGERIA

INTRODUCTION

Leptospirosis is a major disease of swine characterized primarily by abortion, stillbirths and neonatal mortality (Blood and Henderson, 1968). Fetal losses occur mainly in sows infected after mid-pregnancy (Fennestad and Borg-Petersen, 1966). Leptospira pomona is the most common serotype isolated from pigs; others include L. canicola (Williams et al., 1971), L. grippotyphosa (Hanson et al., 1970), L. icterohemorrhagiae (Schnurrenberger et al., 1970) and L. australis (Hanson, 1972).

In Africa, leptospirosis has been reported in Zaire, Ethiopia, Morocco and Senegal. In Zaire, Van Riel and Van Riel (1954) found 4.6 per cent of 283 pigs seropositive to L. icterohemorrhagiae or L. grippotyphosa. In Ethiopia, Mech et al. (1975) found 57 per cent of pigs tested had leptospiral antibodies. A survey in Senegal included 66 pigs with 9 per cent reactors (Van Riel et al., 1971). In Morocco, Blanc et al. (1961) reported that 23 of 31 pigs (74.2%) tested were seropositive for leptospirosis. Swine

leptospirosis has not been reported in Nigeria.

Reported here are the results of serologic testing of pig sera for leptospirosis from Kaduna and Kano abattoirs.

MATERIALS AND METHODS

The serologic procedures were described in Part II.

Sera were collected from pig slaughtered at Kaduna and Kano abattoirs during July to September, 1976.

RESULTS

A total of 105 sera was tested and 46.7 per cent were positive for leptospirosis (Table 1). The female: male ratio of the reactors was 2:1. The distribution of leptospira serotypes, titers and incidence are given in Table 2 and Fig. 1. Serotypes _icterohemorrhagiae_, _pyrogenes_ and _canicola_ had the highest incidence (Appendix 3; Table 15). _L. pomona_ incidence was low.

DISCUSSION

This preliminary survey confirmed the presence of swine leptospirosis in Nigeria. It appears to be more important in Kaduna state than in Kano state where only two out of 12 sera reacted. It is worth noting that extensive pig farming does not exist in the northern part of Nigeria even though a large piggery is in operation at Kano. The difference in reactor rates between the two states could be attributed to geographical location. Most of the pigs killed at the Kaduna abattoirs were either bred at Kakuri or brought up from the humid and irrigated area of Kafanchan; both areas are located in the southern savanna zone. Kano, on the other hand, is located in the drier sudan savanna zone.

Surveys for leptospirosis conducted in the sahelian zone have indicated the presence of L. australis and L. butembo in Ethiopia, and L. cynopteri and L. pyrogenes in Senegal (Van Riel et al., 1971; Koch et al., 1975). Since cynopteri and butembo belong to the seregroup Cynopteri, the present study confirmed the presence of L. pyrogenes

infection in swine and in addition, indicated that L. canicola and L. icterohemorrhagiae infection are present and that L. pomona and L. grippotyphosa infections are not as common as that of the above three serotypes. The findings also indicate that swine leptospirosis may be a public health problem in Nigeria especially in the more humid regions.

This investigation is continuing and it is planned to extend the survey into the more humid southern zones of Nigeria and to combine it with isolation studies.

SUMMARY

A total of 105 pig sera was tested for leptospirosis by the microscopic agglutination test and 46.7 per cent were positive. The female: male ratio of reactors was 2:1. The leptospira serotypes in decreasing incidence were: icterohemorrhagiae (37.1%), pyrogenes (24.8%), canicola (19.0%), pomona (2.8%) and grippotyphosa (1.9%). This preliminary survey confirmed the presence of leptospirosis in pigs in Nigeria.

TABLE 1: Results of Leptospira Testing on Pig Sera Collected from Kaduna and Kano Abattoirs between July and September, 1976.

State	No. Tested			No. Positive			% Incidence
	Female	Male	Total	Female	Male	Total	
Kaduna	49	43	92	32	15	47	51.1
Kano	8	5	13	1	1	2	15.4
Total	57	48	105	33	16	49	46.7

TABLE 2: Titer Distribution of Leptospira Serotypes Among Pig Seroreactors; from Kaduna and Plateau States of Nigeria.

Serotype	Titer						Total	% Incidence
	20	100	200	400	800	1600/+		
L. grippotyphosa	1	1	-	-	-	-	2	1.9
L. icterohaemorrhagiae	23	10	1	1	-	-	35	37.1
L. pomona	2	1	-	-	-	-	3	2.8
L. canicola	6	2	1	3	4	4	20	19.0
L. pyrogenes	6	8	1	4	4	3	26	24.8
Total	38	22	3	8	8	7	86*	

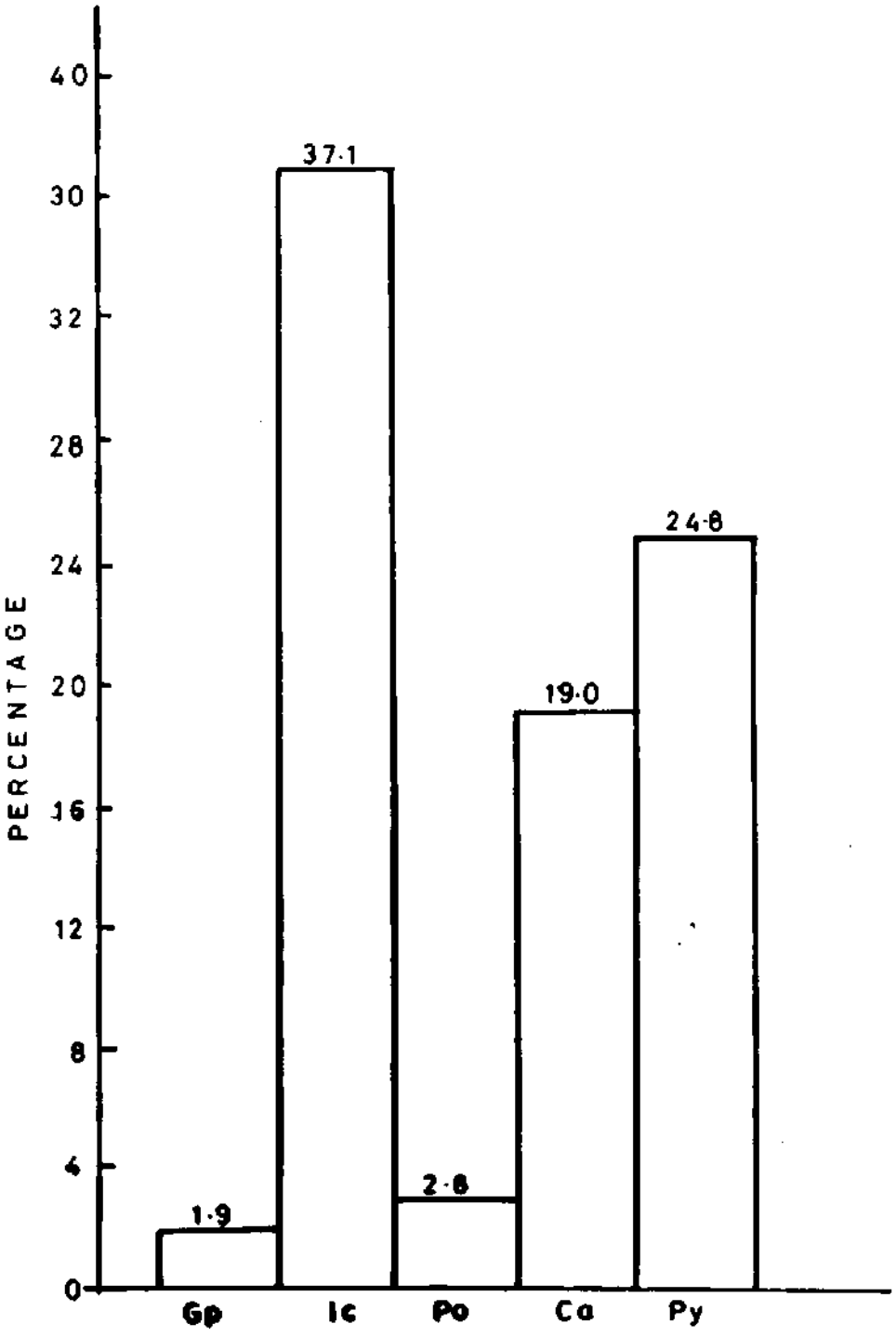
*Includes multiple reactors

TABLE 3: Leptospira Serotype Detected in Livestock in Eight Northern States of Nigeria - 1975/1976.

Serotype	State								
	Bauchi	Borno	Gongola	Kaduna	Kano	Plateau	Niger	Sokoto	Total
L. grippotyphosa	0	0	0	11	1	1	7	2	22
L. icterohemorrhagiae	3	2	0	15	12	2	6	4	44
L. hardjo	1	3	2	8	4	1	5	6	30
L. hebdomadis	2	7	2	11	13	4	5	7	51
L. pomona	2	3	9	6	13	3	7	10	53
L. canicola	1	0	0	5	1	2	2	0	11
L. pyrogenes	2	1	1	16	5	6	9	1	41
L. tarassovi	0	1	0	16	1	5	3	2	28
L. australis	0	0	2	17	1	1	0	0	21
Total	11	17	16	105	51	25	44	32	301



Fig. 1 Incidence of Leptospira serotypes in pigs in Kaduna and Kano states of Nigeria. (Gp = grippotyphosa; Ic = icterohemorrhagiae; Ca = canicola; and Py = pyrogenes).


PERCENTAGE
40
30
32
28
24
20
16
12
8
4
0
37·1
24·8
19·0
2·8
1·9
Gp
Ic
Po
Ca
Py

PART VI

BACTERIOLOGIC SURVEY FOR LEPTOSPIROSIS

INTRODUCTION

The leptospiral serotype distribution lists compiled by Sulzer (1975) indicates that leptospira serotypes have been isolated in only five African countries. In Tunisia, Leptospira icterohemorrhagiae has been isolated from Rattus norvegicus (Bakos and Cladli, 1966). In Egypt, L. canicola was isolated from a dog (Maronpot et al., 1971). Extensive survey of wildlife and domestic animals as potential reservoirs of leptospirosis in Kenya and Uganda was undertaken by Ball (1966) and Krauendonk et al. (1969), and resulted in isolation of L. icterohemorrhagiae, L. canicola, L. pyrogenes, L. autumnalis, L. australis, L. hebdomadis, L. bataviae and L. tarassovi. Baylet and Van Riel (1971) and Joyce-Clarke and Michaelis (1968) isolated L. icterohemorrhagiae in Senegal and in South Africa, respectively.

To the above list must be added that of pioneer leptospirologist Van Riel and his associates (1946, 1953, 1954, 1956) who isolated many leptospira serotypes including the following serotypes which are now reference strains: L.

nwoglo, L. ndambari and L. ndambukuje in icterohemorrhagiae serogroup, L. bafani and L. kamituga in the canicola group, L. butembo in the cynopteri group, L. jules, L. nona, L. kambale and L. kabura in the hebdomadis group, and L. kisuba in the tarassovi group.

While serological surveys have found that leptospirosis exists in all African countries where they have been undertaken, few epidemiologic surveys have been done so far. The findings of the above authors attest to the fact that there is still much to learn about leptospirosis in Africa.

Reported here is a study of potential reservoir hosts of leptospirosis in Nigeria.

MATERIALS AND METHODS

Rodents

Brown field rats (Arvicanthus niloticus[1]) gerbils (Gerbillus spp.), hedgehogs (Atelerix albiventris), white-

1. Identified by Mrs. Mary Dyer, Department of Biological Sciences, Ahmadu Bello University, Zaria.

tailed rats (Cricetomys gambianus), and house mice (Mus musculus) were caught around the premises of Ahmadu Bello University from November 1974 to December 1976. This area included the brush around the student hostels and along the Kubani river, the Kubani dam site, the NAPRI[2] research station at Shika, and University farm lake.

The rodents were brought to the laboratory, anaesthetized and bled aseptically by cardiac puncture with a 2 ml syringe and 21 gauge needle[3]. Sera were separated after clotting and stored at -20°C until tested.

Cultural procedures

After bleeding, the rodents were sacrified and a kidney was removed aseptically and squashed through a 2 ml syringe into 5 ml phosphate buffered saline (PBS). From this suspension, 0.5 ml was inoculated into 5 ml of Ellinghausen-McCullough medium (EM) containing 400 mcg/ml of 5-Fluoruradil. From this diluted medium, 0.5 ml was removed and

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2. National Animal Production Research Institute.

3. Terumo Corporation Tokeyo, Japan.

inoculated into a second tube of 5 ml EM medium.

The original FBS suspension was examined immediately after bleeding and sacrifice. Cultures were stored at 28 - 30°C in an incubator[4] and examined for 45 days.

Cattle

Kidneys were obtained from apparently healthy cattle after slaughter at Zaria and Kano abattoirs during January to April 1977.

Individual kidneys were collected in a sterile 400 ml beaker covered with aluminium foil. The kidneys were taken to the laboratory and processed within three hours of collection. Each kidney was cultured by removing the perirenal fat, sterilising the surface with a red hot spatula, aseptically peeling off the capsule, and by removing a 1 x 1 x 1 cm section with sterile scissors and placing it in sterile petri dish containing 5 ml of FBS. The kidney section was minced using sterile scissors and forceps,

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4. Gallenkamp, Essex, England.

allowed to settle and 0.5 ml of the suspension was inoculated into 5 ml of EM medium, and 0.5 ml of this dilution was inoculated into another tube of 5 ml of EM medium. The cultures and original kidney supernatant were then treated the same as the rodent kidneys.

Sewage Effluent

Sewage effluent from the Kano NLMA[5] piggery and pig abattoris were collected five times from July to September, 1976. Sewage effluent from the piggery was taken from 42 stations, including breeding pens, farrowing pens, fattening pens, boars pens, as well as main drainage lines.

At the Kaduna abattoirs sewage was taken from five stations. These were the pool of blood forming after slaughter and then at 10 m intervals along the drainage of the adjoining vegetable garden.

Specimen of sewage were filtered through No. 2 Whatman filter paper and 2 ml added to 2 to 3 ml double strength EM medium. The tubes were incubated at 28 - 30°C for 45 days and examined at weekly intervals.

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5. Nigerian Livestock and Meat Authority.

Identification of Cultures

When growth occurred in any culture, multiple EM tubes were inoculated and one was sent to the WHC/FAO Leptospirosis Reference Laboratory in London for storage and confirmation[6]. The other tubes were kept for identification using Difco[7] antisera by the method described by Turner (1968). The cultures were screened against 16 available Difco antisera and then tested against dilutions of those with positive reactions.

RESULTS

A total of 252 rodents were trapped and examined for leptospirosis; 221 brown field rats (A. niloticus), 13 white-tailed rats (C. gambianus), 5 house mice (M. musculus), 11 gerbils (Gerbillus spp.), and 2 hedgehogs (Atelerix albiventris). The results are given in Tables 1 - 5. Field rats only were positive (4.5%).

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6. Courtesy, Dr. L. H. Turner, Director.

7. Difco Laboratories, Detroit, Michigan, U.S.A.

Titers were obtained against L. icterohemorrhagiae, L. hardjo, L. tarassovi and L. australis. Except for L. icterohemorrhagiae, the titers were 1/100 or more. All positive sera reacted against a single serotype with one exception (Table 3).

Eight isolates were obtained from field rats; five L. australis, one L. ballum and the others were not identified (Table 4).

Of 74 cattle kidneys, 49 were obtained from Zaria abattoirs and 25 from Kano. Five isolates were made and one was identified as L. pyrogenes. Three isolates reacted against antiserum to serotypes ballum, australis, bataviae, canicola and icterohemorrhagiae. The fifth cattle isolate did not react against any of the 16 commercial antisera employed (Table 5).

No leptospira were isolated from the sewage effluents collected at Kano and Kaduna.

DISCUSSION

Galton et al., (1962) recommended inoculating multiple tubes with different types of media to insure optimal recovery of leptospira. Accordingly, most investigators inoculate two or three types of media. In this survey it was materially not possible to inoculate more than two tubes of medium and only Ellinghausen-McCullough's medium was used. It was found easy to prepare and convenient and it eliminated the need for an animal colony. Myers and Jelambi (1975) reported making all their isolations of L. hardjo from cattle on EM medium. They also found no growth occurred in Fletcher's medium.

Cultures were kept for 45 days and discarded. It was found that whether leptospira were seen under darkfield examination of kidney suspension or not, examination of cultures during the first two weeks of incubation was imperative for recovery of any leptospira. The risk of contamination and overgrowth by fungi or other bacteria increased greatly after the second week. This may be one reason why the three isolates were lost.

Serum titrations were not carried beyond 1/1600. Where 4+ reactors were recorded it was assumed that the endpoint

was beyond this dilution. However, readings of 2+ (50% of leptospira agglutinated) were considered endpoint readings.

The results indicate that all positive rats had been infected since they all but one reacted against a single antigen. Titers of 1/1600 were recorded against L. tarassovi and L. hardjo had titers of 1/100. Even though L. tarassovi and L. hardjo were not isolated, the titers recorded suggest that they will be isolated from animal species in the future. The reactions of L. icterohemorrhagiae indicate a possible minor role of this serotype as attested by the serological results of cattle in Zaria (Part II). Two of the rodents that yielded positive cultures had antibody titer. All others were negative. It is known that animal species can be carriers and seronegative and vice versa. The low rate of seroreactors and low isolation rates suggest the possibility of low grade infection of short duration in a susceptible animal population.

Contrary to the rodent population, cattle around Zaria had antibody titers against L. grippotyphosa, L. tarassovi and L. pyrogenes. Serotypes hardjo, hebdomadis and pomona

were found exclusively in slaughter cattle. The isolation of L. pyrogenes provides evidence of the public health significance of cattle in Kaduna state.

So far isolation of L. pyrogenes from bovine species has only been reported in South-East Asia in water buffaloes (Szatalowicz et.al., 1969) and in Peru (Sulzer, 1975). Other isolations have been limited to man, dogs, rodents and pigs. It is also significant that L. pyrogenes antibodies accounted for 25 per cent of the human seroreactors and 24 per cent of the swine seroreactors in Kaduna state (Part III and V).

L. australis and L. ballum were recovered from field rats (A. niloticus) and none of the other rodents surveyed. This rodent has been found to be a carrier for L. icterohemorrhagiae in Egypt (McGuire and Myers, 1957). In Kenya and Uganda, Ball (1966) failed to isolate any leptospira from the kidney cultures of 26 A. niloticus rats. It is significant that this is the first time L. australis and L. ballum have been reported in this species in Africa. The interaction of this species with other rodents in this region is apparent although they were found negative for leptospirosis

by serology and culture. The latter may be due to the small number sampled.

Mus musculus has been found to carry L. icterohemorrhagiae, L. ballum, and L. djatzi (Alexander, 1960; Noguchi, 1917). The field mouse Apodermus sylvaticus is also common in this area but none were caught. L. ballum and L. grippotyphosa have been isolated from this species (Fraya de Azevedo and Palmeira, 1961).

Rats, Rattus norvegicus and R. rattus, are established carriers of L. icterohemorrhagiae and L. canicola (Inada et al., 1916; Brown and Gorman, 1960; Roth et al., 1961a; Rademan et al., 1964).

The white-tailed rat (Cr. gambianus) has only recently been found to be a carrier of L. icterohemorrhagiae in Senegal (Baylet and Van Riel, 1971).

The epidemiologic importance of hedgehogs and gerbils has been shown by Berg-Petersen (1962) and Van der Hoeden and Shenberg (1962).

Man as well as animals is affected by the three sero-

types recovered. Sugar cane cultivation and small scale irrigation are important farming practices in the alluvial valleys of Kaduna state and hence potential public health hazards for human leptospirosis.

In Australia Johnson (1950) has associated L. australis with infections occurring in cane fields. L. ballum causes anicteric non-fatal type illness in man (Borst, 1948). L. pyrogenes infections are also known to cause non-fatal anicteric illness with conjunctival congestion and sometime meningitis. Workers in rice and sugar cane fields are reported to be infected with this serotype.

In Africa, Mailloux and Kolochine-Erbert (1961) reported two cases of L. australis infection in Pointe Noire. The patients had antibody titers of 1/100,000 and no isolation was made.

Recovery of L. ballum and L. australis from rodents and the high frequency of L. australis antibodies in cattle, further points to the epidemiologic importance of rodents and the promiscuous ecological relationship between rodent carriers and cattle grazing over the northern savanna zones.

This also suggests that in future L. ballum be part of any screening battery for leptospirosis in man and animals in Nigeria. This was the first epidemiologic survey undertaken and it is a preliminary to a larger investigation especially in the more moist savanna zones of Nigeria.

SUMMARY

A total of 252 rodents were trapped in the environs of Ahmadu Bello University, Zaria, over a two-year period: 221 brown field rats (A. nilcticus), 13 white-tailed rats (Cr. gambianus), 5 house mice (M. musculus), 11 gerbils (Gerbillus spp.), and 2 hedgehogs (Atelerix albiventris). Field rats only were found positive (4.5%). Titers were detected against L. australis, L. icterohemorrhagiae, L. tarassovi, and L. hardjo. All positive sera except one reacted against a single serotype. Eight isolates were obtained from field rats; 5 L. australis, 1 L. ballum, and 2 unidentified.

Five isolates were recovered from bovine kidneys; 1 L. pyrogenes and 4 unidentified. One unidentified isolate

failed to react with any of the 16 screening leptospira antisera and another was considered tentatively to be L. ballum.

No leptospira were isolated from the sewage effluents collected at Kano and Kaduna.

TABLE 1: Rodent Species Trapped in Zaria and Tested for Leptospirosis (Nov. 1974 - Dec. 1976).

Species	Total Sampled	Total Positive	
		Serology	Culture
A. niloticus	221	10	8
Cr. gambianus	13	-	-
Mus musculus	5	-	-
Gerbils	11	-	-
Hedgehogs	2	-	-
Total	252	10(3.9%)	8(3.2%)

TABLE 2: Rodents Sampled in Zaria from November 1974 to December 1976.

Year	Month	No.	Positive	
			Serology	Culture
1974	November	2	-	-
	December	2	-	-
1975	January	34	-	-
	February	31	1	-
	March	0	-	-
	April	10	-	-
	May	16	-	-
	June	24	-	-
	July	-	-	-
	August	26	1	-
	September	17	3	3
	October	20	5	4
	November	4	-	-
	December	-	-	-
1976	January	14	-	-
	February	7	-	-
	March	14	-	-
	April	-	-	-
	May	1	-	-
	June	-	-	-
	July	5	-	-
	August	-	-	-
	September	1	-	-
	October	-	-	-
	November	15	-	-
	December	4	-	1
Total		252	10	8

TABLE 3: Antibody iter of Brown Field Rats (*A. niloticus*) Trapped in Zaria and Tested Against Ten Leptospira Serotypes.

Rodent No.	Sex	Antibody Titer			
		ictero	*hardjo*	*tarassovi*	*australis*
WR 59	M	-	100	-	-
WR 128	F	-	-	1600+	-
WR 148	F	-	-	-	1600+
WR 158	M	-	-	-	1600+
WR 161	F	-	-	-	1600+
WR 163	M	-	-	-	1600+
WR 169	M	20	-	-	-
WR 174	F	20	-	-	-
WR 175	F	20	-	-	100
WR 176	F	20	-	-	-

ictero = *icterohemorrhagiae*

TABLE 4: Leptospira Titer and Isolates from Brown Field Rats (*A. nilcticus*) Trapped in Zaria, Nigeria.

Rodent No.	Sex	Serology		Culture
		Titer	Serotype	
WR152	F	-	-	*L. australis*
WR158	M	1600	*australis*	" " *
WR160	M	-	"	" "
WR163	M	1600	"	" "
WR175	M	-	-	" "
WRK27	F	-	-	*L. ballum*
WR164	F	-	-	unidentified **
WR172	F	20	ictero.'	"

* died before confirmation

** died before typing

.' ictero.: *icterohemorrhagiae*

TABLE 5: **Results of Agglutination of Cattle and Rodent Leptospira Isolates.**

Source	Isolate	Titer ballum	australis	batavine	canicola	icterc	pyrogenes	Identification
Cattle	CT 2b	-	-	-	-	-	3200	L. pyrogenes
	CT 3(1b)	200	100	100	100	20	-	not identified*
	CT 4(a)	100	100	20	100	100	-	" "
	CT 5(a)	3200	3200	3200	3200	3200	-	" "
	CT 6a	-	-	-	-	-	-	" "
Rodent	WRK 27	400	200	100	20	-	-	L. ballum
	WR 152	-	800	-	-	-	-	L. australis
	WR 160	-	800	-	-	-	-	L. australis
	WR 163	-	800	-	-	-	-	L. australis
	WR 175	-	800	-	-	-	-	L. australis

* L. ballum tentatively

** Died before typing was performed.

TABLE 5: (Cont'd)

Source	Isolate	Titer						Identification
		bellum	*australis*	*batavia*	*canicola*	*icterc*	*pyrogenes*	
Rodent	WR 164**							
	WR 172**							
	WR 158**							

* *L. bellum* tentatively

** Died before typing was performed

PART VII

PUBLIC HEALTH SIGNIFICANCE OF LEPTOSPIROSIS IN NORTHERN NIGERIA: GENERAL DISCUSSION

The problems encountered in surveys of this type in a developing country are complex and can be summarized into three main groups: organization, communication, and execution.

It was planned 5 to 10 herds to be sampled in each locality. This would have allowed collection of over 6,000 sera but only 4,940 were collected, and 93.4 per cent arrived at the laboratory. The sampling procedures were predetermined so as to allow for tracing back herds and owners at a later date for monitoring and determining exact incidence and prevalence rates. Organization was also complicated by the time factor. An ideal sampling would be when all species were present in sufficient numbers in an area at the time of collection. Hence, the plan was to start in the north and proceed southwards until the beginning of the rains, when the nomadic Fulbe cattle rearers would be migrating back northwards. For that purpose, sampling was started in July, 1974 and went in full scale during the period of December, 1975 to July, 1976.

In Nigeria methods of rearing cattle differ from those

of goats, sheep, and donkeys or horses. The cattle are driven to grazing areas and migrate during the dry season (December to May) from the Sudan savanna zone through the northern Guinea savanna zone to the more humid southern savanna zone. Sheep and goats are usually kept in and around the living compounds. They feed on small vegetation leaves and act more like scavengers. Donkeys are heavily utilized during the pre-rainy season and at the time of harvest they are used mainly for carrying loads. During the rest of the year, the donkeys are left to themselves.

This means that donkeys, goats and sheep are more exposed to humans than cattle and horses; ownership of the latter two is more restricted. Whereas one could plan on herds of cattle, sheep and goats were usually sampled in small groups of three to five per compound and one donkey per household. Sampling procedures had, therefore, to be carefully planned to obtain at least a "meaningful" number for each village or town.

During the survey, more than 30,000 km were covered. As the area surveyed was extremely vast, it was not possible to be away in the bush for more than a week at a time.

Communication and execution are two related factors under the survey conditions. Methods of rearing and keeping of animals in Nigeria differ from most countries. The Fulbe herdsman is attached to his cattle and is reluctant to have any blood drawn from his animals. While letters addressed to authorities made them aware of the work being conducted, it did not necessarily convince herdsmen why blood should be collected. This simple fact explains a number of discrepancies in collection technique and number of samples obtained. These factors were beyond our control.

It is well-known that reservoir hosts of most important zoonoses are various species of domestic and wild vertebrate animals (Schwabe, 1969). Infection in these species can occur independently of man. Human involvement usually occurs through occupational exposure. Leptospirosis figures as one of the diseases principally acquired through agricultural and agriculturally-related occupations (Anon., 1962).

The potential for becoming infected with leptospira

organisms as a result of other activities, however, is being increasingly recognized (Geistfield, 1975). In the United States, during 1971 - 1973, outbreaks of leptospirosis not connected with occupational exposure were reported. In Texas, an outbreak occurred in children who had played with infected dogs in pools of water after heavy rain (Barkin and Glasser, 1973). In Missouri, infected dogs were found shedding leptospira in their urine even though they had been vaccinated for leptospirosis (Feigin et al., 1973). In Oregon, an outbreak was attributed to contact with urine of infected cattle.

In the epidemiology of human leptospirosis in northern Nigeria it is more difficult to define occupational disease as the population is predominantly farming (90%). Even though occupational groups and agricultural communities are high risk entities, the incidence of leptospirosis can only be determined by investigation. Turner (1967) stated that it is no longer advisable to assume that leptospirosis does not exist because the so-called "classical symptoms" are unknown in a particular country. Accordingly, serological investigations can be conducted in livestock

and human populations to determine incidence and prevalence rates for leptospirosis. The results indicate presence or absence of leptospirosis in a population in a given area.

Conditions favorable for maintaining and spreading leptospirosis exist in Nigeria. The practice of digging earth for construction results in large craters on the outskirts of villages and towns. These open trenches eventually enlarge with alternating dry and wet seasons, become year round pools of stagnant water, which serve as wash areas and sources of water for domestic animals. In the bush these pools are part of the landscape and are established for providing water for cattle during the dry season and are also utilized by wildlife.

This serological survey identified three main animal groups as potential reservoirs for leptospirosis and therefore of public health importance in Nigeria: (a) cattle, dogs and rats; (b) donkeys, horses and swine; and (c) sheep and goats. The percentage incidence rate for leptospirosis for each species is shown in Fig. 1.

Cattle, dogs and rats have extensive human contact

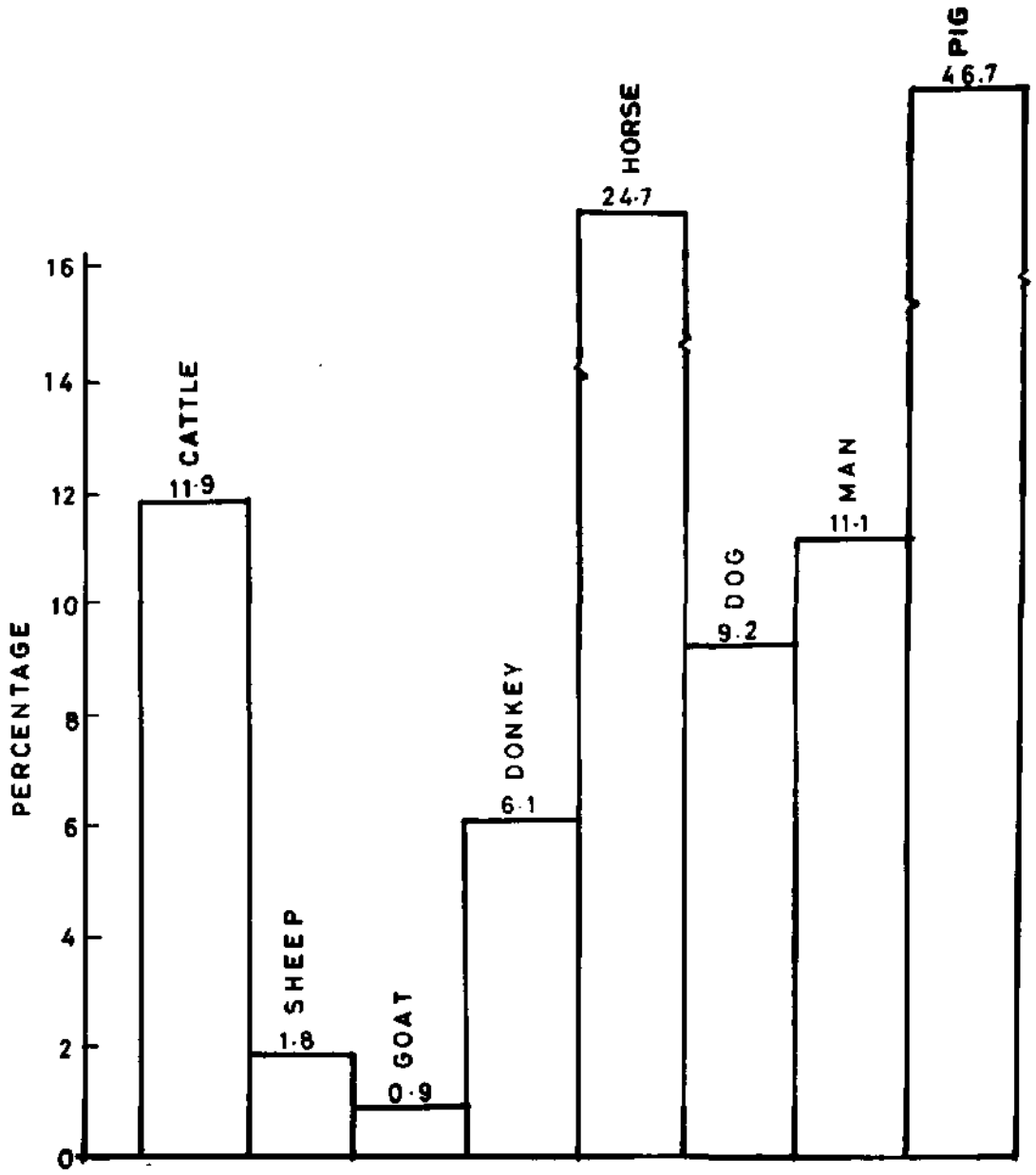


Fig. 1 Incidence of leptospirosis in domestic animals and man in northern Nigeria in 1975 - 76.

and they are known carriers of leptospira. The dogs are actually the most important source of home acquired leptospirosis except when the area is infected with rats (Meyer, 1964). In farm animals, pigs and cattle are common sources of leptospirosis. Dogs in Zaria and Jos had high titer serological reactions which suggested actually infection.

Isolation of L. pyrogenes and L. ballum from cattle and L. australis and L. ballum from rodents indicates the public health importance of these animals in Nigeria. Serreactions to these same serotypes were found to be prevalent in animal species originating from Zaria. This also adds to the epidemiology of leptospirosis in that it represents the first isolation of L. pyrogenes from cattle in Africa and probably the first isolation of australis and L. ballum from Arvicanthus spp. L. ballum is a serotype recognized to infect field mice and is known to be pathogenic to man (Borst, 1948). The recovery of this serotype from a bovine kidney indicates a relationship between cattle and rodent species.

Although swine, horses and donkeys have high titer reactions, this group's role as a public health hazard is

limited. Swine rearing is restricted to two large government piggeries. They are traditionally reared only in the southern part of Kaduna state where one also finds reactions to the same serotypes in man and other livestock. Exposure of horses is limited and donkeys have a low incidence of leptospiral antibodies.

Sheep and goats are capable of transmitting leptospirosis, but the low incidence found in this survey suggests they play a minor role in the epidemiology of human infection in Nigeria. Continuation of the survey in conjunction with isolation attempts from abattoir samples could probably yield more definite results.

Results obtained are significantly lower than those obtained by the team of students from the Royal Veterinary College, London, (Turner et al., 1975) who found 68.9 per cent prevalence of leptospira reactors in semi-nomadic cattle herds. This can be accounted for by the fact that the survey was always discontinued during the heavy rains and was concentrated around Zaria which is more accessible, whereas Turner's team collected their data during the month of July and August, 1975. They also noted a significant difference

between permanent government farms and the semi-nomadic herds, which they ascribed to management practices. The same can be said of the nomadic herds sampled in the present survey. Cattle are driven through grazing reserves and continue their journeys north or south depending on the time of the year. They were not concentrated in particular areas except in the night enclosures where they are tied in pairs. These camp sites are important during the rainy season when they become muddy quagmires they are approximately 10 cm deep.

An examination of incidence in different states indicates that georgraphical regions within Nigeria are important in the distribution of leptospirosis. Incidence varied from 4.7 per cent in Kano state to 15.7 per cent in Niger state. All nine serotypes were found to occur in man and livestock. Surveys conducted in other African countries in the same latitude revealed the same serotypes (Van Riel et al., 1971; Shigidi, 1975; Koch et al., 1975). Further, where large enough samples were obtained the incidence was high in Plateau and Kaduna states, whereas it was low in the more northern drier states of Borno and Sokoto. Finally, more

serotypes were found in states like Niger, Plateau and the southern part of Kaduna state, all approximately in the same latitude.

Incidence in human population was about the same as in cattle. There is no doubt that leptospirosis is underdiagnosed in Nigeria, first because most clinicians did not suspect it, secondly, because most laboratories are ill-equipped to diagnose leptospirosis. The results described here provide information of value to public health and clinical medicine in the areas surveyed. They emphasize that leptospirosis should be considered in the differential diagnosis of F.U.O., hepatitis and of suspected viral infections of the central nervous system.

In a serologic survey of veterinarians in Nigeria for brucellosis, leptospirosis and Q. fever, two (7.7%) were positive for leptospirosis (Ayiver et al., 1975). This represented evidence of infection in two recent graduates in a high risk occupation for leptospirosis and other zoonoses.

It is concluded from this study that leptospirosis is present in the eight northern states of Nigeria and that

rodents, cattle and dogs are the principal carriers and are potential public health hazards.

It is considered that continuous monitoring for leptospirosis over a period of years will be needed, and will permit isolation and identification of the serotypes present in Nigeria. Along this line the following work has been planned:

1. An epidemiologic investigation of leptospirosis in the environs of Zaria involving dogs, rodents, and small ruminants.

2. Continue isolation studies of sewage effluents from local slaughter establishments.

3. Investigate the possible role of leptospirosis hepatitis in F.U.O. and aseptic meningitis at Ahmadu Bello University Teaching Hospital.

4. Antigenic analysis of isolated serotypes.

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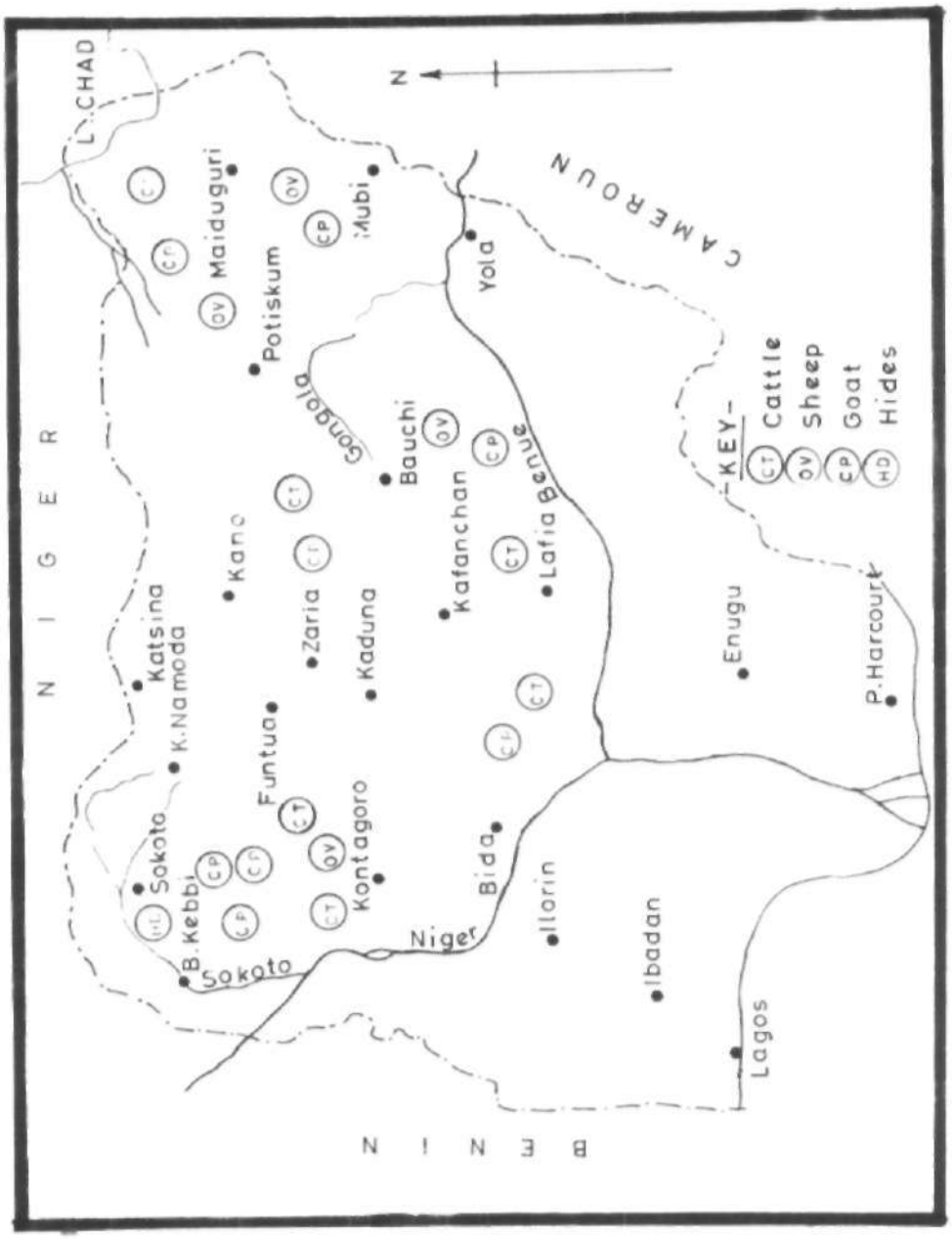
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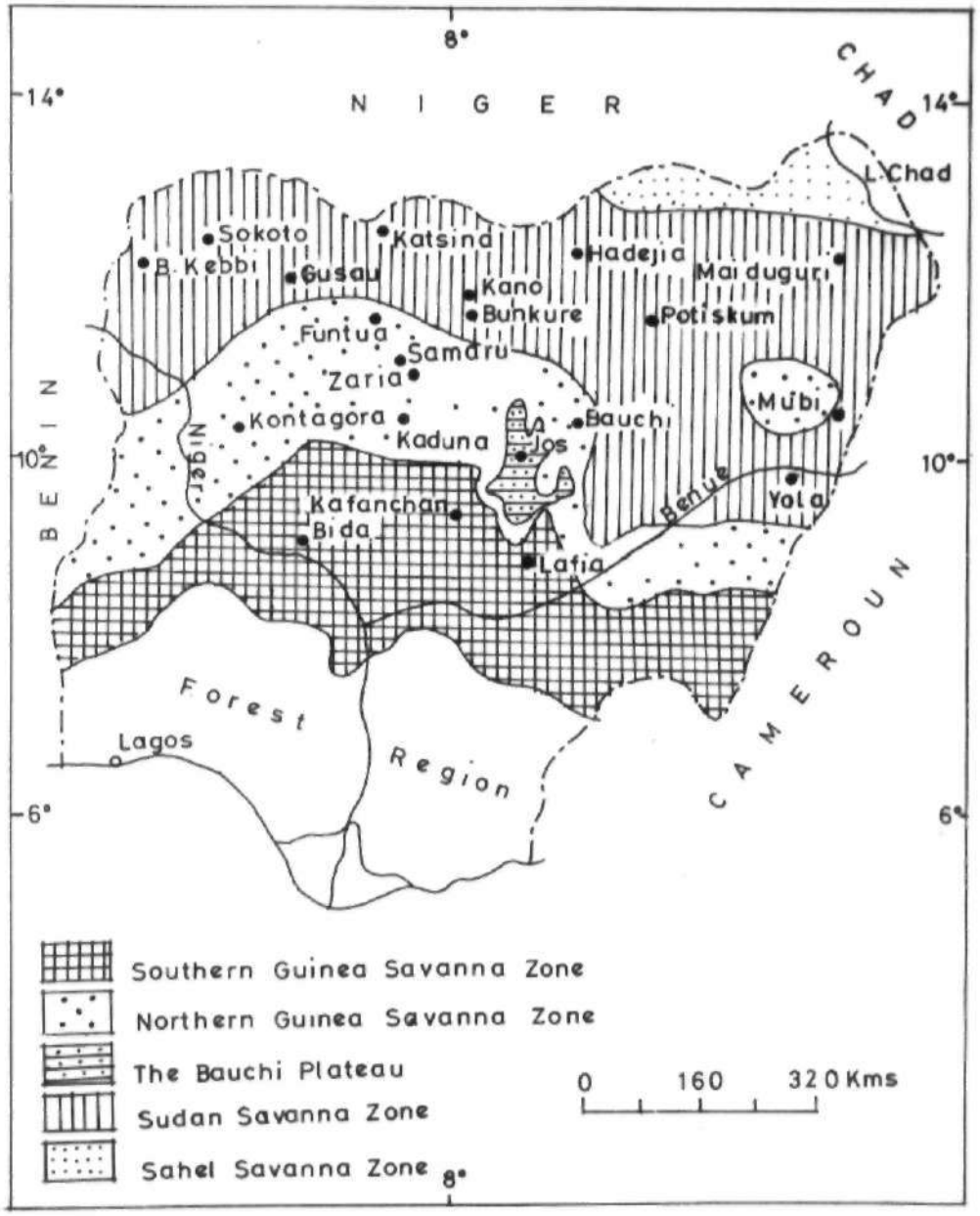
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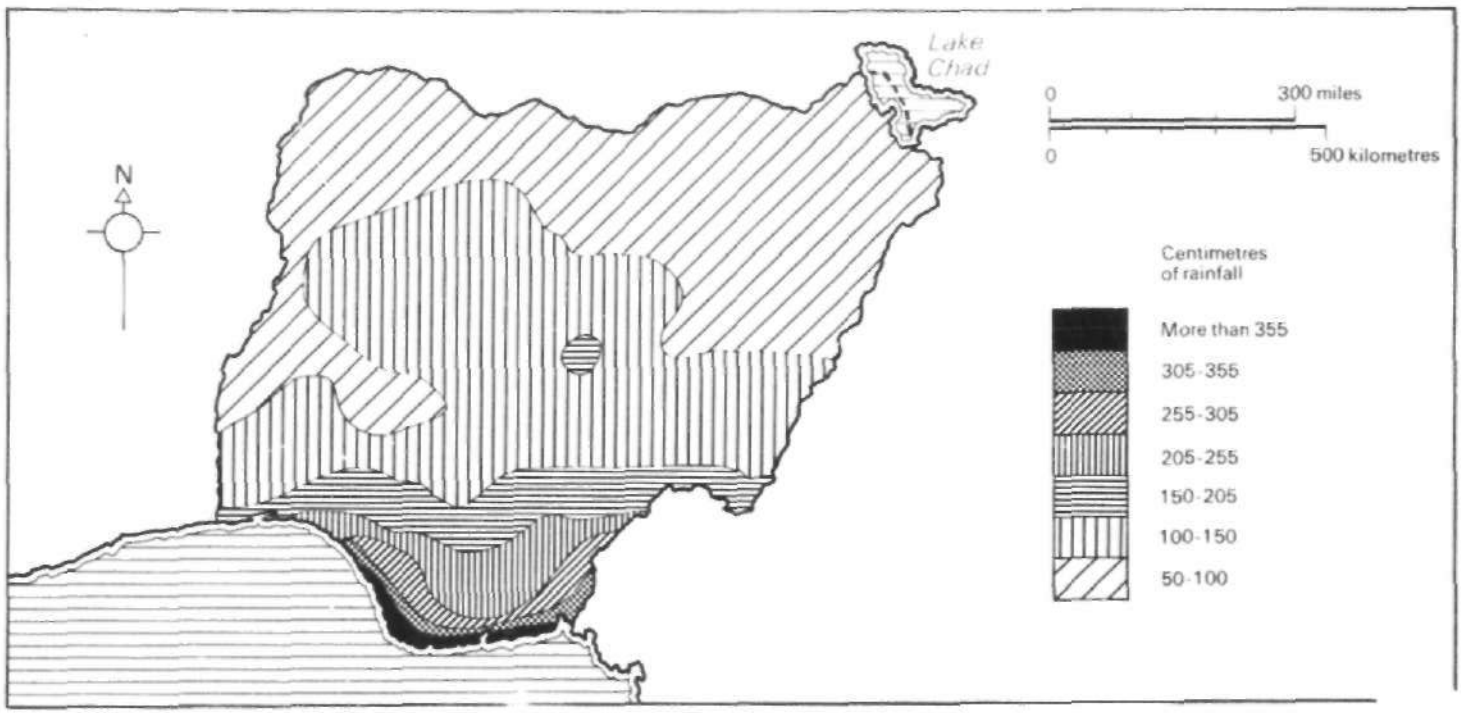
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APPENDIX 1

Maps of Nigeria


L. CHAD
N
CAMEROUN
NIGER
BENIN
Maiduguri
Potiskum
Mubi
Yola
Gongola
Bauchi
Kafanchan
Lafia
Benue
Kano
Zaria
Kaduna
Katsina
K.Namoda
Funtua
Sokoto
B.Kebbi
Kontagoro
Bida
Niger
Sokoto
Ilorin
Ibadan
Lagos
Enugu
P.Harcourt
KEY-
CT Cattle
OV Sheep
CP Goat
HD Hides


8°
14°
N I G E R
CHAD
14°
L. Chad
Sokoto
Katsina
Hadejia
B. Kebbi
Gusau
Kano
Maiduguri
Bunkure
Potiskum
Funtua
Samaru
Zaria
Mubi
Kontagora
Bauchi
Kaduna
Jos
10°
BENIN
Niger
Benue
Yola
Kafanchan
Bida
Lafia
CAMEROUN
Forest
Region
Lagos
6°
Southern Guinea Savanna Zone
Northern Guinea Savanna Zone
The Bauchi Plateau
Sudan Savanna Zone
Sahel Savanna Zone
0 160 320 Kms
8°



Total rainfall

APPENDIX 2

Provisional List of Leptospira Serotypes

Provisional list of Leptospira serotypes adapted from Turner (1976).

1. Icterohemorrhagiae Serogroup

icterohemorrhagiae	sarmin	weaveri
copenhageni	birkini	hualien
mankarso	smithi	gem
naam	ndambari	monymusk
mwogolo	ndahambukuje	tonkini
dakota	budapest	bog-vere

2. Canicola Serogroup

canicola	sumneri	benjamin
bafani	brocmi	malaya
kamituga	bindjei	portland-vere
jonsis	schueffneri	galtoni

3. Pyrogenes Serogroup

pyrogenes	biggis	manilae
zanoni	hamptoni	camlo
myocastoris	alexi	varela
abramis	robinsoni	guaratuba

4. Pomona Serogroup

pomona	mozdok	dania
kennewicki	tropica	tsaratsovo
monjakov	proechimys	

5. Grippotyphosa Serogroup

grippotyphosa	ratnapura	rattus
valbuzzi	vanderhoedeni	

6. Hebdomadis Serogroup

hebdomadis	szwadjizak	polonica
nona	georgia	saxkoebing
kambale	peraneles	haemolytica
kremastos	hardjo	recardi
worsfoldi	recreo	geeyaweera
jules	medanesis	guaricurus
maru	wolffi	gorgas
borincana	trinidad	beye
kabura	sejroe	tabaquite
mini	balcanica	goiano

N.B. nero is now considered to be identical with saxkoebing

7. Tarassovi Serogroup

tarassovi	atachafalaya	tunis
bakeri	chagres	langati
atlantae	gatuni	kaup
guidae	osetane	vughia
kisuba	darien	moldaviae
bravo	viouous	

8. Panama Serogroup

panama	cristobali

9. Australis Serogroup

australis	bratislava	pina
lora	fugis	nicaragua
muenchen	bangkok	hawain
jalna	peruviana	wewak (?)

10. Javanica Serogroup

javanica	coxi	anhoa
poi	sofia	waskurin (?)
sorex-jalna	ceylonica	

11. Autumnalis Serogroup

autumnalis	erinacei-auriti	gurungi
rachmati	mooris	weerasinghe
fort-bragg	sentot	alice
sumatrana	louisiana	lanka
bulgarica	orleans	srebana
bangkinang	djasiman	

12. Cynoptei Serogroup

cynopteri	canalzonae	butembo (?)

13. Bataviae Serogroup

bataviae	balboa	belorussiae
paidjan	claytoni	habaki
djatzi	brasiliensis	
kobbe	argentiniensis	

14. Ballum Serogroup

ballum	castellonis	arboreae

15. Shermani Serogroup

shermani

16. Ranarum Serogroup*

ranarum evansi

17. Bufonis Serogroup*

carlos

*Two new proposed serogroups

APPENDIX 3

Serologic Survey for Leptospirosis
Tables 1 - 15

TABLE 1: Cattle Seroreactors to Leptospirosis From Borno, Bauchi and Gongola States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer ictero*	hardjo	hebdo*	pomona	canicola	australis
Borno	Maiduguri	45	A**	F	-	-	100	-	-	-
		46	A	F	-	-	100	-	-	-
		47	A	F	-	200	20	-	-	-
		51	A	F	-	-	20	-	-	-
		61	A	F	-	-	-	100	-	-
		64	A	F	-	-	-	100	-	-
		69	A	F	-	-	20	-	-	-
	Potiskum	81	A	F	100	20	200	-	-	-
		82		F	-	-	-	200	-	-
		89		F	100	20	100	-	-	-
Bauchi	Bauchi	7	3	F	20	-	-	-	20	-
		21	2½	F	-	-	20	400	-	-
		39	2	F	-	200	20	100	-	-
		103	-	-	20	-	-	-	-	-
Gongola	Nubi	134	2	F	-	-	-	20	-	-
		136	3	F	-	20	20	-	-	-
		138	5	F	-	-	-	20	-	100
		146	7	M	-	-	-	100	-	-
		162	3	F	-	-	-	20	-	-
		163	7	F	-	20	-	100	-	-

* icterohemorrhagiae and hebdomadis

** Adult

TABLE 1: (Cont'd)

State	Town	No.	Age (yrs.)	Sex	Titer					
					*ictero**	*hardjo*	*hebdo**	*pomona*	*canicola*	*australis*
	Mubi	169	8	F	-	-	-	-	-	100
		172	6	F	-	-	20	100	-	-
	Yola	265	2	F	-	-	-	800	-	-
		288	6	F	-	-	-	100	-	-
		290	6	F	-	-	-	20	-	-

* *icterohemorrhagiae* and *hebdomadis*

** Adult.

TABLE 2: Cattle Seroreactors to Leptospirosis From Kano and Sokoto States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer ictero*	hardjo	hebdo*	pomona	tarassovi
Kano	Hadejia	10	7	F	20	-	-	-	-
		11	8	F	20	-	-	-	-
		1	2	M	20	-	-	-	-
		2	4	F	20	-	-	-	-
		5	7	F	-	-	100	100	-
		6	7	F	-	-	100	-	-
		7	6	F	-	-	100	-	-
		9	6	M	-	-	-	20	-
		10	6	F	-	20	-	100	-
	Kadawa	1	A**	F	-	-	20	-	-
	Bunkura	1	7	F	-	-	200	200	-
		7	2	F	-	-	100	400	-
		9	4	F	-	-	20	-	-
		13	2	F	-	-	200	-	-
		14	5	M	-	-	-	-	200
		22	3	F	-	-	100	-	-
		24	7	F	-	-	-	20	-
		27	1½	M	-	-	200	-	-

* icterohemorrhagiae and hebdomadis

** Adult

TABLE 2: (Cont'd)

State	Town	No.	Age (yrs.)	Sex	Titer ictero*	hardjo	hebdc*	pomona	tarassovi
Kano	Metro	13	3	F	-	-	200	400	-
		16	6	F	-	-	-	200	-
		22	1	M	-	-	-	100	-
		27	8	F	-	100	20	-	-
		28	7	F	-	-	-	20	-
		32	7	F	-	-	-	200	-
		45	8	F	20	-	-	-	-
		60	4/12	F	-	20	-	-	-
		67	5	F	-	20	20	-	-
		69	9	F	-	-	-	20	-
Sokoto	Birnin Kebbi	6	5	F	-	-	-	100	-
		19	6	F	-	200	200	1600	-
		20	7	F	-	20	-	200	-
		24	14	F	-	-	20	400	-
		26	14	F	-	-	20	400	-
	Gusau	4	6	F	-	20	20	100	-
		13	3	F	-	800	200	1600	-
		22	8	F	-	20	-	100	-
		23	7	F	-	-	-	-	100
		27	3	F	100	-	200	-	-
		29	2	F	-	20	-	-	-
	Sokoto Metro	7	6	F	-	-	-	400	-
		16	4	F	-	-	20	100	-

* icterohemorrhagiae and hebdomadis ** Adult

TABLE 3: Cattle Seroreactors to Leptospirosis from Plateau and Benue States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	grippo*	ictero*	hebdo*	pomona	cani*	pyrog*	taraas*
Plateau	Jos	1	10	F	-	-	-	20	-	-	-
		2	10	F	-	-	-	-	-	-	100
		4			-	-	-	-	-	-	100
		5	4	F	-	-	100	800	-	-	-
		33	7	F	-	-	-	-	-	-	100
		40	9	F	-	-	-	-	-	-	20
		46	5	F	-	-	-	-	400	-	-
		47	6	M	-	-	-	-	400	-	-
Benue	Lafia	3	11	F	-	-	100	-	-	-	-
		5	8	F	20	-	-	-	-	-	-
		10	10	F	-	-	100	-	-	-	-
		31	9	F	-	20	-	-	-	100	-

* grippotyphosa, icterohemorrhagiae, hebdomadis, canicola, pyrogenes and tarassovi

TABLE 4: Cattle Seroreactors to Leptospirosis from Niger State, Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	ictero*	hardjo	hebdo*	pomona	cani*	pyro*	tarass*
Niger	Abuja	3	4	M	-	20	-	-	20	-	-	-
		4	13	F	-	100	-	-	-	-	-	-
		16	6	M	-	-	100	20	-	-	-	-
		17	5	F	20	-	-	-	-	-	-	-
		36	2	F	100	-	-	-	-	-	-	-
		44	5	F	-	-	-	-	-	-	400	-
		54	4	F	20	-	-	-	-	-	-	-
		56	4	M	-	20	-	-	-	-	-	-
	Bida	14	10	F	-	-	20	-	100	-	-	-
	Kontagora	1	6	M	-	-	-	-	-	20	-	-
		3	3	M	-	-	-	-	-	-	-	20
		15	2	M	-	-	-	100	400	-	-	-
		16	4	F	-	-	-	20	1600	-	-	-
		20	2	M	-	20	400	-	-	-	-	-
		27	3	M	-	-	-	-	-	-	400	-

* grippotyphosa, icterohemorrhagiae, hebdomadis, canicola, pyrogenes, and tarassovi

TABLE 5: Cattle Seroreactors to Leptospirosis From Kaduna State, Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	ictero*	hardjo	hebdo*	pomona	cani*	pyrog*	tarass*	WR.
Kaduna	Kafanchan	3	9	F	-	-	-	-	20	-	-	-	-
		6	9	F	-	-	20	-	-	-	-	-	-
		7	7	F	-	200	20	20	-	-	-	-	-
		13	4	M	-	-	-	-	-	-	20	-	-
	Katsina	9	2½	M	-	-	-	100	-	-	-	-	-
		10	6	F	-	-	400	20	-	-	-	-	-
		14	3	M	-	-	-	20	-	-	-	-	-
		16	2	F	-	-	200	-	-	-	-	-	-
		17	4	F	-	-	-	1600	-	-	-	-	-
		26	5	F	-	-	20	-	-	-	-	-	-
		28	6	F	-	-	-	-	-	-	-	400	-
	Funtua	17	8	F	200	-	-	-	-	-	-	-	-
		20	4	F	-	-	-	-	100	-	-	-	-
		22	6	F	20	-	-	-	-	-	-	-	100
		24	5	F	-	-	100	-	-	-	-	-	-
		35	2	F	-	-	-	-	-	-	-	-	20
		42	5	M	-	-	-	-	-	20	-	-	-
	Zaria	1196		F	-	-	-	-	-	-	-	-	200
	Shika	??		F	-	-	-	-	-	-	-	-	200
		1009		F	-	-	-	-	-	-	-	800	-
		1016		F	-	-	-	-	-	-	-	20	-
		1027		F	400	-	-	-	-	-	-	400	100
		1042		F	-	-	-	-	-	-	-	400	100

* grippotyphosa, icterohemorrhagiae, hebdomadis, canicola, pyrogenes, and tarassovi

TABLE 5: (Cont'd)

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	ictero*	hardjo*	hebdo*	romona	cani*	pyrog*	tarass*	WR.
	Shika	1054		F	-	-	-	-	-	-	-	1600	400
		1060		F	200	-	-	-	-	-	-	-	-
		1159		F	-	-	-	-	-	-	-	800	200
		183		F	100	-	-	-	-	-	-	-	-
		496		F	-	-	-	-	-	-	-	20	-
		813		F	-	-	-	-	-	-	-	-	400
		874	?	F	-	-	-	-	-	-	-	-	400
		901		F	-	-	-	-	-	-	-	200	-
	Abattoir	1	-	-	100	-	20	-	-	-	100	-	20
		3	-	-	-	20	-	-	-	-	-	-	-
		4	-	-	100	-	-	-	-	-	20	-	-
		5	-	-	-	-	-	-	-	-	100	-	-
		6	-	-	-	-	-	-	-	-	-	20	-
		7	-	-	-	-	-	20	100	-	-	20	-
		8	-	-	-	-	-	20	100	-	-	800	-
		9	-	-	-	-	-	-	20	-	-	800	-
		10	-	-	-	-	-	20	20	-	-	-	-
		12	-	-	20	-	-	-	-	-	-	-	-
	Samaru	14	-	-	-	-	-	-	-	-		800	100
	Abattoir	15	-	-	-	-	-	-	-	-	100	-	20
		17	-	-	-	20	-	-	-	-	-	-	-
		18	-	-	-	20	-	-	-	-	-	-	-
		23	-	-	-	-	-	-	-	20	-	-	-

* grippotyphosa, icterohemorrhagiae, hebdomadis, canicola, pyrogenes and tarassovi

** WR= australis

TABLE 6: Sheep Seroreactors to Leptospirosis From Kaduna, Plateau, Bauchi and Niger States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	hebdo*	pomona	pyrog*	tarass*	Aust*
Kaduna	Kafanchan	6	3	F	-	-	-	20	-	100
		20	3	F	-	-	-	100	-	-
		10	2	M	-	-	-	20	-	-
		18	3	F	-	-	-	-	20	-
Plateau	Jos	15	1	F	-	200	1600	-	-	-
Bauchi	Bauchi	9	-	-	-	-	-	20	-	-
Niger	Abuja	5	9/12	F	100	-	-	-	-	-
		12	A**	F	20	-	-	-	-	-
		13	A	F	20	-	-	-	-	-
		14	A	F	-	-	-	20	-	-

* grippotyphosa, hebdomadis, pyrogenes, tarassovi and australis

** Adult

TABLE 7: Goat Seroreactors to Leptospirosis From Kaduna and Kano States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer ictero*	canicola	pyrogenes
Kaduna	Katsina	6	4	F	-	-	-
	Kafanchan	6	5	F	100	1600	1600
Kano	Kano	6	2	F	-	-	20
		8	A**		200	-	20

* icterchemorrhagiae

** Adult

TABLE 8: Horse Seroreactors to Leptospirosis From Bauchi, Borno, Kaduna and Gongola States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer ictero*	canicola	pyrog*	tarassovi
Borno	Potiskum	2	A**	M	-	-	-	20
		4	A	M	-	-	20	-
Gongola	Yola	2	8	M	-	-	20	-
Bauchi	Bauchi	2	7	M	-	-	20	-
Kaduna	Katsina	1	5	M	20	-	20	-
		4	8	M	100	-	20	-
		6	7	M	-	-	20	-
		9	6	M	-	-	20	-
	Kafanchan	2	8	M	20	-	-	-
	Funtua	8	7	M	-	100	20	-
		1	5	M	100	-	-	-
					-	20	-	-

* icterohemorrhagiae and pyrogenes

** Adult

TABLE 9: Horse Sero-reactors to Leptospirosis From Kano, Plateau, Sokoto and Niger States of Nigeria.

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	ictero*	hardjo	hebdo*	pomona	cani*	pyrogenes	tarass*
Kano	Kano	1	5	M	20	20	-	-	-	-	-	-
		6	6	M	-	1600	-	-	-	-	-	-
		10	11	M	-	20	-	-	-	-	-	-
		11	5	M	-	200	-	-	-	-	-	-
	Wudil	3	8	M	-	200	-	-	-	-	-	-
		10	6	M	-	200	-	-	-	-	-	-
Plateau	Jos	2	8	M	-	-	20	20	20	-	-	-
		8	6	M	-	20	-	-	-	-	20	-
	Lafia	2			-	-	-	-	-	-	20	-
Sokoto	Gusau	2	8	M	20	-	-	-	-	-	-	200
		5	4	M	100	20	-	-	-	-	-	-
		11	4	F	-	20	-	-	-	-	-	-
Niger	Abuja	2			-	-	-	-	-	-	-	-
	Bida	1			-	-	-	-	-	-	100	-
		4	6	M	-	-	-	-	-	-	100	-
		7			-	-	-	20	-	100	-	-

* icterohemorrhagiae, griprotyphosa, hebdomadis, pyrogenes, canicola and tarassovi

TABLE 9: (Cont'd)

State	Town	No.	Age (yrs.)	Sex	Titer grippo*	icterо*	hardjo	hebdo*	pomona	cani*	pyrogenes	tarass*
	Kontogora	1	8	M	-	-	-	-	-	-	20	-
		8	2	M	-	-	-	-	-	-	-	100
		14	5	M	-	-	100	-	20	-	-	-
		16	17	M	-	-	100	-	-	-	20	-
		17	4	M	-	-	-	20	-	-	-	-
		18	-	-	100	-	-	-	-	-	-	20
		19	2	M	-	-	-	-	20	-	-	-

*icterohemorrhagiae, grippotyphosa, hebdomadis, pyrogenes, canicola and tarassovi

TABLE 10: Donkey Leptospiral Seroreactors in Kaduna, Kano, Bauchi, Niger and Sokoto States From 1975 to 1976

State	Town	No.	Age (yrs.)	Sex	Titer								
					grippo*	ictero	hardjo	hebdo	lomona	canicola	pyrog	tarass	australis
Kaduna	Kafanchan	18	7	M	-	20	-	-	-	-	-	-	-
		2	8	M	-	-	-	-	-	-	-	-	1600
	Katsina	5	7	M	-	20	-	-	-	-	400	-	100
		1	6	F	-	-	-	-	-	-	-	20	-
		9	5	F	20	-	-	-	-	-	-	-	-
	Zaria	9	5	M	100	-	-	-	-	-	-	-	-
		10	5	M	-	-	100	20	-	-	-	-	-
		11	5	M	-	20	-	400	-	-	-	-	-
		13	4	M	-	20	-	-	-	-	20	-	20
		15	5	M	-	-	-	100	-	-	-	-	-
		30	5	M	-	20	-	-	-	-	-	-	-
		32	A	M	100	20	-	-	-	-	-	-	-
Kano	Kano	3	3	M	-	-	-	-	-	-	20	-	-
		5	5	M	-	-	-	-	100	-	-	-	-
		24	2	M	-	-	-	-	-	-	20	-	-
	Rano	3	5	M	-	-	-	-	-	20	100	-	-
Bauchi	Bauchi	1	-	-	-	20	-	-	-	-	-	-	-
Niger	Kontagors	2	6	M	-	100	-	-	-	-	-	-	-
		27	4	M	-	-	-	-	-	-	20	-	-

* grippotyphosa, icterohemorrhagiae, hebdomadis, pyrogenes, tarassovi, australis

TABLE 10: (Cont'd)

State	Town	No.	Age (yrs.)	Sex	Titer								
					grippo*	ictero	hardjo	hebdo	pomona	canicola	pyrog	tarass	australis
	Kontagora	37	3	M	-	-	-	-	-	-	100	-	-
		40	4	M	-	200	-	-	-	-	-	-	-
		41	5	M	-	-	-	-	400	-	-	-	-
Sokoto	Argungu	7	4	M		-					20		
		13	5	F		20					-		

* grippotyphosa, icterohemorrhagiae, hebdomadis, pyrogenes, tarassovi, australis

TABLE 11: Human Leptospiral Seroreactors in Bauchi and Gongola States From 1975 to 1976.

State	Town	Serum No.	Age (yrs.)	Sex	Titer							
					grippo*	hardjo	hebdo	romona	canicola	pyrog	patoc	tarass
Bauchi	Bauchi	13	A	M	20	-	-	-	-	-	-	-
		14	A	M	200	-	-	-	-	-	-	-
		16	A	M	-	-	-	200	-	-	-	-
		22	A	M	20	-	-	-	-	-	-	-
		28	27	F	-	100	200	200	-	-	-	-
		30	20	M	-	-	-	100	-	-	-	-
		35	A	M	-	-	-	-	-	-	-	400
		39	20	M	-	-	-	-	-	-	-	100
		46	A	M	-	-	20	100	-	-	-	-
		47	A	M	-	-	-	-	-	200	-	-
		54	21	F	-	-	-	-	20	-	-	-
Gongola	Mubi	9	20	M	-	-	-	-	-	100	-	-
		15	17	M	-	-	-	-	-	-	-	200

* grippotyphosa

TABLE 12: Human Leptospiral Serореactors in Kaduna State From 1975 to 1976.

State	Town	Serum No.	Age	Sex	Titer grippo*	ictero	hardjo	hebdo	pomona	canicola	pyrog	patoc	tarass	WR.
Kaduna	Kaduna	25	25	M	-	-	-	-	-	-	-	-	20	-
		10	19	M	-	-	-	-	-	20	-	-	-	-
		36	38	M	20	20	-	-	-	-	-	-	-	-
		35	A	F	-	-	-	-	-	-	-	20	-	-
		38	15	M	-	-	-	-	-	-	-	20	-	-
		40	A	F	-	-	-	-	-	-	-	20	-	-
		46	86	M	-	-	20	20	-	-	-	20	-	100
		48	23	F	-	-	-	-	-	-	-	20	-	-
		54	30	F	-	-	-	20	-	-	-	-	-	-
		62	28	F	-	-	-	-	20	-	-	-	-	-
		76	38	M	-	-	-	-	-	-	-	20	-	-
		77	20	M	-	-	-	-	-	-	-	100	-	-
		83	29	M	-	-	100	-	20	-	-	-	-	-
		84	A	F	-	-	-	-	-	-	-	20	-	-
		88	30	M	-	-	20	20	-	-	200	-	-	-
		89	A	M	-	20	-	20	100	-	-	-	-	-
		91	28	M	-	-	-	20	20	-	-	-	-	-
		92	30	M	-	20	-	-	-	-	-	-	-	-
		104	22	F	-	-	-	20	-	-	-	20	-	-
		108	27	F	-	-	-	100	-	-	-	-	-	-
		111	26	F	-	20	-	20	-	-	-	20	-	-
		115	18	F	-	-	-	-	-	-	20	-	-	-
		121	25	F	-	-	-	-	-	-	-	20	-	-

* grippotyphosa ** WR = australis

TABLE 12: (Cont'd)

State	Town	Serum No.	Age	Sex	Titer grippo*	ictero	hardjo	hebdo	pomona	canicola	pyrog	patoc	taraas	WR.**
	Kaduna	123	24	F	-	-	-	-	-	-	-	20	-	-
		124	21	F	-	-	-	-	-	-	100	20	-	-
		140	20	M	-	-	20	20	100	-	-	-	-	-
		158	18	M	-	-	-	-	-	-	20	-	-	-
		162	30	M	-	-	-	20	-	-	-	-	-	-
		166	27	M	-	-	-	-	-	100		-	-	-
		168	30	M	100	-	-	-	-	-		-	-	-
		171	31	M	-	-	-	-	-	-		-	20	-
		177	21	F	-	100	-	-	-	-		20	-	-
		178	14	F	-	-	-	-	-	-		100	20	-
		181	22	F	-	-	-	-	-	-		-	-	100
		182	20	F	-	200	-	-	-	20		-	-	-
		184	17	F	-	-	20	Trace	100	-		-	20	-
	Kafanchan	64	22	F	-	-	-	-	20	-		-	-	-
		75	16	F	-	Trace	-	-	-	-		-	-	-
	Zaria	119504	A	F	-	20	-	-	-	-		-	-	-
		524294	A	F	-	-	-	-	-	-		-	-	100

* grippotyphosa,

**WR = australis

TABLE 13: Human Leptospiral Seroreactors in Kano, Niger and Sokoto States from 1975 to 1976

State	Town	Serum No.	Age	Sex	Titer grippo	ictero	hebdo	pomona	canicola	pyrog	patoc	tarass
Kano	Kano	K 960	26	F		-	-	-	-	-	20	-
		K1714	A	F		100	-	-	-	-	-	-
		K1818	A	F		100	-	-	-	-	-	-
		K 943	23	M		20	-	-	-	-	20	-
		C 500	A	M		100	-	-	-	-	-	-
	Hadejia	9	25	M		-	-	-	-	-	-	20
		20	25	F		20	-	-	-	-	-	-
		22	32	M		20	-	-	-	-	-	-
		35	21	F		-	-	-	-	-	20	-
Niger	Kontagora	16	20	M		-	-	-	20	800	-	-
	Abuja	28	15	M		20	-	-	-	-	-	-
		34	15	M	100	-	-	-	100	-	-	-
Sokoto	Argungu	5	15	M	-	-	-	-	-	200	-	-
		6	8	M	-	20	-	-	-	-	-	-
		25	22	F	-	-	-	-	-	-	20	-
		27	40	F	-	-	-	-	-	-	-	200
	BKB.	6	19	F	-	-	200	-	-	-	-	-
		8	25	F	-	-	20	-	-	-	-	-
		16	22	F	-	-	400	-	-	-	-	-
		18	21	F	-	-	-	-	-	-	-	400
		32	22	F	-	-	-	-	-	-	-	20

TABLE 13: (Cont'd)

State	Town	Serum No.	Age	Sex	Titer							
					grippo	ictero	hebdo	pomona	canicola	pyrog	patoc	tarass
	Gusau	3	13	F	-	-	-	-	-	-	-	-
		16	20	F	-	20	-	-	-	-	-	200
		30	20	F	-	-	-	20	-	-	-	-
		39	20	F	-	-	-	-	-	-	-	-
	Sokoto	27	A	F	-	-	100	-	-	-	20	-
		32	A	F	-	-	-	-	-	-	-	-
		37	A	F	-	-	-	-	-	-	20	-
											20	-

TABLE 14: Human Leptospiral Seroreactors in Jos, Plateau State From 1975 to 1976.

State	Town	Serum No.	Age	Sex	Titer								
					grippo*	ictero	hardjo	hebdo	pomona	canicola	pyrog	patoc	tarass
Plateau	Jos	692	45	M		20		-	-	-	20	100	
		694	A	F		-		-	-	-	-	20	
		708	50	M		-		-	-	-	-	20	
		740	A	F		-		-	20	-	-	-	
		BIO 795	A	F		-		-	-	-	20	100	
		BIO 794	24	F		-		-	-	-	100	-	
		BIO 792	A	F		-		-	-	-	-	20	
		K 749	A	M		-		-	-	-	-	20	
		K 772	24	F		-		20	-	-	-	-	
		K 795	A	M		20		-	-	-	-	-	
		K 791	A	M		-		-	-	-	20	-	
		K 775	A	F		-		20	-	-	100	20	
		K 757	A	F		-		-	-	-	-	20	
		K 795	30	F		Trace		-	-	-	-	100	
		K 797	25	F		Trace		-	-	-	-	20	
		K 796	25	F		Trace		-	-	-	-	-	
		K 780	45	F		-		-	-	Trace	-	100	
		K 752	A	M	Trace	-		20	-	-	-	100	
		BIC 765	A	M	-	-		800	-	-	-	800	
		K 747	A	M	-	-		-	-	-	-	-	20
		K 748	A	M	-	-		100	-	-	-	-	-
		K 787	37	F	-	-	1600	100	1600	-	-	100	-
		K 792	25	F	-	-	-	100	-	-	-	20	-

TABLE 14: (Cont'd)

State	Town	Serum No.	Age	Sex	Titer grippo*	icter.	hardjo	hebdo	pomona	canicola	pyrog	patoc	tarass
	Jos	K 762	29	F	-	-	100	-	-	-	20	20	-
		CLA 5	A	F	-	100	Trace	800	100	-	-	-	-
		CLA 6	A	M	100	100	-	20	-	-	-	20	-
		CLA 8	A	M	-	20	Trace	100	-	-	-	100	20
		CLA 11	A	F	-	20	-	20	-	-	-	20	-
		OLA 21	60	M	100	100	-	800				100	20
		OLA 12	27	F	20	20		100				-	-
		OLA 19	20	F	-	20		-			20	20	-
		OLA 2	A	M	-	100		-			20	20	-
		CLA 14	38	F	-	-		-			-	20	100
		CLA 13	29	F	100	20		-			20	-	-
		OLA 25	28	M	-	-		-			-	-	-
		CLA 26	32	F	-	-		-	20		-	-	-
		OLA 27	35	M	-	-		-	20		-	-	-
		BIO 852	A	M	-	100		-	-		-	20	-
		BIO 922	50	M	20	-	20	100	-		-	20	-
		BIO 965	30	M	-	20	-	-	-		-	-	-
		BIO 967	11	F	-	-	-	-	-		20	-	-
		BIO 970	20	M	-	-	-	-	-		-	20	-
		BIO 850	A	M	-	-	-	20	-		-	-	-
		K858	A	F	-	-	-	20	-		-	-	-
		K834	A	M	-	-	-	100	100		-	-	-
		K861	24	F	-	-	-	20	-		20	-	-
		K824	A	M	-	-	-	-	-		20	100	-

TABLE 14: (Cont'd)

State	Town	Serum No.	Age	Sex	Titer grippo*	ictero	hardjo	hebdo	pomona	canicola	pyrog	pateo	taraas
	Jos	BIO 886	24	F	20	-	-	-	-		-	-	20
		BIO 899	A	M	-	-	-	-	-		20	-	-
		K890	A	M	-	-	-	-	-		-	20	20
		BIO 968	15	M	800	-	-	-	-		-	20	-
		K848	A	F	-	-	-	-	-		-	-	20
		K906	A	F	-	-	-	20	-		-	-	-
		K1092	25	F	100	-	-	-	-		-	-	-
		K1102	A	M	-	-	-	-	-		20	-	-
		K1103	A	M	-	-	20	-	-		20	-	-
		K1104	A	M			100		20		800		
		K1105	A	M			20		20		-		
		K1106	A	M			-		-		20		
		K1107	A	M			-	20	-		-		
		K1108	A	M			100	20	-		400		
		K1110	A	F	1600		-	-	-		-		
		K1112	A	M	-		-	-	-	20	-		
		K1119	14	F	-		20	20	100	-	-		
		K1131	A	M	-		20	20	400	-	-		
		BIC1110	A	M	100		-	-	20	-	-		
		BIO1119	35	F	-	20	100	100	20	-	-		
		BIO1127	25	F	-	-	20	-	-	-	-		
		BIC1131	35	F	-	-	20	-	-	-	-	400	
		BIC1175	A	M	-	-	-	-	-	-	20	-	

TABLE 15: Swine Sercreactors to Leptospirosis From Kaduna and Kano States of Nigeria.

Town	No.	Age	Sex	Titer icter*	grippo*	pomona	canicola	pyrog*	australis*
Kaduna	1	A	F	20	-	-	-	-	-
	2	A	F	20	-	-	-	-	-
	3	A	F	20	-	100	800	100	100
	4	A	F	20	-	-	-	-	-
	5	A	F	20	-	-	-	-	-
	6	A	M	100	100	-	-	400	-
	8	Y	F	20	-	-	20	-	-
	9	A	F	20	-	-	-	20	-
	10	Y	M	100	-	-	-	100	-
	11	A	F	20	-	-	-	20	-
	12	A	M	100	-	-	-	-	-
	13	Y	F	20	-	-	-	-	-
	14	A	F	-	20	-	-	-	-
	15	A	M	20	-	-	-	-	-
	18	Y	M	-	-	-	20	-	-
	19	Y	F	100	-	-	-	-	-
	20	Y	F	-	-	-	20	-	-
	21	A	F	-	-	-	-	100	-
	22	A	F	20	-	-	100	-	-
	29	Y	F	-	-	-	20	20	-
	30	Y	F	-	-	-	-	20	-
	31	A	F	20	-	-	-	-	-
	32	A	F	-	-	-	20	-	-

* icterohemorrhagiae, gripptyphosa, pyrogenes and australis

TABLE 15: (Cont'd)

Town	No.	Age	Sex	Titer					
				icterо*	grippo*	pomona	canicola	pyrog*	australis*
Kaduna	36	Y	F	20	-	-	-	-	-
	38	A	M(T)	-	-	-	-	100	-
	39	Y	M	100	-	-	1600	-	-
	40	A**	M	100	-	-	800	400	-
	41	A	F	400	-	-	1600	1600	-
	42	A	F	20	-	-	400	400	-
	44	A	F	100	-	-	400	400	-
	46	Y	F	-	-	-	-	20	-
	47	A	M	-	-	-	-	100	-
	48	A	M	20	-	-	-	-	-
	49	A	F	20	-	-	800	1600	-
	55	A	F	20	-	-	400	800	-
	69	Y	F(T)?	-	-	-	-	200	-
	72	A	F	20	-	-	-	100	-
	73	A	F	200	-	-	-	-	-
	75	A	F	20	-	-	-	-	-
	84	A	F	100	-	-	200	800	-
	87	Y	M	20	-	-	-	-	-
	88	Y	M	100	-	20	1600	1600	-
	91	A	M	-	-	20	1600	800	-
	101	A	F	-	20	--	20	20	-
	102	A	M	-	20	-	800	800	-
	103	A	M	-	-	-	-	100	-
	104	A	M	-	-	-	100	100	-

PUBLIC HEALTH SIGNIFICANCE OF LEPTOSPIROSIS IN NIGERIA

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AN ABSTRACT OF A THESIS
Submitted to
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Leptospirosis is a disease affecting man and animals and is a major zoonosis. It is primarily an occupational disease of livestock owners and attendants, veterinarians, slaughtermen, sewage workers and others engaged in related activities. The etiologic agent is a spirochete of the genus leptospira, tentatively speciated into two complexes: parasitic (interrogans) and saprophytic (biflexa). The interrogans complex has more than 140 serotypes divided into 18 serogroups. Although leptospirosis appears to be worldwide, knowledge of its geographical distribution is incomplete. The present study was undertaken to investigate the public health significance of leptospirosis in eight northern states of Nigeria by a serological survey of livestock and man and a bacteriological survey of potential reservoirs.

4,613 sera was collected and tested for leptospiral antibodies to serotypes grippotyphosa, icterohemorrhagiae, hardjo, hebdomadis, pomona, canicola, pyrogenes, tarassovi and australis by the microscopic agglutination technique.

In a total of 2,852 sera tested in livestock, the

incidence rates for the eight states in decreasing order were: Niger (13.0%), Kaduna (8.7%), Kano (8.0%), Borno (7.3%), Plateau (6.4%), Gongola (5.7%), Sokoto (4.4%) and Bauchi (3.0%). The overall species incidence rates were: horses (24.7%), cattle (11.9%), donkeys (6.1%), sheep (1.8%), and goats (1.1%). All nine seretypes were found in cattle, horses and donkeys, and in sheep (grippotyphosa, hebdomadis, pomona, pyrogenes, and tarassovi) and goats (pyrogenes, icterohemorrhagiae and canicola).

Twenty-six (9.2%) of 286 dogs from Zaria and Jos were positive for one to three seretypes; 61.5 per cent had titers of 1/100 or more. L. canicola and L. icterohemorrhagiae were the predominant seretype with 5.7 and 4.3 per cent incidence, respectively. Two dogs reacted with L. pyrogenes and one with L. australis. There was no difference in the leptospirosis incidence in the canine population of Zaria and Jos.

A total of 1,374 human sera collected from hospitals were tested for leptospiral antibodies to the nine seretypes and bifluxa vatec 1 during 1975 - 76. The incidence rates for the eight states in decreasing order were: Plateau

(31.8%), Bauchi (16.7%), Gongola (19.5%), Kaduna (9.4%), Sokoto (5.1%), Kano (4.5%), Niger (3.0%) and Borno (nil). Antibodies to all ten serotypes were detected in Plateau and Kaduna states only. Serotypes were detected in the following decreasing frequency: bifloxa 52, icterohemorrhagiae 33, hebdomadis 28, pyrogenes 27, pomona 24, hardjo 20, tarassovi 19, grippotyphosa 18, canciola 8, and australis 3. There was an age but no sex diffeence among the seroreactors; adults 77.6 per cent incidence rate, aged 4.6 per cent, and children (< 10 years) 0.7 percent.

In a two-year period 221 brown field rats (A. niloticus), 13 white-tailed rats (Cr. gambianus), five house mice (M. musculus), 11 gerbils (Gerbillus spp.) and 2 hedgehogs (Atelerix albiventris) were trapped and examined for leptospirosis. Field rats only were found positive (4.5%). Titers were detected against L. australis, L. icterohemorrhagiae, L. tarassovi and L. hardjo. All positive sera reacted against a single serotype. Eight isolates were obtained from field rats; 5 L. australis, 1 L. ballum and two unidentified.

Five isolates were recovered from bovine kidneys, 1

L. pyrogenes and four unidentified. One unidentified isolate failed to react with any of the 16 screening leptospira antisera and another was considered tentatively to be L. ballum.

No leptospira were isolated from sewage effluents collected at Kano and Kaduna.

It is concluded from this study that leptospirosis is present in the eight northern states of Nigeria and that rodents, cattle and dogs are principal carriers and are potential public health hazards.