A COMPARATIVE SERO-EPIDEMIOLOGICAL SURVEY FOR THE PREVALENCE OF *LEPTOSPIRA* ANTIBODIES IN DOMESTIC ANIMALS AND MAN IN NYANDARUA AND TURKANA DISTRICTS OF KENYA.

BY

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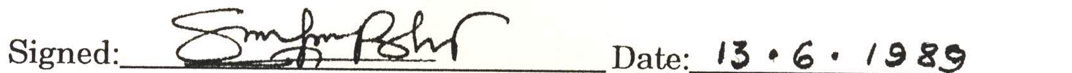

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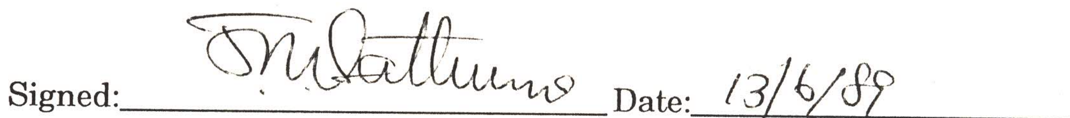

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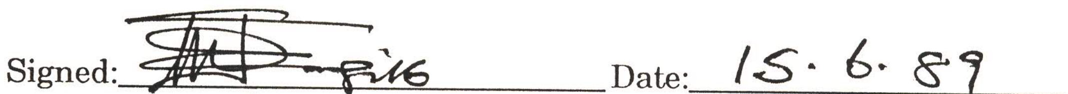

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DEDICATION

To my parents **Harrison Macharia** and **Peris Wanjiru**, my wife **Elizabeth Wanjiku**, my two sons **Martin Macharia** and **George Githaiga** and finally to all **my educators**.

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SUMMARY

Leptospirosis in domestic animals and man is a worldwide problem of public health and economic significance. Of economic importance are the losses from abortion, still-births, infertility, reduced weight gains, decreased production and deaths. Man is susceptible to all pathogenic leptospires and the disease in domestic animals may be a source of infection to man.

Leptospirosis has been detected in all countries where adequate investigations have been conducted. Detailed investigations have been carried out in Europe, Australia, New-Zealand, North and South Americas and South-East Asia. Relatively less has been done in Africa and thus, relatively little is known about *Leptospira* in African domestic animals and man.

In Kenya, leptospirosis has been shown to affect cattle, pigs, sheep and goats, wild and pet animals, as well as man. The actual prevalence rates of leptospirosis in domestic animals and man are, however, not known since previous studies did not involve extensive surveys to determine these rates, especially in man, sheep and goats. There have not been any attempts to determine which serovars are prevalent in these animal species and man. No information is available on the epidemiological factors which could be contributing to the distribution of leptospirosis in the animal species most commonly associated with man.

The purpose of this study was, therefore, to compare the prevalence rates of leptospirosis in domestic animals (cattle, sheep

and goats) and man, and to investigate the possible epidemiological determinants of the disease in these animal species for Nyandarua and Turkana districts of Kenya, and also to determine which leptospiral serovars are involved.

A total of 2,172 cattle, sheep and goat, and human sera were collected from both Nyandarua and Turkana districts.These included 326 cattle, 357 sheep and goat and 315 human sera from Nyandarua and 439 cattle, 369 sheep and goat and 366 human sera from Turkana districts. Each serum sample was screened against the following eleven leptospiral serovars:- *L. copenhageni, L. mankarso, L. autumnalis, L. sejroe, L. hardjo, L. grippotyphosa, L. pomona, L. canicola, L. australis, L. patoc 1 and L. wolffi* using the microscopic agglutination test (MAT). The positive sera were then titrated to determine the end point titre.

Leptospiral antibodies were detected in 49.0%, 55.0% and 7.6% of the cattle, sheep and goats and human sera tested respectively from Nyandarua District, with 34.0%, 34.2% and 0% of the sera from the respective animal species showing microscopic agglutination (MA) titers of 1:200 and above, which was considered indicative of infection. On the other hand, antibodies were detected in 44.0%, 24.0% and 14.0% of cattle, sheep and goats and human sera from Turkana District, with 22.3%, 3.8% and 4.6% of the sera from the respective animal species showing titers ≥ 1:200.

The most prevalent serovars in all the animal species considered in this study were those of the *Hebdomadis* serogroup, which included serovars *hardjo* and *wolffi*. The respective prevalence rates of antibodies to serovar *hardjo* in Nyandarua and

Turkana districts were; cattle (14.7%, 9.4%), and sheep and goats (22.4%, 0.0%). Human sera from Turkana had a prevalence rate of 1.6%; Nyandarua District did not have any positive human sera. The respective prevalence rates of antibodies to serovar *wolffi* in Nyandarua and Turkana districts were; cattle (27.2%, 8.0%), sheep and goats (2.0%, 0.3%), and human sera from Turkana District showed a prevalence rate of 1.1%. Antibodies to *Leptospira autumnalis* appeared to be common in sheep and goats from both districts, occurring at 3.9% and 1.4% in Nyandarua and Turkana districts respectively. Antibodies to serovars of the *Hebdomadis* serogroup, which were the most prevalent in cattle, sheep and goats were the same as those detected in man.

Sex appeared to influence the prevalence rate of antibodies to leptospirae in cattle from Nyandarua District while age did not. Both factors did not seem to influence the prevalence rate in sheep and goats from the district. Unlike in Nyandarua, age, and not sex appeared to have an influence on the prevalence rate of leptospiral antibodies in cattle from Turkana District. All the sheep and goats sampled in Turkana were adults and only two were females, one of which was positive for leptospiral antibodies. Information on the sex and age of individuals from whom human sera were obtained in Turkana was not available.

Except in humans, the prevalence rates in cattle, sheep and goats were higher in Nyandarua District, which falls in ecological zone II, a wetter area compared to Turkana District in ecological zones V-VI, which is relatively a much drier area.

It was concluded that antibodies to leptospirae in cattle, sheep and goats occurred at a higher prevalence rate in the wetter, high potential area compared to the drier, low potential area. High rainfall pattern, high relative humidity, moderate temperature range, a high moisture index and soil pH close to neutrality were associated with Nyandarua District which had higher prevalence rates of antibodies to leptospirae in cattle, sheep and goats. Reactors in human sera were detected in Turkana and not in Nyandarua District though. Male cattle from Nyandarua showed higher prevalence rates as opposed to those in Turkana. This did not appear to be the case in sheep and goats from either district. Age appeared to influence the level of leptospirosis in the dry Turkana District, with adult animals showing more reactors. Although serovars *hardjo* and *wolffi* were the most prevalent in human, cattle, sheep and goats, reactor rates to *L. autumnalis* and *L. australis* were detected in sheep and goat sera.

On the basis of the serological findings in this study, there is need therefore for an attempt to isolate the organisms, especially in the wetter areas, in order to determine the appropriate control measures that would need to be instituted for effective control of leptospirosis.

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1: INTRODUCTION

Leptospirosis is a widely distributed disease of both animals and man, caused by various serotypes belonging to the genus *Leptospira* . It has been considered the world's most wide-spread contemporary zoonosis (van der Hoeden, 1964), occurring in man, his pets (dogs and cats), livestock (cattle,pigs, sheep and goats) and in a wide variety of wild animals (Burdin, 1963; Kranendonk *et al.* 1968; D'Souza, 1983). The disease cannot always be easily diagnosed clinically on the basis of clinical signs, early signs or symptoms being either not specific and/or common to different diseases or disorders. In order to confirm the diagnosis of leptospirosis, identification of antibodies in the serum and isolation of the organisms are required (WHO., 1982).

The presence of moisture, warmth (about 25°C), and the pH of the soil or surface waters around neutrality (pH 6.2-8.0) are optimal for the survival of leptospires. Factors that hamper survival of leptospires outside the animal host include salinity, chemical pollution and certain absorptive properties of clay soil (Turner, 1967; WHO., 1967; 1982).

The disease is of economic importance as it causes losses from abortions, still-births, infertility, reduced weight gains, decreased production and deaths (Morse, 1955; Hanson, 1976; Brugge and Dreyer, 1985; Ellis *et al.*, 1985).

Leptospirosis is a febrile disease attributable to any one of a large number of serologically distinct members of spirochaetal species, *Leptospira interrogans* and may present itself in a variety of clinical

syndromes in animals and man (Michna, 1970; Feigin and Anderson, 1975; WHO., 1982; Blood *et al.*, 1985). The other currently recognised species, *Leptospira biflexa*, comprises of non-pathogenic leptospires commonly found in natural waters. The serovar (serotype) is the basic taxon and has been used in the classification of *Leptospira*.

Work carried out on leptospirosis in other parts of the world outside Africa indicates that clinical cases in both man and animals are not commonly recognised, but serological evidence has revealed that the infection is prevalent (Hanson, 1981). It has been shown that the disease is an important public health problem, influenced by agricultural practices and level of sanitation in many countries. It has been detected in all countries where adequate investigations have been conducted. Such detailed investigations have been carried out in Europe, Australia, New-Zealand, North and South Americas and South-East Asia (Gsell, 1984). Relatively little has been done in Africa (D'Souza, 1983). Thus, relatively little is known about *Leptospira* in African domestic animals and man (Amatredjo and Campbell, 1975).

Comparatively little is also known about leptospirosis in domestic animals and man in Kenya. The history of leptospirosis in this country dates back to 1948 when the first occurrence of the disease was reported in a dog in Nanyuki (Piercy, 1951), and the first serious outbreak was also observed in Nanyuki in 1956 affecting cattle, sheep and goats (Anon, 1979). Forty percent of the affected animals died during this outbreak, and the causative agent was found to be *Leptospira grippotyphosa* (Burdin *et al.*, 1958). More cases were reported from various parts of Kenya between 1957 and 1968 (D'Souza, 1983). A new leptospiral serovar in the *Australis* serogroup and two others belonging to the *Hebdomadis* serogroup

were isolated and reported (Dikken *et al.*, 1978; 1979). During 1967 and 1968, Njenga isolated 275 leptospiral strains from rodents in Kenya (Kranendonk *et al.*, 1968). Three new serovars, represented by the strains *Kanana*, *Lambwe* and *Njenga* were described (Dikkhen *et al.*, 1981). The annual reports of the Kenya Government, Veterinary Department do not contain any mention of leptospirosis in Kenya between 1968 and 1975. There were reports of several outbreaks involving hundreds of cattle from various parts of the country every year between 1976 and 1982. Of particular interest was an outbreak in 1977 at Gicheha Farm, a well managed dairy and beef farm in Rongai area (Nakuru District) of the Rift-Valley province of Kenya (Anon, 1978), where dairy cattle were affected and the organisms were isolated from milk. The disease later spread to other parts of Nakuru, Nyandarua, Baringo, Rumuruti, Thika and Naivasha areas. *Leptospira grippotyphosa* was isolated (Anon, 1978).

Renal histopathology of *Leptospira grippotyphosa* infection in farm animals in Kenya has been studied (Burdin, 1963). Ball (1966) reported positive serological and cultural results from several wild and domestic animals in Kenya and Uganda. Tabel and Losos (1979) reported a localized outbreak of bovine leptospirosis due to *Leptospira grippotyphosa* in Kiambu area. A serological survey by Stirling and Lhermette (1980) showed a high incidence of bovine leptospirosis in Kenya. D'Souza (1983), in his work involving the screening for *Leptospira* antibodies in bovine sera collected from different ecological zones of Kenya against major *Leptospira* antigens, showed a high prevalence (41%) of *Leptospira* antibodies. He found *Leptospira hardjo* to be the most frequent serovar affecting cattle. This serovar is a common human pathogen.

The first record of leptospirosis in goats and sheep in Kenya was in 1958 (Burdin *et al.*, 1958). *Leptospira grippotyphosa* was incriminated and in a later study, a prevalence rate of 12% to the antigens of serogroups *Hebdomadis* and *Icterohaemorrhagiae* were recorded in goats (Ball, 1966). In a recent preliminary serological survey for leptospiral agglutinins in sheep and goats in Kenya a prevalence of 18.25% was found (Wanyangu *et al.*, 1988). The survey also demonstrated a wide-spread prevalence of the disease throughout the country.

Forrester *et al.* (1969) and De'Geus *et al.* (1969; 1977) reported the presence of human leptospirosis in Kenya caused by serovars other than *Leptospira grippotyphosa* ; these included serovar *hardjo* in the *Hebdomadis* serogroup, *canicola, pyrogenes, australis, autumnalis* and *tarassovi*. In studies among human populations in tropical countries, particularly in Africa, prevalence rates of 17-26% were recorded among the rural community in Kenya (De' Geus, 1971) while prevalence rates of 33-39% have recently been recorded among the rural community and cocoa farmers in Ghana (Hogerzeil *et al.*, 1986).

From these previous studies, the actual prevalence rates of leptospirosis in domestic animals and man are not known since the studies did not involve extensive surveys to determine these rates, especially in man, sheep and goats. There has not been any attempt to determine which serovars are prevalent in these species and man. No information is available on the epidemiological factors which could be contributing to the distribution of leptospirosis in the animal species most commonly associated with man. Nor has any work been carried out to determine to what extent the disease is an occupational hazard in Kenya and its economic importance, taking

into account its prevalence in these domestic animals and man. The purpose of this study was, therefore, to compare the prevalence of leptospirosis in the different animal species and to investigate the possible epidemiological determinants of leptospirosis in these animal species for Nyandarua and Turkana districts of Kenya. The work is intended to provide information on the possible role played by these animal species in the epidemiology of leptospirosis as a zoonosis of public health importance in Kenya.

2: LITERATURE REVIEW

2:1 HISTORY:

Leptospirosis is a disease caused by spirochaetes belonging to the genus *Leptospira*. It is not a new disease. Acute and often fatal infections of man and animals, characterized by pyrexia, haemorrhage, haemoglobinuria and jaundice have been attributed to leptospirae for many years (Michna, 1970).

The history of the disease dates back to 1886 when Weil (Gsell, 1984) described a severe icteric disease in man (Weil's disease) which appeared to be a unique clinical illness whose aetiology remained obscure until 1915 when Inada *et al.* (1916) successfully cultivated the causal agent, a spirochaete which he named *Spirochaeta icterohaemorrhagiae*. Stimson had earlier, in 1907, reported finding *Leptospira* in the renal tubules of a man who had died from a febrile illness complicated by jaundice; he named the organism *Spirochaeta interrogans* and described its shape as resembling a question mark (Stimson, 1907; Wilson and Miles,1975a).

Studies carried out by Noguchi (1918) on the causal agent for Weil's disease and on Stimson's original preparation showed that the organisms were morphologically different from all known genera of spirochaetes, and concluded without doubt that they were *Leptospira*.

Following the identification of the organism, reports of the disease in man and other animals were recorded in various parts of the world. The significance of the rodent as a reservoir of *L. icterohaemorrhagiae* became apparent in many parts of the world

shortly after the identification of *Leptospira icterohaemorrhagiae* in man. The first isolation of the organisms from rats in USA was reported in 1917 (Noguchi, 1918), and the first case of human Weil's disease associated with rat exposure was reported in 1922 (Wadsworth *et al.*, 1922). To-date, wild animals, and in particular the rodents, have been reported to be the main reservoirs of *Leptospira* organisms (Palmer and Waitkins, 1987). Waitkins *et al.* (1985) were able to isolate leptospires from the coypu (a rodent). *Icterohaemorrhagiae* and *Hebdomadis* serogroups were found to be the prevalent leptospiral serogroups in these rodents. This work indicated the possible significance of the coypu in the spread of leptospirosis to domestic animals.

Numerous serotypes of leptospirae involving man and animals were discovered throughout the world (Gsell, 1984) following the initial identification of the causative agent for Weil's disease as indicated below. *Leptospira autumnalis,* the causative agent for "autumn fever" was discovered in Japan in 1925. *Leptospira pyrogenes* and *L. bataviae* were discovered in Indonesia in 1923, where clinicians also described the so called "spirochaetal fever", occurring without icterus. In Russia, *L. grippotyphosa* in humans and field mice was reported in 1928 and a new species, *L. andamana*, also in Russia was discovered in 1933. In 1937, *L. australis* A and B were discovered in sugar-cane workers, and in the same year, *L. pomona* was also discovered in Australia. In Denmark, *L. sejroe* (1939), *L. saxkoebing* (1944) and *L. ballum* with various carriers were isolated from human illnesses. During this period, *L. bataviae* and other serotypes were seen as the cause of "rice-field fever" in Italy and Spain. In 1944, *L. pomona* was found in Switzerland to be the cause of

"swine-herd" disease, with the pig being the carrier. Then in 1948, *L. tarassovi* (*L. hyos*) was found as another leptospirae in pigs with human cases in Switzerland and Argentina.

Various names, other than Weil's disease (Gsell, 1984), were used to define leptospirosis in various animal species during those early days. These included: "Seven-day fever" caused by *L. hebdomadis*, "autumn fever" caused by *L. autumnalis*, "swamp/mud fever " caused by *L. grippotyphosa*, and "rice fever" caused by *L. bataviae*. In veterinary medicine, the terms "yellows" in the dog, "infectious haemoglobinuria" in cattle and "swine herd fever" in pigs have been used to describe infections due to *L. icterohaemorrhagiae*, *L. grippotyphosa* and *L. pomona,* respectively. Following these descriptions, various leptospiral infections associated with different animal reservoirs were reported. These have been reviewed by various workers (Babudieri, 1958; Michna, 1970; Ryu, 1971; Feigin and Anderson, 1975).

World Health Organisation experts (WHO, 1967; WHO, 1982) have published a list of 20 serogroups and over 120 leptospiral serotypes occurring in all parts of the world since 1960. Rats, mice, dogs, pigs, cattle, sheep, goats, horses, bats, silver foxes, jackals and various other animals have been retrospectively associated with a typical leptospiral serotype.

Pet animals such as the dog and other domestic animals, especially cattle, may harbour the organisms after becoming infected by direct contact with leptospiral serovars specific to their host species. The reservoir, or the maintenance host varies to some extent with the infecting serovar and geographical location (Terpstra, 1987).

2:2 AETIOLOGY:

2:2:1.CLASSIFICATION AND NOMENCLATURE:

The genus *Leptospira* is one of the five genera (*Spirochaeta, Critispira, Treponema, Borrelia* and *Leptospira*) belonging to the family spirochaetaceae and the order spirochaetalis (Turner, 1974; WHO, 1982; Johnson and Faine, 1984). The genus is comprised of two species: *L. interrogans* whose strains are parasitic and pathogenic for man and animals and, whose normal habitat is the proximal convoluted tubules of the mammalian kidney and *L. biflexa*, which consists of the so called "saprophytic" leptospires (Turner, 1974; Johnson and Faine, 1984),and are predominantly free living spirochaetes occurring in fresh surface waters and associated soils and occasionally in sea water where several strains have been isolated from these marine environments (Russell, 1984).

Unlike *L. interrogans*, the strains of *L. biflexa* are rarely, if ever, associated with infection in man or other mammals and are also avirulent for laboratory animals. The species therefore has generally been considered to comprise saprophytic and non-pathogenic strains of *Leptospira*. Leptospires belonging to the *Interrogans* species are more fastidious in their growth requirements and they do not grow at 13°C, or in the presence of 225μg/ml 8-azaguanine, a purine analogue, unlike those belonging to the *Biflexa* species (Johnson and Rogers, 1964). *Leptospira interrogans* are also more susceptible to the growth inhibitory activity as compared to the *L. biflexa*. *Leptospira biflexa* possess a constant lipase activity, a property which is variable among the *L. interrogans* serovars. The two species differ in their

serological characteristics too. A genus specific antigen is found in the *L. biflexa* serogroup *Semaranga* serovar *patoc 1.*

Recent work indicates that saprophytic leptospires have little or no catalase activity (Banfi *et al.*, 1981). A possible third species which is phenotypically similar to *L. biflexa* but remarkably different from all the other leptospires in its DNA characteristics has been isolated (Russel and Faine, 1979; WHO, 1982). Accordingly, this species was classified provisionally as *L. illini*, species *in certae sedis* (species of uncertain position) while awaiting additional studies as proposed by the taxonomic sub-committee on *Leptospira* (TSCL) (WHO, 1982).

Included within each of the species *L. interrogans* and *L. biflexa* are a large number of serological types designated as serovars (serotypes). These serovars consist of strains which cross-agglutinate at high titers with one another's antisera. Those showing common antigenic structures are combined and assigned to the same serogroup (Alexander, 1976). These serogroups have not as yet been defined accurately; hence they lack official taxonomic status, though they remain as a necessary component of the classification system (Dikken and Kmety, 1978; WHO, 1982), in that the term "serogroup" does conveniently aid taxonomists in describing leptospires which are closely related as demonstrated by the present agglutination tests.

The serovar has been considered the basic taxon. Each serovar is represented by a reference strain, to which the description of the serovar is attached. The basic method used to differentiate serovars is the agglutination-adsorption test (Dikken and Kmety, 1978). The cross-agglutination, and variations in agglutinability of different strains, enable the determination of only the serogroup which is probably

concerned. Thus, to reach a more definite diagnosis, agglutination-adsorption studies are essential. However, these are impracticable on isolates from routine specimens. Newer methods, however, have been developed over the past few years that can be used in the classification of leptospires. Monoclonal antibodies have been developed for identification and serotyping (Kobayashi *et al.*, 1984; Jost *et al.*, 1987; Korver *et al.*, 1987). Bacterial restriction endonuclease DNA analysis techniques (BRENDA) have proved more useful tools than conventional agglutination-absorption methods in the classification of leptospires (Marshall *et al.*, 1981; Hookey *et al.*, 1985; Thiermann and Ellis, 1986).

Sub-division into sero-groups, serovars (serotypes) and subserotypes (strains) is based upon both cross-agglutination and agglutination-adsorption techniques. Cross-reactivity can be observed within each *Leptospira* species as well as between the two species (Czekalowski *et al.*, 1953; Wolff, 1954; Cox *et al.*, 1957).

Approximately over 180 serovars have been isolated and classified under *L. interrogans*, on the basis of the antigenic composition. These serovars have been arbitrarily assembled into 20 serogroups, on the basis of common cross-reacting agglutinogens. On the other hand, about 65 serovars have been classified under *L. biflexa* (WHO, 1967; 1982; Russell, 1984). It has been suggested that the saprophytic strains can be divided into 11 serogroups and that it is likely that more new serogroups may be discovered as more investigators extend their investigations on water leptospires (Russell, 1984). Appendix 1 shows the list of *Leptospira interrogans* reported to have been isolated from man and animals (WHO., 1982).

As the name serovar suggests, identification of leptospires has relied upon serological methods. Marshall *et al.* (1981) described a means of differentiating isolates of *L.interrogans* by restriction endonuclease analysis of DNA. They suggested that this new method had advantages over serological testing. This has been supported by the work of Rubinson *et al.* (1982), who indicated that some serovars of *L. interrogans* can be subdivided by means of bacterial restriction endonuclease DNA analysis (BRENDA), and that such subdivision probably correlated with epidemiological relationships between strains and their hosts and may necessitate the nomination of new reference strains to the present subtypes within serovars *L. hardjo, L. balcanica* and *L. tarassovi*. These newer methods have found great use in recent times in the identification and serotyping of leptospires (Jost *et al.*, 1987; Korver *et al.*, 1987). They have been used with accuracy and ease to differentiate various serogroups of *L. interrogans* (Marshall *et al.*, 1981; Rubinson *et al.*, 1982; Thiermann and Ellis, 1986). This has led to more accurate and reproducible classification (Hookey *et al.*, 1985), which permits more understanding of the relationship between strains and their hosts.

Hookey *et al.* (1985) have indicated that numerical treatment of data obtained from DNA restriction endonuclease patterns is possible and can be applied to the identification of strains of *Leptospira*. Using this method, the majority of *Leptospira* strains could clearly be identified to the serogroup level.

The fact that leptospirae possess many antigens, some of which seem to be common to all strains in both complexes of the genus, led to the discovery of an antigen which is capable of reacting with antibodies elicited by a wide range of leptospiral strains in man,

notably the *Biflexa* strain *patoc 1*. It has been used in both the complement fixation test and the indirect immunofluorescent test to screen for leptospiral antibodies in human sera irrespective of the infecting serovar (Wilson and Miles, 1975b).

2:2:2.MORPHOLOGY:

Leptospirae are very fine slender organisms whose morphology can be studied satisfactorily only by dark-ground illumination, phase-contrast, and electron-microscopy. They do not stain readily with aniline dyes or giemsa stain and therefore are not visible under a bright-field microscope (Russell, 1984). At magnifications of x100 or greater, they are readily visible by dark-field microscopy, but are less clear by phase-contrast microscopy (Torten,1979).

They are 0.1 by 6-20μm in size, tightly coiled, tend to be gram negative and show a very active flexous motility as a result of an axistyle that is regarded as a flagella analogue by its function and chemical composition (Turner, 1974; Russel, 1984). There are two flagellae (axial fibrils), one at each end of the cell. The axial fibril is considered to be the locomotor organnelle since it produces an electrical impulse. A long filamentous spiral cell characterises their basic morphological entity. Their cell walls are structurally and chemically similar to other bacteria (Yanagawa and Faine, 1966; Turner, 1974). The helical conformation of *Leptospira* is clockwise (right handed) (Carleton *et al.*, 1979; Hovind and Hougen, 1986).

The appearance of living leptospirae varies with the milieux in which they are observed, which in turn influences the movements of the organisms. Three types of movements have been observed and

may occur together; a to-and-fro progression; rotational or oscillation about the long axis; and flexion (Wilson and Miles, 1975a). One or both ends of the cell is bent or hooked. Straight forms also occur, which rotate and move slower than the hooked form. When moving in free liquid, one cell end is straight and the other is hooked, the movement being in the direction of the straight end. In semi-solid medium, they display a serpentine type of movement. Unlike other bacteria with external flagella, the to-and-fro progression movement of leptospirae is enhanced as the viscosity of the milieux increases (Russell, 1984). They move rapidly, their bodies being somewhat rigid (Babudieri, 1961). Unlike other spirochaetes which show infinite flexibility in semi-solid environments, there is an immediate return to the straight form in liquid medium.

Some of the basic morphological features the leptospirae share with other spirochaetes are an outer envelope enclosing an inner protoplasmic cylinder. The protoplasmic cylinder consists of a peptoglycan layer, a cytoplasmic membrane and the cytoplasmic contents of the cell. Between the outer envelope and the protoplasmic cylinder are located the axial fibrils.

The different serovars belonging to both the parasitic *L. interrogans* and the saprophytic non-pathogenic *L. biflexa* species cannot be distinguished from one another morphologically (Russell, 1984).

Their flexibility, unique motility combined with their narrow diameter and shape, enables them to pass through sterilizing (0.1-0.45μm pore size) filters and to migrate within media solidified with upto 1% agar. Few other non-spirochaetal bacteria have these

capabilities. Species belonging to *L. interrogans*, and not *L. biflexa*, rapidly converts to spherical forms in hypertonic environments.

2:2:3.CULTURAL CHARACTERISTICS:

Leptospirae are obligate aerobes that produce oxidase and/or peroxidase. They have a preference for micro-aerophilic conditions (Czekalowski *et al.*, 1953; Ellinghausen, 1960; Russell, 1984). Their nutritional requirements are unique, though simple. Vitamin B_1 and B_{12} and long chain fatty acids are the only organic compounds known to be required. These must be supplied bound to albumin or in a non-toxic esterified form due to their toxicity as free fatty acids. Carbohydrates are not a good source of carbon. Ammonium salts are effective sources of nitrogen (Ellinghausen and MacCullough, 1965). Amino acids are utilized to only a small extent towards this goal. Non-essential nutrients (pyruvates) enhance initiation of growth of parasitic leptospires especially, *L. hardjo* and *L. ballum*.

Leptospires incorporate purine bases instead of pyrimidine bases into their nucleic acids, hence, they are resistant to antibacterial activity of the pyrimidine analogue, 5-fluorouracil, a compound used in the preparation of selective media for isolation of leptospires from contaminated sources.

Since free fatty acids are toxic to leptospires, serum or serum albumin is incorporated into culture media to bind the fatty acids in an available but non-toxic form (Johnson and Wilson, 1960; Johnson and Gary, 1963). Anion-exchange resins also bind fatty acids, and they can serve as a substitute for albumin for the cultivation of

Leptospira (Johnson and Wilson, 1960). Bey and Johnson (1978) described a protein free medium composed of charcoal-detoxified Tweens (polysorbates), Vitamins B_{12} and B_1, inorganic salts and organic buffer which supported the growth and could also be used in the subculturing of both pathogenic and saprophytic *Leptospira*.

Although they can be grown in a variety of media, the three commonly used are: Media based on a solution of inorganic salts and buffered with phosphate, media supplemented with pooled rabbit serum (Fletcher, 1928; Korthof, 1932; Staurt, 1946) and media with bovine serum albumin fraction V and Tween 80 (Ellinghausen and MacCullough, 1965 and modified by Johnson and Harris, 1967).

Most serovars will grow on synthetic media comprised of chemically defined ingredients (Shenberg, 1967). Such artificial media, when enriched with 10% rabbit serum or 1% bovine serum albumin-Tween 80, provide easy cultivation of the leptospires.

Leptospires are slow growers. They reach maximum growth after 7-15 days at an incubation temperature of 28-30°C (Babudieri, 1961). The optimal temperature for *in vitro* propagation is 28-30°C. A rapid multiplication for 1-2 days may be observed at 37°C. Average generation time is 12 hours when cultivated in a suitable aerated medium with a pH of 7.4 and at 30°C (Johnson and Faine, 1984), resulting to an yield of 6-8x10^{9} cells/ml. Exposure to 40°C is deleterious and 56°C and above, lethal for pathogenic strains (Turner, 1966). They are extremely sensitive to dehydration and therefore die within minutes of exposure to dry conditions. In the laboratory the organisms are maintained in liquid or semi-solid media such as

Fletcher's or Korthof's. Use of liquid nitrogen provides the long term storage of cultures (Torney and Bordt, 1969).

Growth is easily initiated in most media and is usually visualized as one or more rings of dense growth some mm to cm below the surface of tubed liquid media. This zone showing more turbidity signifies an area of greatest growth and is referred to as Dinger's zone. In semi-solid medium (liquid medium + 1% agar), growth also occurs below the surface to give diffuse to discrete non-pigmented colonies, which are visible within 7-14 days for most serovars. The shape of the colony of the various strains changes with time and for a given strain, the colony size is directly related to the agar concentration. Media with pyruvate is necessary for colonial growth of the more fastidious strains. Although various colonies have been described, the significance of their difference is not known and are therefore not a useful differentiating characteristic (Wilson and Miles, 1975a; Russell, 1984). Growth is best in liquid or semi-solid media, but colonies will form on solid media too, especially Cox's agar medium (Cox and Larson, 1957; Amstrong and Goldberg, 1960; Faine and van der Hoeden, 1964).

2:3 TRANSMISSION:

Although the particular conditions which facilitate interspecies trasmission of leptospires are largely unknown (Hathaway *et al.*, 1984), the commonest mode of transmission is indirect contact with environments (soil, mud, water, etc.) contaminated with virulent leptospires shed in the urine of convalescent or reservoir hosts (Turner, 1969). Many outbreaks of leptospirosis in humans have

resulted from bathing in waters contaminated with urine of carrier animals; contaminated foodstuffs, pastures, soils, and premises assist considerably in the dissemination of animal leptospirosis (Michna, 1970). Wet, low-lying paddocks and muddy pastures, coupled with the ability of leptospires to survive for long periods in water, result in favourable conditions for the transfer of virulent leptospires between infected materials and susceptible hosts (van der Hoeden, 1958; Amatredjo and Campbell, 1975). Under favourable conditions, surface waters into which the organisms are excreted may remain infectious for several weeks (Gillespie and Ryns, 1963). Survival in badly polluted waters is limited though. The infectivity of contaminated rapidly flowing waters has been demonstrated, and downstream dissemination of leptospires from contaminated foci documented (Alston and Broom, 1955). Sporadic outbreaks of the disease in cattle have been thought to be as a result of contamination of rivers and canals by infected cattle on distant upstream farms (Waitkins *et al.*, 1985).

The sources of infection are direct or indirect contact with water and soils contaminated with urine of leptospiruric animals or tissues from infected animals. Mucosal membranes of the conjuctiva, nose, mouth, reproductive tract and broken skin serve as entry sites. Natural and experimental infections by mouth would indicate that the pathogen is able to pass through mucosae of the mouth, oesophagus and intestines and it may also invade through the skin, especially if it is abraded, diseased, or softened as a result of prolonged contact with water or mud (Michna, 1970; Russel, 1984). Entry can also be by inhalation of droplets of fluids containing leptospires.

Direct contact with hosts, their carcasses, organs or urine is generally less common except in certain occupations. Handling of infected animals or their organs at or after slaughter, mopping of urine, and accidental bites by carrier rodents, ferrets and dogs may result in transmission from animal to man (Michna, 1970). However, infections through bites are probably coincidental as leptospires are not excreted in the saliva (Wilson and Miles, 1975b).

Venereal transmission and transplacental infection of the foetus have been reported in man and are important in some animals (van der Hoeden, 1958; Sleight and Williams, 1961; WHO, 1967; Turner, 1967; Coghlan and Bain, 1969). The presence of leptospires, and in particular *L. hardjo,* in the genital tract of naturally infected cattle may be an indication of the possibility of venereal transmission in these animal species (Ellis and Thiermann, 1986). Isolation of related strains from bulls supports this view (Kiktenko, 1976). Kiktenko *et al.* (1976) were able to isolate leptospires belonging to the *Hebdomadis* serogroup from the semen of bulls. More evidence of venereal transmission in cattle has been suggested by the work of Ellis and his colleagues (1986).

Insects have been shown to be able to carry leptospires on their integument and in their alimentary canal and have therefore been incriminated in the transmission of leptospirosis (Callow, 1967). Blood-suckling arthropods can also carry viable leptospirae. Krepkogorskaia and Rementsova (1957) isolated *L. grippotyphosa* from ticks removed from cattle and van der Hoeden (1958) found *L. canicola* in a tick taken from a hedgehog which proved to be a carrier of the same serotype. The revelations and the observation that ticks

seem to maintain for a long time viable *Leptospira* taken with a blood-meal (WHO, 1967) supports Callow's suggestion.

Although leptospires are shed in milk, whole milk has been shown to be leptospiracidal, and since the organisms only survive for a few hours, transmission is therefore less likely by drinking milk (Kirshner and Maguire, 1955, Kirshner *et al.*, 1957), A few workers, however, have been able to isolate some strains of *Leptospira* from milk (Ellis *et al.*, 1976; Thiermann, 1981).

2:4 PATHOGENESIS OF LEPTOSPIRAL INFECTION IN ANIMALS:

Hamsters and guinea-pigs are used as indicators of virulence and pathogenicity of leptospires (Torten, 1979; Britol *et al.*, 1979). Young hamsters and guinea-pigs are highly susceptible to leptospirae irrespective of the route of inoculation (intraperitonially, subcutaneously, cutaneously or orally). Gerbils (van der Hoeden, 1953) and chicks 1-2 days old (Wilson and Miles, 1975a) are likewise very susceptible. Rabbits, mice, rats, and voles are only slightly susceptible (Wilson and Miles, 1975a). Adult rabbits are completely resistant, hence, they do not become carriers of leptospires and are, therefore, the animals of choice for antibody production (Torten, 1979). Mice do not become clinically ill but become active carriers and shedders of live virulent leptospirae (Vinh Tu *et al.*, 1982).

Naturally acquired infection by leptospires does not cause a local inflammatory reaction or lesion at the site of entry.

The pathogenesis of leptospirosis can broadly be divided into two phases: leptospiraemia and leptospiruria (Turner, 1967). The first phase of leptospiraemia is after *Leptospira* enters into the host. Once in the blood stream, they migrate to visceral tissue where they multiply and from there they are transported to all tissues of the body. There is no evidence that *Leptospira* multiply in organs such as the spleen or liver and are then released into the blood stream (Burnstein and Baker, 1954). This leptospiraemic phase is rapid and lasts 4-8 days and its end coincides with the appearances of antibodies in the blood (Ellis, 1978). Pregnant animals may have their foetus/foeti infected during this phase. The pathogenesis and pathological changes in the foetus resemble those of the adult animals but are relatively more severe owing to low immunity in the foetus. Foetal leptospirosis results from *Leptospira* crossing the placenta (Dacres and Kiesel, 1958; Fennestad and Borg-Petersen, 1958; Fennestad, 1963). The leptospirae in the foetus may result in stillbirth or death of the foetus and abortion (Te Punga and Bishop, 1953; Furguson *et al.*, 1957; Michna, 1970; Ellis *et al.*, 1976; Ellis and Michna, 1977; Hathaway *et al.*, 1983). Calves infected with serovar *pomona* have shown very obvious morphological changes in red blood cells (RBCs) which are concurrent with haemoglobinemia (Thompson and Marshall, 1986). The alteration of the cell cytoplasm appeared to be the preliminary change while RBC sequestration and erythrophagocytosis were pronounced within the spleen, liver and bone-marrow.

Following entry into the body tissues, most of the *Leptospira* are got rid of through phagocytosis (Burnstein and Baker, 1954) and as the antibody response to the infecting serovar develops, leptospires are eliminated from the host with the exception of the eye and kidney

where they persist for varying periods of time. Phagocytosis of leptospires *in vitro* by both macrophages and polymorphonuclear leucocytes (PMN) from different animal species, ranging from those susceptible to those highly resistant to infections with *L. interrogans* was demonstrated by the work of Faine and van der Hoeden (1964). Leptospires also persist in the brains of naturally infected animals and may also do so in humans (Russel, 1984). In the liver they may cause hepatitis in addition to haemolytic and haemorrhagic complications.

The leptospires get into the lumina of the kidney's convoluted tubules by traversing the inter-tubular spaces and the tubular epithelial cells or their junctions (Michna, 1970) where they are protected from serum antibodies. They multiply in the convoluted tubules from where they are subsequently shed intermittently as the second phase of leptospiruria which may last for 8 days - 5 weeks, while in some animals, it may last from 3 months to the rest of the animal's life-time. This phase of leptospiruria is important in the spread of the disease. It is believed to be the means by which the infection is maintained in a wide variety of animal species (Turner, 1967).

The animal reservoirs may be serologically negative although culturally positive. This phenomenon was explained by Babudieri (1958) in his theory of ectoparasites in which he stated that the initial leptospiraemia is followed by an immunized state. The organisms accumulate in the secondary convoluted tubules of the kidney and then cease to be internal parasites. The organisms then stop acting as antigens and do not stimulate further antibody production. The blood antibodies then drops below detectable levels. Leptospiruria may then

persist for life, and these carrier animals become a potentially dangerous source of infection (Babudieri, 1958).

The presence of leptospirae in other tissues like the brain, and the eye is thought to elicit hypersensitivity reactions which are responsible for lesions in these tissues (Hoag and Bell, 1954; Stoenner *et al.*, 1963).

Lesions occur when there is a threshold concentration of leptospires in the body. The primary lesions appear to be a result of damage to the endothelial lining of capillaries, migration of leptospires into tissues, local anoxia and later, secondary ischaemic damage to organs, e.g. kidneys, liver and adrenals. Renal tubular necrosis and uremia then follow. Severe cases result in haemoglobinuria and haemoglobin casts may be seen. Liver cell necrosis leads to liver dysfunction with consequent jaundice. Haemorrhages are present in almost all organs. In severe terminal haemorrhagic illness, pathological changes resemble those of disseminated intra-vascular coagulopathy (DIC). Previous suggestion that DIC is responsible for thrombocytopaenia (a well documented complication of leptospirosis) has been refuted (Charles *et al.*, 1986). Hence, its pathogenicity remains uncertain. The pathological changes in kidneys of carriers differ according to the animal species. Leptospirosis is usually associated with renal failure (Russel, 1984) and death may follow.

Although the histopathological appearances seem to result from some toxic action, there is no definite evidence of exo- or endo-toxin and therefore, leptospirae have been considered not to produce any toxins (Wilson and Miles, 1975a). This disagrees with the suggestion that haemolysis associated with leptospirosis is an intravascular

event due to phospholipase-like activity of leptospiral toxins upon red blood cell membranes (Kasarov, 1970; Kemenes, 1974). Later work, however, has continued to point with high possibility the presence of leptospirae associated toxins in some serovars. The ultrastructural lesions which have been reported within the endothelial, hepatic and renal cells of hamsters and guinea pigs with serovar *pomona* and *icterohaemorrhagiae* (Britol *et al.*, 1979) have been considered to be indicative of the action of an unspecified "toxin". The role of these toxins in haemolysis appear to vary between species (Thompson and Manktelow, 1986), a fact that was deduced from their work where serovar *pomona* infection was found to cause red blood cell destruction in cattle but not in hamsters, while serovar *ballum* caused red blood cell destruction in hamsters but not in cattle. Variation in phospholipid contents of red blood cell membranes in different species making such species more or less susceptible to certain phospholipase like leptospiral toxins was suggested as a possible explanation to this interspecies variation. The red blood cell metabolism and eventually their morphology were thought to be a result of the effect of these leptospiral toxins. The *in vitro* mechanism responsible for red blood cell destruction is thought to be different from that which brings about haemolysis during infection (Thompson and Marshall, 1986).

Leptospiral organisms with or without the presence of recognisable disease may be detectable in urine of carrier animals of all species. In the carrier hosts, leptospires may continue to survive in the kidney as well as in the anterior chamber of the eye for months despite high serum antibody titers.

2:5 EPIDEMIOLOGY OF LEPTOSPIROSIS:

Leptospira infection pathway is from an infected animal to other animals and from animals to man by water or food contaminated with infectious urine. Transmission among cattle and between cattle and man is facilitated particularly under intensive managemental conditions (Christmas *et al.*, 1974). Of epidemiological significance, leptospirosis involves shedding of *Leptospira* in the urine of animal carriers, the ability of leptospirae to survive in wet areas, the spread of the infection to other animals and from animals to man. The number and types of serovars present vary with the geographical area. The incidence and prevalence of leptospirosis, therefore, vary considerably between countries and within localities in the same geographical environment.

Infections in temperate zones are often occupational and a relatively small number of serovars are involved. In tropical countries, however, the disease is more wide-spread in the population and the infectious agent may be one of many serovars carried by a large variety of different hosts (Russel, 1984).

Serovars are frequently associated with certain animal species but can infect many different animal species. Some of the domestic animals are able to shed as many as three serovars, which emphasizes the public health significance of these infections in domestic animals and man. Some serovars are universal, others are regional or local.

Although a wide variety of *Leptospira* serovars have been isolated from domestic animals, relatively few are repeatedly associated with each host. Serovars mostly associated with cattle are *L. hardjo, L.*

pomona, and L. grippotyphosa, while in horses, *L. pomona, L. icterohaemorrhagiae and L. autumnalis* are the commonest ones. *Leptospira pomona* and *L. tarassovi* have been associated with swine, while *L. pomona* and *L. grippotyphosa* have been associated with sheep and goats. *Leptospira canicola* and *L. icterohaemorrhagiae* have commonly been associated with the dog. Man is susceptible to all pathogenic leptospires.

The leptospires endemic in regional wildlife, however, may also be reflected in the serovars that are present in domestic animals of that geographical region. In some countries, vaccination has apparently reduced the incidences of the homologous serovars, thereby allowing other regional serovars to increase in incidence.

In the past, people in occupations associated with water or sewage were considered to be at particular risk from leptospirosis as they often worked in rat infested conditions and in water polluted with leptospiral infected urine. Hence, leptospirosis was quite common in sewer workers and miners. Today, with modern pest control measures, the use of protective clothing, and the presence in water of detergents, which rapidly destroy leptospires, the epidemiological pattern of leptospirosis in both man and animals has changed (Torten, 1979; Waitkins, 1985). Such changes observed in incidence, prevalence and host-parasite relationships may be attributable partly to a variety of factors such as; increased awareness of the disease and changes in the pattern of human recreational activities (Feigin and Anderson, 1975), and changes in the herd management practices of domestic animals which have also been implicated as causes of change in the prevalence and incidence of leptospirosis in both man and animals (Christmas *et al.*, 1974; Waitkins, 1983; Ellis *et al.*, 1985).

Age and immune status of the animals, vaccination procedures and milking practices, are also responsible for different courses of the disease in different countries and even within countries in different areas (Terpstra, 1987).

The major occupational risk today is among farm workers whose work or ways of life bring them close to animals. Protective measures similar to those used by water authorities to protect sewer men cannot easily be applied to current farming practices due to the high expenses involved. Nevertheless, awareness of the risks is often helpful.

Other water associated activities that have often been shown to be risk factors for leptospirosis in man are, bathing in fresh water ponds and streams, canoeist and leisure activities such as "raft racing" which is a highly competitive sport where cuts and bruises are easily sustained, thus providing easy entry of leptospires into the body (Feigin and Anderson, 1975; Torten, 1979; Waitkins, 1983). Their favourable environments for survival are moist soil, stagnant or slow moving waters which are slightly alkaline and at temperatures of 22°C and above (Michna, 1970). Survival in these waters is also dependent on salinity and pollution.

Leptospirosis is mostly acquired during or shortly after the warm rainy season. It requires a wet environment for transmission. Hellstrom and Blachmore (1979) indicated that the total level of moisture in the environment is the parameter most strongly associated with the establishment of an outbreak of bovine *L. hardjo* infection.

The natural reservoirs of leptospirosis have been reviewed by Turner (1967) and Michna (1970). Serovars of *L. interrogans* have a wide host range, infecting as many as 160 different species (Russell, 1984); many kinds of domestic and wild animals such as rodentia, carnivora, insectivora, marsupilia, chiroptera, artiodyctyla and lagomorpha harbour them (Michna, 1970). The reservoir or maintenance host varies to some extent with the infecting serovar and geographical location. The primary laboratory animals used to test for pathogenicity and virulence of leptospirae are hamsters and guinea pigs (Russell, 1984).

2:6 CLINICAL MANIFESTATIONS:

The severity of the illness depends partly on the virulence of the infecting strain of *Leptospira*, which may belong to any of the serogroups of the *Interrogans* complex and partly on the host's general health, existing state of various organs and the ability to produce specific antibodies (Wilson and Miles, 1975b).

The disease in all animal species may be subclinical to fatal, a considerable percentage of these leptospiral infections being sub-clinical (Turner, 1969). Symptoms and signs may occur in a wide variety of combinations, thus giving a variety of syndromes whose clinical manifestations are not pathognomonic, and therefore making the disease very insidious and difficult to diagnose. This frequently leads to misdiagnosis of leptospirosis in the field (Russel, 1984). These clinical signs may be acute, sub-acute or chronic, with varying degrees of frequency (Hanson, 1977). These varieties of manifestations in different combinations, which may also mimic other acute

infections, add to the difficulties in diagnosis from the clinical picture alone (Turner, 1967; Michna, 1970).

Successful entry of leptospires through broken skin and mucosal surfaces in both human and domestic animals, results in an acute systemic disease characterized by an abrupt onset of high fever, chills, myalgia, severe headache, anorexia and conjuctival vascular congestion, thereafter followed by signs related to damage inflicted on the kidneys, liver and the gastro-intestinal tract. Jaundice is not an invariable feature, and meningial involvements may result from infections by either of the various serotypes (Michna, 1970; Russel, 1984).

Although the clinical response to natural infection by different serotypes varies from host to host, animal leptospirosis may generally exist in at least four forms (Michna, 1970), which include; sub-clinical infections, acute or sub-acute infections, reproductive disorders or as an ocular disease.

2:7 LEPTOSPIROSIS IN ANIMALS AND MAN:

2:7:1 LEPTOSPIROSIS IN ANIMALS:

Variability of the clinical response to infections by different *Leptospira* serovars occurs from one host to another (Michna, 1970).

2:7:1:1 CATTLE:

The first isolation of leptospires from cattle was done in 1946 in the Union of Soviet Socialist Republics and Israel (WHO, 1982). Considerable work has been done elsewhere in the world and the

prevalence of bovine leptospirosis is now known to be worldwide (WHO, 1982). The most common serovars isolated from bovines are *L. hardjo* and *L. pomona*. *Leptospira grippotyphosa* and other serovars; those of the *Hebdomadis* serogroup, have especially been detected with increasing frequency in recent years. Bovine leptospirosis due to *L. grippotyphosa* has been described in Kenya (Burdin *et al* ,1958). D'souza (1983) reported *L. hardjo* to be the most prevalent serovar in Kenyan cattle.

Cattle are thought to be the natural carriers of *L. hardjo* and are currently the only recognised maitenance hosts for the serovar *hardjo* (Ellis *et al.*, 1981). Persistent carrier animals maintain infection in the kidney (Hart *et al.*, 1984). *Leptospira hardjo* has been found to be a major factor in the aetiology of bovine abortions (Ellis et al., 1982a&b, 1985; Brugge and Dreyer, 1985), and it has been possible to isolate the organism from foetal kidney by careful application of dilution culture technique (Ellis *et al.*, 1982b). This agrees with earlier work by Ellis (1978) in which the *Hebdomadis* serogroup, and in particular serovar *hardjo,* was incriminated as a possible cause of bovine abortion in many parts of the world despite difficulties in practical diagnosis. Little *et al.* (1980) indicated that the diagnosis of leptospiral abortion in cattle is difficult unless a very detailed history of the herd is available. Otherwise, it seemed likely that leptospires are associated with at least some of the large number of abortions in cattle that at present have no attributable cause.

Endemic infection of cattle with serovar *hardjo* is now recognised in many countries (Amatredjo and Campbell, 1975) and routine isolations from fetuses have been reported (Hathaway *et al.*, 1982). In their work, Ellis and Michna (1977) had pointed out the usual reasons

for failure to isolate leptospires from aborted fetuses in most cases, the main reason being autolysis of the fetus which also makes histological examination of the fetus and flourescent staining of leptospires difficult. Despite these hinderances, Ellis *et al.* (1976) were able to isolate a *Hebdomadis* serogroup strain from such an aborted fetus. Cows infected with serovar *hardjo* have been found to shed viable leptospirae in their milk (Ellis *et al.*, 1976). The emerging hazard of cattle infection with serovar *hardjo* is now often referred to as cattle associated leptospirosis (Waitkins, 1985).

The clinical signs of bovine leptospirosis include an incubation period of about 4-10 days followed by pyrexia of 104°F to 107°F, which may last 6-48 hours (Fennestad, 1963), anorexia, jaundice, haemoglobinuria, mastitis, abortions and death (Michna, 1970). Morbidity and mortality rates in calves infected by leptospires, and in particular *L.pomona*, are very variable but jaundice, haemoglobinuria and sudden death are common findings (Hathaway *et al.*, 1984). The clinical picture of experimental infection with serovar *hardjo* in pregnant cattle is characterised by abortion, mummification, still-birth, premature and term birth of weak calves and full term birth live, apparently healthy calves (Ellis *et al.*, 1986). High prevalence of infection in cattle has, presumably, led to an increase in cattle associated leptospirosis in man.

Mastitis characterized by a sudden fall in milk yield and uniformly flaccid udders is associated with leptospirosis in general and infection by *L. hardjo* in particular (Blood and Henderson, 1983). Cases of agalactia generally occur within a week or so of infection, but abortions usually do not follow for four to eight weeks (Hart *et al.*, 1984).

2:7:1:2 SHEEP AND GOATS

The world-wide distribution of leptospirosis in sheep and goats has been described for a variety of different sero-groups of *Leptospira interrogans*. Clinical leptospirosis due to *L. pomona* has been described in sheep by Hartley (1952) and to *L. grippotyphosa* in both sheep and goats by van der Hoeden (1953) and Burdin *et al.* (1958). Various serovars of *L. interrogans* have been isolated from goats in many parts of the world including China, India, Kenya, Israel, Portugal, Turkey,USA (Schollom and Blackmore, 1981) and Australia where Sullivan (1974) reported infections with *L. pomona*. These serovars were found to belong to the *Australis, Grippotyphosa, Hebdomadis* and *Pomona* serogroups. Michna (1967) showed that abortions in sheep were associated with a high prevalence of *L. sejroe* antibodies while Ellis *et al.* (1983) demonstrated a high morbidity, abortions, still births and neonatal deaths in sheep infected with *L. hardjo*. Leptospirosis in bovines has been shown to be a disease of economic importance; this is also likely to apply to sheep and goats (Wanyangu *et al.*, 1988). Serological evidence that sheep play a significant role in the maintenance of *L. hardjo* infections for bovines has been shown by the work of Hathaway and his colleagues (1982). In addition, experimental infections of sheep with *L. hardjo* by Marshall *et al.* (1979), cultural isolations of *L. hardjo* from naturally infected sheep by Bahaman *et al.* (1980) and histological demonstrations of *L. hardjo* in sheep kidneys by Mitchell and Leonard (1985) have reinforced the importance of sheep as a maintenance host.

Leptospirosis in goats and sheep in Kenya was first recognised by Burdin *et al.* (1958) during an outbreak of *L. grippotyphosa* infection in cattle.

Infections with *L. hardjo* in sheep and goats have shown pyrexia and antibody titers to be consistent with the disease, leptospiraemia occurred and the clinical signs were found to be so mild as to pass unnoticed under field conditions, although excretion of leptospires in urine could be detected (Andreini *et al.,* 1983).

The disease in sheep and goats has been shown to be characterized by hemolytic icterus, haemoglobinemia and haemoglobinuria as well as abortions (Kingscote, 1985), still births and neonatal deaths (Ellis *et al.*, 1983). Chronic interstitial nephritis, renal tubular dilatation and focal hepatic necrosis are also a sequele to sheep and goat leptospirosis (McCaughan *et al.*, 1980).

Sheep and goats seem to be less susceptible to infection than cattle. Sheep are most frequently reactive to *L. sejroe* serogroup whereas goats react more to the *L. pyrogens* serogroup (Moite and Myers, 1986).

2:7:1:3 MAN:

Leptospirosis in man is a general term for a range of clinical syndromes caused by infection with any member of the *Interrogans* serogroups (Michna, 1970). The clinical signs of the disease vary considerably from sub-clinical to acute fatal infections, the symptoms in humans resembling those of influenza, and is characterised by fever, chills, headache, myalgia, nausea and vomiting. More severe cases are characterized by icterus, haemoglobinuria, haemorrhages, nephritis and meningitis (Michna, 1970). The few icteric cases may be fatal largely due to renal failure (Russel, 1984). Human infection with

L. hardjo is thought to be higher than is presently assumed and has been shown to have a prolonged period of recovery (Hart *et al.*, 1984).

The high prevalence of infection in cattle has, presumably, led to an increase in cattle associated leptospirosis in human, in whom the disease has been diagnosed commonly in recent years (Christmas *et al.*,1974; Waitkins, 1983, 1985). Cattle associated leptospirosis is a milder form, usually presenting itself as a "flu-like" illness, with fever, severe headache and often mental confusion. Full recovery may take several months in untreated patients. Lethargy is the most common symptom during the convalescence period. In a few cases the infection progresses to lymphocystic meningitis, occasionally hepatorenal failure, and rarely death (Waitkins, 1983). The few cases of meningeal involvement, manifest as aggressiveness and hyperexcitability and an anxious facial expression (Stoenner *et al.*, 1963). In the majority of human cases, however, the illness is usually self-limiting and without sequale (White *et al.*, 1981).

In addition to the potentially large economic losses associated with bovine leptospirosis, this disease also constitutes a significant zoonotic threat (Milner *et al.*, 1980). The majority of human cases result from contact with water and soil contaminated with urine of leptospiruric animals (Russel, 1984). The ultimate contact between producers and sheep at lambing, and the exposure of handlers to urine during transportation of sheep, provide opportunity for transmission of infection to man. Today, rat contamination of water in sewers, wells, etc.. remain an important mode of transmission of leptospirosis to man (Beck *et al.*, 1974). Infections in man have been reported in milkers. The milker works at udder level and is thus liable to be contaminated by urine, either by direct facial splashes or from

aerosols generated when the urine falls to the floor (Hart *et al.*, 1984). Ryan *et al.*(1982) reported high incidences of the disease in dairy farm workers in New Zealand and showed that vaccination of the animals greatly reduced the occupational hazard for these workers.

A serological survey amongst healthy persons in Kenya by Kranendonk *et al.* (1968) revealed a wide-spread infection caused by a large variety of different serogroups. The clinical picture was mild, with some patients becoming jaundiced. Besides the occupational hazards of the veterinary profession, sewer or abattoir work, mining, meat handling or fish trading and farming should also be regarded as significant in the epidemiology of human leptospirosis. Kennel personnel, rodent exterminators and gut washers are also at risk. The possibility of man to man infection has been reported, and the spread by insect vectors cannot be ruled out (Michna, 1970). The possibility of transmission through human milk has been reported in a breast feeding mother (Calore and Koellner, 1988). The infant had acquired the infection from the mother who had been diagnosed positive for leptospirosis.

2:8 DIAGNOSIS:

A variety of diagnostic methods have been used and include bacteriological culturing, direct demonstration of *Leptospira* and immunological procedures. Thus, a combination of the history, clinical signs, direct examination of specimens by dark field microscopy, direct cultures from blood during the acute phase (leptospiremic stage) or urine during the convalescence phase (leptospiruric stage), inoculation into laboratory animals (guinea pigs

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or hamsters), serology and post-mortem can be used to arrive at a diagnosis. Laboratory investigations are needed to confirm the diagnosis. Isolation of leptospires remains the most definite diagnosis of leptospirosis (Rubin *et al.,* 1981). Appendix 2 shows a list of representative strains of leptospires whose antisera are useful in the identification of isolates.

The laboratory procedures, the type of materials required for the examination at different phases of the disease, and the approximate chances of confirmation of leptospirosis are important considerations (Michna, 1970). The outcome of these procedures and their reliability depend very much upon their application at the right time in the cause of the disease, as well as on proper collection and handling of suitable specimens. The isolation of leptospiral organisms from animal tissues is however delicate, tedious and requires skilled techniques. Great importance must be placed on the proper cleaning of glassware to remove any traces of detergent; the use of a small inoculum size of tissue and subsequent serial dilutions to minimize the inhibitory effect of antibodies and antileptospiral substances such as lipids found in tissues and finally, the use of high enrichment media for successful isolation (Turner, 1970 ; Hathaway *et al.*, 1982a ; 1982b)

Many strains of *Leptospira* can be isolated by direct culture in serum-free media (Ellinghausen and McCullough, 1965). Cultural techniques are a better tool for epidemiological survey methods for leptospirosis in domestic animals (Wanyangu *et al.*, 1986). However, a special problem of culturing is that it is too slow when a rapid diagnosis is needed, and some strains are very hard to grow, which emphasizes the need for alternatives, immunological or molecular

methods, for the detection of leptospires in clinical samples (Terpstra, 1986).

Leptospirae are also readily visible with dark field illumination at low magnifications (x100-120) even without the use of oil or water (Turner, 1967). Isolation of leptospires from dead animals had been reported to be a worthless and often unsuccessful exercise. It is generally assumed that autolysis which occurs after death rapidly kills leptospires and renders them unsuitable for culture (Smith *et al.*, 1967; Ellis and Michna, 1977). However, work by Ellis *et al.* (1982b) demonstrated that *L. hardjo* could be isolated from a 4-day dead bovine foetus which was subjected to enviromental temperatures of 15°C for one day and 4°C for the next three days. Wanyangu *et al.* (1986) were also able to isolate *L. icterohaemorrhagiae* from a one week dead coypu preserved at the prevailing weather conditions.

Many workers have tried to isolate *Leptospira* by culturing tissue specimens in various media, especially from aborted foetuses with little success (Kenzy *et al.*, 1961). However, other workers and in particular Dacres *et al.* (1958), Ellis and Michna (1977), Ellis (1978) and Little *et al.* (1980), were successfully able to isolate *Leptospira* from such aborted foetuses. Presently, most of the difficulties have been overcome and it is now possible to diagnose infection of aborted fetuses by a combination of fetal serology, incident light microscopy and culture of fetal kidney tissue (Ellis *et al.*, 1982b). The apparent difficulty of isolation may have been due to loss in viability and ineffectivity of the organisms as a result of degenerative changes due to the time that the foetus remains in the uterus after death (Fennestad and Borg-Petersen, 1958; Kenzy *et al.*, 1961; Ellis and Michna, 1977).

Isolation of the organisms from urine has also been used for diagnosis. This is done because of the possibility that the suspect is shedding *Leptospira* in urine. The urine may be injected subcutaneously into hamsters or guinea-pigs and then later blood or kidney from such laboratory animals is cultured in Ellinghausen's liquid or semi-solid media. Dilution of the urine has been found to improve chances of recovery of *Leptospira* (WHO, 1967). However, culturing from urine may be complicated by the presence of antibodies in the urine which may inactivate the *Leptospira* (Killinger *et al.*, 1971).

Although leptospires can be shed through milk, very few workers have been able to recover *Leptospira* from milk since milk is leptospiracidal (Ellis *et al.*, 1976). Direct leptospiral isolation from bovine milk samples was first reported by Ellis *et al.* (1976). Thiermann (1981) reported the successful isolation of *L. hardjo* and *L. szwajizak* from the milk of experimentally infected cows only when using bovine albumin polysorbate-80 solid medium (BAP-80). Attempts to isolate these organisms using other methods were unsuccessful. The solid BAP-80 plate medium should however be used in conjuction with semi-solid media in any attempts to culture *Hebdomadis* organisms particularly from milk.

Direct demonstration of *Leptospira* in infected materials can be achieved by microscopy, silver impregnation methods and fluorescent antibody techniques (Wilson and Miles, 1975a). Histological examination of kidney and liver specimens for the presence of leptospires is performed using haematoxylin and eosin (HE) and Warthin-Starry silver impregnation stain, based on the original method of Warthin and Starry (1920).

These techniques, however, have disadvantages: the organisms must be present in large numbers, artefacts could easily be mistaken for leptospirae; and some drugs used in treatment may distort the organism. Results from these observations must therefore be regarded as provisional pending results of attempts to isolate the strain or of serological tests (Turner, 1968; Michna, 1970). But even the best of the serological methods still has a limited detection capacity and unfortunately the number of missed cases cannot be reliably estimated (Terpstra, 1987).

Dark field microscopic examination of a drop of urine, if the animal has leptospiruria, can be used to demonstrate presence of *Leptospira*. To demonstrate leptospires in blood requires the blood to be collected in an anticoagulant. The blood cells are then deposited by low-speed centrifugation after which the supernatant is removed and centrifuged at high speed to deposit the leptospires. The material in the "button" is then examined by dark field microscopy. Using this method Wolff (1954) was able to demonstrate leptospires in the blood of 32% of patients in whom leptospiraemia was proved by isolation.

The immunological tests that have been used include: Haemagglutination (HA) and Haemolytic (HL) tests. Complement fixation test (CFT) and the Enzyme Linked Immunosorbent Assay technique (ELISA) (Biancifiori and Cardaras, 1983; Wanyangu *et al.*,1987). Immunodiffusion test with various leptospiral antigens has been achieved and information to this effect accumulated (Yanagawa and Faine, 1966; Yanagawa and Takashima, 1972; Yanagawa *et al.*, 1974). Yanagawa *et al.* (1974) indicated the possibility of identifying leptospires by immunodiffusion, using the antigens extracted from

leptospiral cells with sodium dodecyl-sulfate (SDS). Using this method, they were able to demonstrate a genus-specific reaction.

Much attention has recently been focused on techniques that use enzyme markers conjugated to antigen/antibody instead of radioactive isotopes or fluorescent dyes. The introduction of these enzyme-immunoassays by Engvall and Perlmann (1972) has resulted in extensive use of the tests in a wide range of diagnostic and epidemiological procedures. These rapid serodignostic techniques based on enzyme immunoassays have undergone a lot of advancements (Adler *et al.*, 1980; Terpstra *et al.*, 1980; Zochowski and Waitkins, 1986). The methods are simple, inexpensive, objective and versatile (WHO, 1982). In their work, Biancifiori and Cardaras (1983) showed that leptospirosis can be diagnosed easily and rapidly by ELISA, utilizing purified antigenic fractions. They also showed that ELISA had a higher sensitivity and can be read faster than MAT and offered the advantage of handling a non-infectious antigen. ELISA for leptospirosis diagnosis allowed testing a large number of sera in less than two hours. The IgM-specific dot ELISA technique has been shown to be comparable to the classic MAT in its ability to detect recent exposure to leptospires, it is also rapid and simpler to use (Pappas *et al.*, 1985). Both tests proved suitable for use in the general diagnostic laboratory and both effectively diagnosed acute leptospirosis. More recent work indicates that the dot ELISA technique is more difficult to perform but easier to interpret results (Watt *et al.*, 1988). Only one dilution is required in dot ELISA and no electrical equipments are needed, whereas, a darkfield microscope and several dilutions are needed in MAT.

DNA hybridization is rapidly gaining importance as a diagnostic tool in several infectious diseases, leptospirosis included (Terpstra, 1986). But the handling of radioactive probes and the elaborate extraction procedures used have been the main obstacles to widespread application of the technique. This has been overcome by use of the more stable biotin-labelled probes instead of the radioactive probes (Terpstra *et al.*, 1987), but it is limited to only specially equipped laboratories (Langer *et al.*, 1981).

In the work by Terpstra *et al.* (1987), the applicability of this safer technique was demonstrated in blood, urine and liver smears of fixed whole leptospiral cells. *In situ* DNA hybridization enabled the direct observation and identification of pathogenic leptospires in clinical samples of various origins.

Earlier, Terpstra *et al.* (1986) had observed cross-hybridization between serovars of pathogenic *L. interrogans*. Very little cross-hybridization was however observed with saprophytic *L. biflexa* serovars.

While it is impractical to test for *in situ* cross-hybridization between all of the more than 160 pathogenic serovars recognised so far, the results of the work by Terpstra *et al.* (1987), suggest that a DNA probe prepared from a single serovar can probably be used for the detection of all pathogenic serovars. The use of a mixture of DNA probes from different serovars might, perhaps make the test more useful and preparation of serovar-specific probes may be useful for epidemiological studies.

Although these tests have been available for many years and have recently been modified, they have not been as widely used as the

Micro-agglutination test (MAT), which is more specific and sensitive, except when used in parallel with the MAT (WHO, 1982; Wanyangu *et al.*, 1987).

A positive serological reaction in MAT does not necessarily indicate a current infection as MAT titers may persist for at least 7 years following infection (Roberts, 1958), but MAT is still the standard reference test for serological diagnosis of leptospirosis and is the basis of presumptive classification of *Leptospira* (Turner, 1969; WHO, 1982). The basic principle of the test is agglutination of live or formalinised leptospires by titrated amounts of animal or human serum, but the disadvantages of maintaining a battery of live cultures for the performance of the MAT are well documented (Bragger and Adler, 1976).

Although the MAT is the reference test for the serodiagnosis of leptospirosis, it does not differentiate infection titers from vaccine titers, is tedious, time consuming and can usually be performed only in reference laboratories (Rubin, *et al.,* 1981). It is recommended that a 4-14-day-old fluid culture be used that has been incubated at 30-32^{0}C and contains a density of 1-2x10^{8} leptospiral organisms/ml (WHO., 1982). The antigen should be free from "breeding nests", which are conglomerates of leptospires. The density can be determined by direct counting or by nephelometry. Living cultures or cultures killed with formalin may be used.

The rapid slide agglutination test based on the original method of Galton *et al.* (1958) can also be used for laboratory serological diagnosis of leptospirosis. Galton's test was commercialized by Difco Laboratories, Detroit, Michigan, USA. The Difco bacto leptospiral antigens are stable suspensions of individual and pooled leptospiral

serotypes. They contain formaldehyde in a final concentration of 0.5% (v/v) glycerol to a final concentration of 20% (v/v) and sodium chloride USP to a final concentration of 12% (w/v). The Difco slide test is widely used for presumptive serological diagnosis of leptospirosis but a technical disadvantage of the test is the use of more than one antigen for screening one serum sample. Although this test is useful in the screening for leptospirosis in man and domestic animals, it is recommended to use it in association with the MAT; the standard reference test (Wanyangu *et al.*, 1987)

The antigens chosen for both serological screening (and in epidemiological surveys) are usually of known serovars which are prevalent within the locality. However, changes in the pattern of serovars within the area and new unknown leptospires may emerge; therefore, representative sera of reactors should be tested with a wide range of serovars. Appendix 3 is a list of serovars (with serogroup) for use as antigens in a battery of strains in MAT for detecting infections by an unknown serovar (WHO., 1982). WHO (1982) recommends a battery of fifteen serovars which cover most of the known cross-reactions of leptospires for use to ensure detection of antibodies which may be provoked by any of the large number of serovars. These include; *copenhageni, poi, canicola, castellonis, pyrogenes, grippotyphosa. wolffi, borincana, szwajizak, djatzi, autumnalis, bratislava, pomona, tarassovi* and *patoc 1.* This proposed list of antigens, however, may be modified according to the local needs.

The demonstration of the humoral immune response may be difficult in the early acute phase, especially by the commonly employed MAT (Turner, 1967). But the demonstration of the cell mediated immune (CMI) response by means of the leucocyte

migration inhibition test (LMI) and its use in the diagnosis of the disease, is a possible alternative since CMI continues to persist at low levels even when the humoral response to past infection decreases and the antibodies eventually become undetectable (Ratnam *et al.*, 1984).

2:9 TREATMENT AND CONTROL:

Effectiveness of antibiotics in the treatment and control of leptospirosis is controversial (Hanson, 1976; Stoenner, 1976). Even in experimental infections, treatment of leptospirosis in animals has yielded conflicting results (Stalheim, 1967; Elwell, 1985; Ellis *et al.*, 1985), but the use of dihydrostreptomycin has been successful in treating leptospirosis cases, although alone it has limited value in the control (Hart *et al.*, 1984). However, during renal tubular infection, dihydrostreptomycin may be useful in the treatment of animals in an attempt to eradicate leptospires from urine, and it therefore has been recommended as the drug of choice in animals (Doherty, 1967; Stalheim, 1961, 1967). In cases of infection with serovar *hardjo*, dihydrostreptomycin has not been always effective in treatment and prevention of leptospiruria in carrier cattle (Ellis *et al.*, 1985). More recent work has shown that leptospires are susceptible to chemotherapeutic concentrations of all antibiotics (Wanyangu, 1988). In particular, amoxycillin and ampicillin with minimum inhibition concentrations (MICs) of 0.002-0.008μg/ml were found to be highly effective in animals while in human leptospirosis, chemotherapy showed a significant reduction in the duration of the disease in patients infected with serovar *hardjo* and not serogroup *icterohaemorrhagiae*.

Ringen *et al.* (1955) found that dihydrostreptomycin given intramuscularly at a dose of 11mg/kg body weight twice daily for 3 days eliminated the carrier state in experimental cattle while Terramycin[R] (Oxytetracycline) only reduced the number of leptospires excreted in urine but did not eliminate them. Stalheim (1967) confirmed that dihydrostreptomycin could be used to successfully treat cattle against renal leptospirosis using 25 mg/kg body weight, given once. Mitchell *et al.* (1959) observed a suppression of antibody response on early antibiotic therapy and they postulated that it could have been due to a limitation in generalization and localization of the infection in the kidney. This antibiotic therapy is efficacious if initiated during the first few days of the disease but is of questionable value thereafter. It does not benefit the patient when, as is usual, it is not began before the third or fourth day of illness.

Penicillin in large doses for severe illness is favourable (Turner, 1967). Amoxycillin has emerged as the preferred oral treatment because it is well absorbed. It has been used empirically in a number of *L. hardjo* infections because the illness is often prolonged, but with only anecdotal evidence that it is effective in shortening the course of the disease. The usual dose is 500 mg three times daily for five days (Hart *et al.*, 1984). Amoxycillin, being a better antibiotic than procaine penicillin G in the treatment of leptospirosis, has been indicated (Munnich and Lakatos, 1976), and its use may result in earlier clinical recovery in cases of serovar *hardjo* as stipulated by Hart *et al.* (1984).

Despite the controversy surrounding antibiotic therapy, the main aim in clinical management of leptospirosis should be to prevent the

organism from invading the tissues, especially the kidneys (Wanyangu, 1988).

The control of the disease is best achieved by good management, combined with vaccination (Stoenner, 1976) using single or multiple serovars prevalent in an area. As a zoonosis, Thonton and Gracey (1974) have indicated the following as some of the control measures necessary: hygienic precautions, reduction of rat population, and prompt treatment of clinical cases in man. Any control programme should be suited to the farm in question. It is essential to study management practices, terrain, waterway, climatic conditions, alkalinity of the soil and water, composition of the herd and the interrelationship between various livestock and wildlife before a control programme is initiated (Morter, 1972). Any new animals introduced in the farm should be isolated, observed and blood tested to avoid herd exposure. Other methods of control include, occupational protection of individuals at risk by use of protective clothing, vaccination of animals and humans, rodent control and drainage of wet areas (Russel, 1984). A change of pasture also reduces exposure to contaminated surface waters (Kenzy *et al.*, 1960). The vaccines available for animals afford clinical protection but do not necessarily prevent infection. Increased awareness of the problem, and the use of protective pest control measures have resulted in a considerable decrease in the number of human cases in the groups at high risks such as sewer workers (Palmer *et al.*, 1987).

3. MATERIALS AND METHODS:

3.1 STUDY AREAS:

The study was carried out in Nyandarua and Turkana districts of Kenya (Figure 1). These two districts represent two different ecological zones (Table 1). Nyandarua District is in Central Province of Kenya and falls within ecological zone II whose climate is tropical in nature; it is humid to sub-humid with a moisture index not less than -10, and the vegetation is forest and bushland. Turkana District, on the other hand, is in the Rift-Valley Province and is within ecological zones V and VI whose climate is semi-arid to arid (Anon.,1970). The moisture index in the semi-arid parts of the district ranges between -42 to -52; the area is characterised by woody vegetation. In the dry parts of the district the moisture index ranges from -51 to -57 and the vegetation is characterised by a rangeland of dwarf shrubs and bushed grassland (Anon,1970).

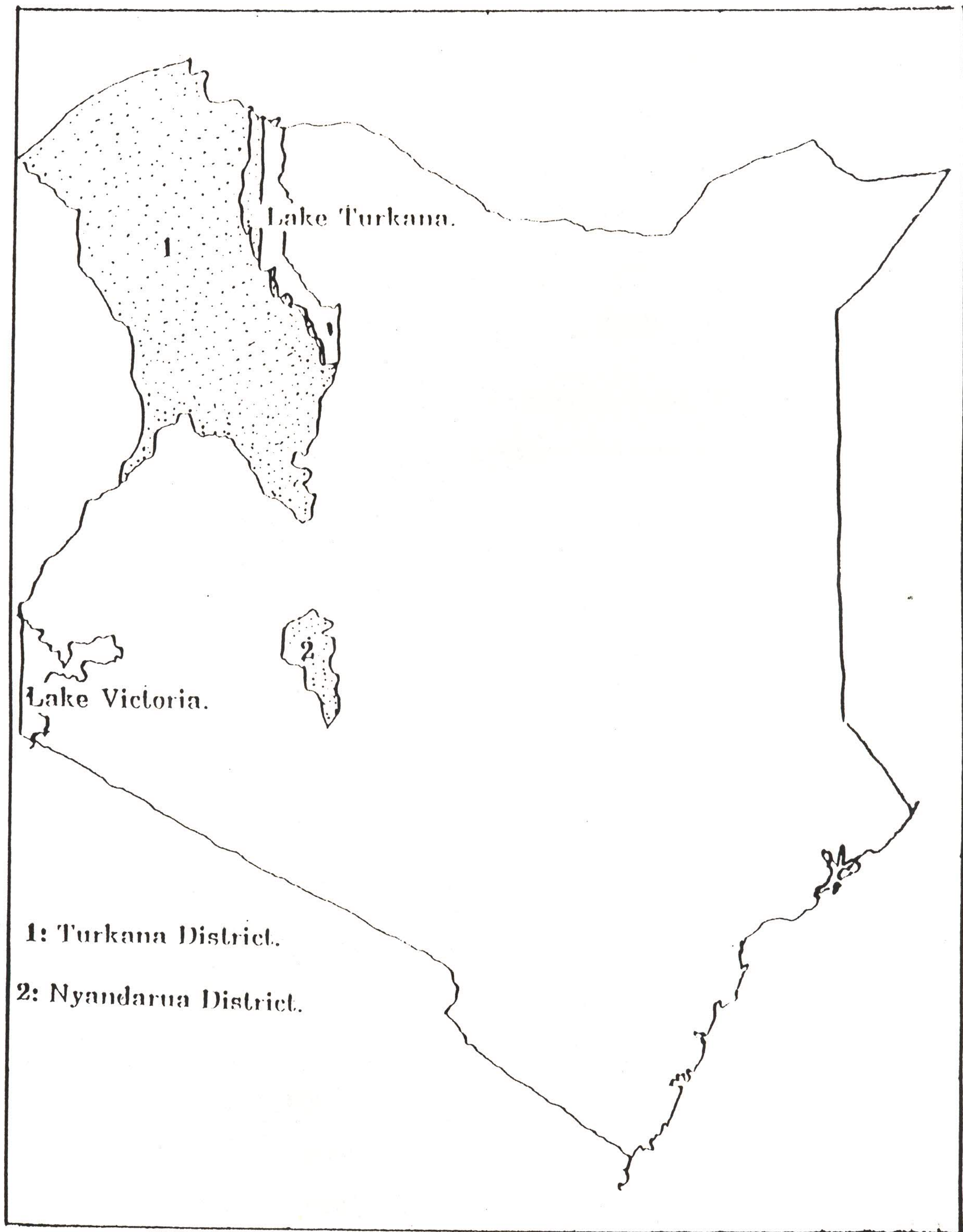

Figure 1: Map of Kenya showing the location of Turkana and Nyandarua districts from which the serum samples were collected.

Source: A study in Physical and Human Geography (Ojany and Ogendo, 1973).

Table 1: Project Areas and a description of the Ecological Zones they occur in.

Zone	Area	Description of the zone
II	Nyandarua	Equatorial climate: Humid to dry sub-humid. Moisture index not less than -10. Forests and bushland.
V&VI	Turkana	Arid-Moisture index -42 to -51. Woody vegetation and very arid. Moisture index - 51 to -57. Range-land of dwarf shrubs and bushed grass-land.

Source: National Atlas of Kenya. Survey of Kenya (Anon., 1970).

Nyandarua District is inhabited mainly by mixed, small and large scale farmers whose main livestock activity is keeping dairy cattle and wool sheep. Goat keeping is not as common in the district. The area is usually wet throughout the year. Turkana District is mainly hot and dry for the greater part of the year. The inhabitants are semi-nomadic pastoralists. Cattle are kept for both beef and milk mainly for subsistence purposes. The goat population is higher than that of sheep. Table 2 indicates the population sizes of cattle, sheep and goats and humans in the two districts.

Table 2: Population sizes of Cattle, Sheep and Goats and Humans in Nyandarua and Turkana districts.

District	Population size		
	Cattle	Sheep and Goats	Humans
Nyandarua	118,222	124,500	247,572
Turkana	220,387	1,089,318	142,066

Source: Livestock and Wildlife population Inventories by districts in Kenya, Technical Report No. 102, Kenya Rangelands Ecological Monitoring Unit (KREMU), (Donald, G. Peden, 1984).

3.2 COLLECTION OF SERUM SAMPLES:

A total of 2,172 serum samples were collected from Nyandarua and Turkana districts. Of these, 998 were from Nyandarua District and 1,174 were from Turkana District. Table 3 is a summary of the total number of sera collected in each district for each animal species.

Table 3: Summary of the total number of serum samples collected from each district.

DISTRICT	CATTLE	SHEEP & GOATS	HUMAN	TOTAL
Nyandarua	326	357	315	998
Turkana	439	369	366	1174
Total	765	726	681	2172

Sampling for sera from the various animal species was done as described below:

3.2.1 LIVESTOCK SAMPLES :

Serum samples from domestic animals (cattle, sheep and goats) were obtained by systematic random sampling which involved cluster sampling in cases where the herd and/ or flock sizes were small as described by Leech and Sellers (1979).

In Nyandarua District, blood (from which serum samples were obtained) was collected from animals in randomly selected farms. In farms with large herds, systematic random sampling was carried out. In farms with small herds, cluster sampling, involving bleeding of all the animals in the farm, was carried out.

In Turkana District, (unlike Nyandarua where sampling was done at the farm level) blood samples were collected from randomly selected herds of animals presented for vaccination at various crushes in the various divisions. These were systematically sampled. The herds were identified by their owners who presented them for

vaccination during the vaccination campaign period. Sheep and goat blood samples were collected at the central slaughter-house at Lodwar. These animals which were brought for slaughter came from all over the district. About 10-15 ml of blood were collected into sterile universal bottles from the severed neck blood vessels during the slaughtering process. Where these animals were not from the slaughter-house, sheep and goats were bled using gauge 18 disposable sterile needles of length 1.5 in. Blood from cattle was collected by bleeding from the jugular vein using gauge 14 or 16 bleeding needles of length 2 in.

Blood samples (10-15 ml) collected into the universal bottles were kept overnight at room temperature to allow serum separation. About 1-1.5 ml of the serum was harvested by decanting into 2 ml plastic vials which were then labelled appropriately and stored in a freezer (-20°C) until they were transported to the laboratory. Where a clot hindered the serum decantation, an applicator stick was used to ease it in order to facilitate decantation. This was done carefully to avoid disturbing the clot.

The serum samples were transported to the laboratory in a cool-box packed with ice-packs. They were kept in a freezer at -20°C until required.

3.2.2 HUMAN SAMPLES :

Human serum samples were provided by the regional hospital's laboratories. These samples had already been processed by the laboratories and packed in 2 ml plastic vials ready for transportation to our laboratory. In Nyandarua District the samples were collected from patients seeking medical attention from Ol-Kalau District hospital and Nyahururu General hospital. These patients came from various parts of the district, and therefore were representative of the district. In Turkana District, 25 of the serum samples were collected at Lodwar District hospital and the rest (also from Turkana) were obtained by courtesy of the African Medical and Research Foundation (AMREF), Nairobi. These too were transported to the laboratory where they were stored in a freezer at -20°C until needed.

3.2.3 Distribution of the number of serum samples from each district for cattle, sheep and goats, and human beings.

Table 4 shows the total number of male and female animals sampled out of the total sample for each species in each of the two districts. Human samples from Turkana District did not have information on sex and age.

Table 4: Distribution of the total number of sera from cattle, sheep and goats, and human beings by sex in Nyandarua and Turkana districts.

	Total		Males		Females		Not specified	
	N	T	N	T	N	T	N	T
Cattle	326	439	68	136	258	303	0	0
Sheep & goats	357	369	72	367	285	2	0	0
Human	315	366	123	N/A	192	N/A	0	366

Key:

N=Nyandarua. T=Turkana. N/A=Information not available.

3.2.4 Distribution of the total number of serum samples from each district for each species by the age-group.

Table 5 shows the total number of adult and young animals from which sera were obtained out of the total sera collected for cattle, sheep and goats, and human beings in each of the two districts. In Turkana District, all the sheep and goat sera were collected at slaughter and were from adult animals only. Human sera from Turkana District were obtained from African Medical Research Foundation (AMREF) and did not have information on sex and age.

Table 5: Distribution of the total number of serum samples from Nyandarua and Turkana districts for each species by the age-group.

	Total		Adults		Young		Not specified	
	N	T	N	T	N	T	N	T
Cattle	326	339	241	226	85	213	0	0
Sheep & goats	357	369	283	369	74	0	0	0
Humans	315	366	205	N/A	110	N/A	0	366

Key:

N=Nyandarua. T=Turkana. N/A=Information not available.

3.2.5 Distribution of the total serum samples from each district for each species by sex and age-group.

Table 6 shows the total number of adult and young animals for each sex, from which sera were collected out of the total sera obtained from cattle, sheep and goats, and human beings in each of the two districts. No young sheep and goats were sampled from Turkana District and as indicated earlier, only two of them were females.

Table 6: Distribution of the total serum samples from Nyandarua and Turkana districts for each species by sex and age-group.

	Total		MALES Adult		MALES Young		FEMALES Adult		FEMALES Young		N/A	
	N	T	N	T	N	T	N	T	N	T	N	T
Cattle	326	439	36	52	32	84	205	174	53	129	0	0
Sheep & goats	357	369	47	367	25	0	236	2	49	0	0	0
Human	315	366	72	N/A	51	N/A	133	N/A	59	N/A	0	366

Key:

N=Nyandarua. T=Turkana. N/A=Information not available.

3.3 SOIL SAMPLES:

Soil samples were collected from the two districts for the purposes of pH determination. In Nyandarua District, the samples were collected from farms that had been selected for sera collection and in Turkana District from the divisions where cattle, sheep, goat and human blood samples were obtained. Approximately 500g of soil was collected into polythene bags and brought to the Department of Soil Science of the University of Nairobi, Kabete. The average pH of soils from each district were calculated and compared (see Section 4.13).

3.4 OTHER INFORMATION GATHERED IN THE STUDY:

At the time of collection of sera, the following information was recorded; the animal species, sex and age group. The following information for the two districts was gathered from the local meteorological stations and the Kenya Meteorological Department, Nairobi; rainfall, relative humidity and temperatures. Means, where applicable, were calculated to enable comparisons for the two districts (see Section 4.14).

3.5 *LEPTOSPIRA* REFERENCE CULTURES AND ANTISERA:

A battery made up of eleven *Leptospira* serotypes (serovars) was used in screening and titrating the serum samples using the MAT (WHO, 1982). These antigens and their corresponding antisera were obtained from Difco Laboratories, Detroit, Michigan, USA through the National Veterinary Research Centre, Kabete. They included; *L. copenhageni, L. mankarso, L. autumnalis, L. sejroe, L. hardjo, L. wolffi, L. grippotyphosa, L. pomona, L. canicola, L. australis and L. patoc 1* (Table 7). These comprise of serovars known to be common in Kenyan animals and which have previously been used in survey studies on leptospirosis in Kenya (Kranendonk *et al.*, 1968; D'Souza, 1983).

Table 7: Battery of *Leptospira* antigens used for serology in the Microagglutination Test.

Serogroup	Serovar	Strain
Icterohaemorrhagiae	*copenhageni*	M20
	mankarso	Mankarso
Autumnalis	*autumnalis*	Akiyami A
	sejroe	M84
Hebdomadis	*wolffi*	3705
	hardjo	Hardjoprajitno
Grippotyphosa	*grippotyphosa*	Moskva V
Pomona	*pomona*	Pomona
Canicola	*canicola*	Hond Utrecht IV
Australis	*australis*	Ballico
Semaranga	*patoc*	Patoc 1

(Source: Veterinary Laboratories, Kabete, Kenya).

3.6 PROPAGATION OF LEPTOSPIRAE, SUB-CULTURING, MAINTENANCE AND ANTIGEN PRODUCTION:

Ellinghausen and McCullough liquid medium (Ellinghausen and MacCullough, 1965), modified by Johnson and Harris (EMJH) (1967) (Appendix 3), was used for routine preparation of the eleven *Leptospira* antigens. The Ellinghausen, MacCullough, Johnson and Harris (EMJH) medium is a Tween-albumin (TA) bovine serum (Tween 80) medium available commercially from Difco Laboratories, Detroit, Michigan, USA. It was supplied as a Bacto-Leptospira Base-EMJH (code 0794) and Bacto-Leptospira Enrichment-EMJH (code 0795) and prepared for use according to the formulations described by Ellinghausen and MacCullough, (1965) as modified by Johnson and Harris (1967).

Semi-solid Fletcher's medium (Agar added), prepared as in Appendix 2 was used for maintenance of stock cultures.

The eleven test antigens used in the study, were produced from 5-7 day-old whole cultures routinely prepared in Difco EMJH medium and incubated at 29-30°C. At the age of 5-7 days the recommended density of approximately 2x10^{8} cells per ml for use in the MAT was achieved. Estimation of the density was made by the method described by Alexander (1976); 0.01 ml (a loopful) of the culture was placed on a clean glass slide, covered with a 22x22 mm cover slip and the preparation examined at x450 magnification. A count of 100-200 *Leptospira* organisms in the field was considered to give a satisfactory density for use (Alexander, 1976). Purity and viability of the whole culture antigens were also determined before use by placing a drop from the Dinger's zone of the cultures on a slide

and examining it at x400 plus oil under a dark-field microscope. After establishing that the culture was pure and free from contamination (mainly by micrococci), and that there was a good density of viable *Leptospira* organisms, 1 ml of the culture was sub-cultured into 10 ml of freshly prepared EMJH medium. The sub-culturing was done at weekly intervals in order to provide 5-7 day-old antigens for use in the tests.

Stock antigens were cultured and maintained in Fletcher's medium in screw-cup tubes. One ml of each serovar drawn from the top part (Dinger-zone) of the medium of origin, and having a satisfactory viability, purity and density test results, was inoculated into 10 ml of fresh Fletcher's medium. These stock cultures were made in duplicate. One set was incubated at 29-30°C and the other kept at room temperature. After signs of growth (visible ringed area of growth at the top of the medium-Dinger's zone), the cultures were transferred into a dark cupboard for storage. Sub-culturing of these stock cultures was done at 6-8 week intervals as they are viable for upto three months and must be sub-cultured before this period. It was important to observe safety measures and to maintain sterile conditions in the working area during these sub-culturing procedures.

3.7 PURIFICATION / DECONTAMINATION OF CONTAMINATED CULTURES:

Any culture that was found to be contaminated with other micro-organisms was purified by use of sterile millipore filters (Swinnex^{R}-13 filter unit: Millipore Corporation, Bedford, Mass.

01730, USA) combined with the addition of 100mg of 5-fluorouracil (5UF) per ml of the medium. Fluorouracil makes the medium selective for leptospires (Johnson and Rogers, 1964). The procedure involved filtration of the contaminated cultures through the above bacteriological filters of pore size 0.45 microns mounted onto a syringe. The filtrate was collected into 2-3 tubes of fresh medium (EMJH and Fletcher's) and incubated as previously described. Four to seven days later, the decontaminated cultures were tested for purity and viability under the dark-field microscope.

Other precautions taken were to avoid use of old cultures (> 7 days-old) since leptospirae tend to form "breeding nests" (conglomerates of leptospires) which are micro-colonies occurring in older cultures. These micro-colonies interfere with the reading of the tests as they consist of densely packed masses of entangled organisms resembling agglutinated leptospirae. They can be removed by differential centrifugation. Very young cultures had to be avoided too as these would give low concentrations. Age and the density of the antigen are among the variables which may affect the reproducibility of the results (WHO., 1982). Antigens derived from an old culture and those with a high concentration of leptospires may lack sensitivity, which is enhanced by antigens with a low density of leptospires.

Use of unsuitable media, and inhibitory substances in the inoculum and antibiotic therapy also affect the viability of leptospirae and were therefore avoided in order to produce pure and viable test antigens.

3.8 MICROSCOPIC -AGGLUTINATION TEST (MAT):

The test was based on the original procedure described by Galton *et al.* (1965) and modified by Cole *et al.* (1973). It has been recommended as the reference method for serological classification, serotyping and identification of isolates, and is useful for testing for antibodies in serum (WHO, 1982). Live antigens were used.

The serum samples were screened at 1:50 dilution. All samples showing 50% agglutination were regarded as positive and titrated further.

3.8.1 MAT TEST PROCEDURE:

3.8.1.1 **Screening:**

The test serum samples were dispensed into flat-bottomed microtiter plates (Limbro Chemical Co. New Haven, Conn. and Dynatech Laboratories, Alexandria, Virginia) and screened at 1:50 dilution against all the eleven *Leptospira* serovars. Each column of the microtiter plate was used for a different serum and separate rows for each of the antigens.

The serum was diluted by mixing 50 μl of the test serum with 1.2 mls of phosphate buffered saline (PBS, pH 7.4) containing 0.4% formalin in labelled plastic vials. This gave a serum dilution of 1:25. To screen the serum, 25μl of the diluted serum was dispensed into each of eleven wells in the appropriate columns using a 25 μl Eppendorf dropper pipette (Eppendorf, West Germany). Twenty-five μl of 5-7 day-old antigen suspension (suspended in liquid EMJH medium) were then discharged into corresponding wells, making a

final dilution of 1:50 in a volume of 50 µl. Separate pipette dropper tips were used for each serum and antigen. Each plate was then tapped gently to mix the contents while covered with a plate cover (Microtest 11, Falcon plastics, Oxnard, California, and Linbro Chemical Co., New Haven, Conn.) to exclude debris and prevent evaporation. The preparations were then incubated at 30°C for 2-3 hours, after which the wells were examined for agglutination by placing the plates on the stage of a dark-field zoom microscope (No. 599-003, E. Leitz, Inc., Rockleigh, N.J.) at x120 magnification. The results were read as either positive or negative. The degrees of agglutination were recorded as follows; 4+ where 75% or more agglutination was observed, 3+ where 50-75% cells agglutinated and many clumps were seen in the field, 2+ where 25-50% cells agglutinated and at least one specific clump was seen in the field or a + where there were occasional small clumps or small stellate aggregations.

The control tests included were; 1.) hyper-immune sera (known positive sera) and each antigen used and 2.) phosphate buffered saline and each antigen as a negative control.

3.8.1.2 **Titration of positive sera:**

Any positive serum samples detected in the screening test were titrated using a two-fold serial dilution of from 1:50 to 1:1600 in separate plate wells against the respective antigens. Six wells were used for each serum (six dilutions from 1:50 to 1:1600) titrated, which allowed sixteen sera to be titrated on a single plate (duplicate sets) against a single antigen. Fifty µl of the diluted positive test serum was put into the first well of each column (8 wells for a different test

serum) in the two halves of the plate using a 50 μl volume Eppendorf dropper pipette. Into each of the remaining wells, 25 μl of phosphate buffered saline with 0.4% formalin was added using a titertek multichannel pipette (8 tips) (Flow Laboratories, Rockville, Maryland, USA); 25 μl of serum was drawn from each well in the first column and dispensed into the succeeding wells to make doubling dilutions of the sera, giving a dilution range of 1:50 to 1:1600. This allowed eight serial dilutions at once. Twenty-five μl of the antigen were then added into each well in the plate, mixed, incubated at 30°C for 2-3 hours and examined for agglutination as previously described. The end-point was taken as the highest dilution of serum in which at least 50% of leptospirae were found agglutinated (Anon, 1967).

The interpretation of the titers was done as stipulated by Carter and Moujen (1981) where an end-point titer of 1:50 to 1:100 was considered suspicious while a titer of 1:200 or more was considered positive.

3.9 ANALYSIS OF DATA:

The data obtained from the two districts for the various animal species were analysed to determine the serological distribution of leptospiral antibodies in these animal species and for the two different districts. The prevalences of these antibodies were compared between and within these animals for the eleven serovars used in this study. The serovars occurring in each district and in each animal species were determined in order to investigate the possible epidemiological role these animals played in the distribution

of leptospirosis in the two districts. The distribution of antibodies by the species, age-group and sex were also determined to see whether these factors had any influence on the spread of the disease. An attempt was made to investigate the possible effects various factors (rainfall, temperature, relative-humidity, soil pH and husbandry) may have on the distribution of leptospirosis in the two districts. Prevalences of each of the eleven serovars in each species of animal were used to determine the serovar(s) commonest amongst these animals, thus attempting to evaluate the possible role each animal species played in the epidemiology of each serovar.

3.9.1 Comparison of the proportions of animals positive for *Leptospira* antibodies between the two districts

The Z-test for independent samples (Remington and Schork, 1985) was used to determine whether the proportions of animals positive for *Leptospira* antibodies differed significantly between Nyandarua and Turkana districts;

The null hypothesis (H_0) was

$H_0 : P_1 = P_2$ and the alternative hypothesis (H_1) was

$H_1 : P_1 \neq P_2$.

Where, P_1= Proportion of animals positive for leptospirosis in Nyandarua District and P_2= Proportion of animals positive for leptospirosis in Turkana District. The results were interpreted at 0.05 level of significance.

3.9.2 Determination of the effect of animal species, sex and age-group on the prevalence rate of leptospirosis in cattle, sheep and goats and humans within Nyandarua and Turkana districts.

The Chi-square test (X_c^2) (Steel and Torie, 1981) was used to test whether there were significant differences between various epidemiological factors and positive animal reactors on MAT for each animal species within each district. For this purpose it was necessary to classify the results for the individual animals in each district into those positive and those negative by species, age group and sex in each district. The results were set out as follows,

Distribution of MAT results of the animal sera from Nyandarua and Turkana districts by species , sex and age-group.

Animal factor (species, sex and age-group).

		F_1	F_2	Totals
MAT	Positive	a	b	a+b
Results	Negative	c	d	c+d
	Totals	a+c	b+d	a+b+c+d =N

$$X_c^2 = \frac{N([ad-bc]-N/2)}{(a+b)\ (c+d)\ (a+c)\ (b+d).}$$

Where, F_1 and F_2 denote any one of the factors (species, sex or age-group) for each of the animal species under consideration in Nyandarua and Turkana districts respectively, and for which the

MAT results are indicated, where a and b are the positives while c and d are the negatives for the two districts respectively.

The differences between the percentages were taken to be significant at the 0.05 level if the value of X_c^2 calculated was equal to or greater than 3.84. (Snedecor and Cochran, 1972).

3.9.3 Testing for differences in the prevalence between cattle, sheep and goats, and humans within each of the two districts.

The chi-square test statistic was used to test whether there was significant difference in the prevalence of *Leptospira* antibodies between cattle, sheep and goats, and humans within Nyandarua and Turkana districts. It was necessary to classify the results of the MAT for each animal species within each district as follows;

	+ve Sera	-ve Sera	Total Sera
Cattle	a	b	a+b
Sheep and Goats	c	d	c+d
Humans	e	f	e+f

The difference in the prevalence between the three animal species within each district was taken to be significant at 0.05 (with a 2 degrees of freedom) level (Putt *et al.*, 1987).

4: RESULTS

4.1 Results of MAT on cattle, sheep and goat and human sera obtained from Nyandarua and Turkana districts.

Two thousand, one hundred and seventy-two (2,172) serum samples were collected from Nyandarua and Turkana districts. These samples included;326 cattle sera, 357 sheep and goats sera, and 315 human sera from Nyandarua District (Table 8), and 439 cattle, 369 sheep and goats, and 366 human sera from Turkana District (Table 9).

The results of MAT for each animal species in these two districts are as shown in Tables 8 and 9 below.

Table 8: Results of MAT on cattle, sheep and goat sera obtained from Nyandarua District.

Species	Total tested	Reactive sera	Sera +ve	Sera ±	Sera -ve
Cattle	326	161	111 (34.0)	50	165
Sheep & Goats	357	196	122 (34.2)	74	161
Human	315	24	0 (0.0)	24	291

Key:

Reactive serra= Total sera reacting at 1:50 screening dilution.

± = Total suspicious sera (reactive at 1:50 to 1:100).

+ve = Total positive sera (≥ 1:200).

Parenthesis = The percentage positive sera.

--ve = Total negative sera.

Table 9: Results of MAT on cattle, sheep and goat sera obtained from Turkana District.

Species	Total tested	Reactive sera	Sera +ve	Sera ±	Sera -ve
Cattle	439	194	98 (22.3)	96	245
Sheep & Goats	369	88	14 (3.8)	74	281
Human	366	51	17 (4.6)	34	315

Key:

± = Total suspicious sera (reactive at 1:50 to 1:100).

+ve = Total positive sera (≥ 1:200).

Parenthesis = The percentage positive sera.

-ve = Total negative sera.

Using the Z-test (Section 3.9.1), the occurrence rates of *Leptospira* agglutinins in cattle, sheep and goats, and humans, were used to determine if there were any significant differences in the prevalence of *Leptospira* antibodies in each of the two animal species and man between the two districts.

Comparison between the percentage (34.0%) cattle sera from Nyandarua District (Table 8) with positive MAT titers to one or more leptospiral antigens with the percentage (22.3%) of the cattle sera from Turkana District (Table 9) with positive MAT titers showed a significant difference ($p < 0.05$). Similarly, comparison between sheep and goat sera which had prevalences of 34.2% and 3.8% in Nyandarua and Turkana districts (Tables 8 and 9), respectively, was significant ($p < 0.05$). The difference between the prevalence of *Leptospira* antibodies in human sera for Nyandarua (0.0%) and

Turkana (4.6%) districts (Tables 8 and 9) was also significant ($p < 0.05$).

4.2 MAT results of the total number of serum samples from each of the two districts for each animal species and man by both sex and age-group.

Tables 10 and 11 show the distribution of the MAT results for serum samples from both districts by sex and age-group. Using the Chi-square test (Section 3.9.2), the various prevalence rates of *Leptospira* antibodies in each animal species were compared by species, sex and age-group to determine if these factors had any effect on the observed prevalence rates. The results were as indicated in tables 10 and 11 below.

Tables 10: Results of MAT on cattle, sheep and goat s, and human sera obtained from Nyandarua District by sex and age-group.

		Males				Females			
	reactive	Adult		Young		Adult		Young	
Species	sera	±	+ve	±	+ve	±	+ve	±	+ve
Cattle	161	6	18	4	14	27	64	13	15
Sheep& Goats	196	121	8	1	8	50	82	11	14
Human	24	7	0	3	0	10	0	4	0

Key:

Reactive sera = Total sera reacting at 1:50 screening dilution.

± = Total suspicious sera (reactive at 1:50 to 1:100).

+ve = Total positive sera (≥ 1:200).

Out of the total cattle sampled from Nyandarua, 20.9% were male and 79.1% were females. In these, 47.1% of the males and 30.6% of the females were positive to one or more leptospiral antigens. The prevalence rate appeared to be dependent on sex ($p < 0.05$). Of this same cattle population, 73.9% were adult and 26.1% young animals. Thirty four per cent (34.0%) of these adult animals and 34.1% of the young were positive to one or more of the leptospiral antigens. Age-group did not appear to be associated with the prevalence rate of *Leptospira* antibodies in cattle within this district ($p > 0.05$). Of the total male cattle sampled from Nyandarua, 52.9% were adult and 47.1% were young. Of these, 50.0% of the male adults and 43.8% of the young males were positive to one or more serovars. Female cattle from Nyandarua consisted of 79.5% and 20.5% adult and young animals respectively. Of these, 31.2% and 28.3% female adult and young animals were positive to one or more leptospiral antigens. Age did not appear to influence the prevalence rate of *Leptospira* antibodies in both male ($p > 0.05$) and female ($p > 0.05$) animals.

Out of the total sheep and goats sampled, 20.2% and 79.8% were males and females respectively. Of these 36.1% males and 33.7% females were positive to one or more leptospiral serovars. In these animals, 79.3% and 20.7% were adult and young, respectively. Out of the adult animals, 35.3% male and 29.7% female were positive to one or more serovars. In both male and female animals, sex ($p > 0.05$) and age-group ($p > 0.05$) did not appear to influence the prevalence rate of *Leptospira* antibodies within the district.

There were no positive sera from human samples in Nyandarua District.

Table 11: Results of MAT on cattle, sheep and goat sera obtained from Turkana District by sex and age-group.

		Males				Females			
	Reactive	Adult		Young		Adult		Young	
Species	sera	±	+ve	±	+ve	±	+ve	±	+ve
Cattle	194	10	20	14	9	37	49	35	20
Sheep& Goats	88	74	13	0	0	0	1	0	0
Human	51	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Key:

Reactive sera = Total sera reacting at 1:50 screening dilution.

± = Total suspicious sera (reactive at 1:50 to 1:100).

+ve = Total positive sera (≥ 1:200).

N/A = Information not available on sex and age.

Out of the cattle sampled from Turkana District, 31.0% were males and 69.0% were females. Of these, 51.5% were adults and 48.5% young. Amongst the samples, 21.3% and 22.8% male and female animals, respectively, were positive, while 30.5% and 13.6% were the positive adult and young animals respectively.While sex ($p > 0.05$) did not appear to influence the prevalence rate, age ($p < 0.05$) did. *Leptospira* antibodies appeared to be more prevalent in adult males and females than in the young males or females in cattle within Turkana. The prevalence rate in both male and female animals within Turkana appeared to be dependent on age; of the total male animals, 38.5% of adult males and 10.7% of young males were positive for *Leptospira* antibodies ($p < 0.05$), while, 28.2% and 15.6%

of the female adult and young animals, respectively were positive ($p < 0.05$).

The associations between the prevalence rate in sheep and goats sampled from Turkana and the sex and age-group were not determined since all the animals sampled were adults and only two samples were from female animals.

Human samples did not have the sex and age-group categories specified; hence the influence of sex and age were not determined.

4.3 MAT results of the total number of sera at 1:200 dilution for each animal species within each of the two districts.

In Nyandarua District, 34.0% of the total cattle sera and 34.2% of the total sheep and goat sera were positive to one or more leptospiral antigens. None of the human sera collected from this district were positive. In comparison, sera from Turkana District were positive to one or more leptospiral antigens as follows; 22.3% of the total cattle sera, 3.8% of the total sheep and goat sera and 4.6% of the total human sera (Table 12).

Table 12: Distribution of the positive and negative number of sera to any one of the eleven leptospiral antigens in each animal species in Nyandarua and Turkana districts.

Area	Animal species	Total sera tested	Sera +ve	Sera -ve	%sera +ve
NYANDARUA	Cattle	326	111	215	34.0
	Sheep & Goats	357	122	235	34.2
	Human	315	0	315	0
TURKANA	Cattle	439	98	341	22.3
	Sheep & Goats	369	14	355	3.8
	Human	366	17	349	4.6

Key:

Sera -ve = All sera that did not show positive reaction at a screening dilution of ≥1:200 to one or more antigens.

Sera +ve = All sera that showed a MAT titer of 1:200 or above to one or more antigens.

Between the three animal species, within each district, Nyandarua ($p < 0.05$) and Turkana ($p < 0.05$), there appeared to be a significant difference in the prevalence of *Leptospira* antibodies between cattle, sheep and goats, and humans within both districts.

4.4 Comparison of prevalence rates of leptospiral antibodies in sera from each animal species for the two districts.

Figures 2 and 3 show the comparisons of the prevalence rate of antibodies to each of the eleven leptospiral antigens (A - K) between cattle, sheep and goats and human in Nyandarua and Turkana districts.

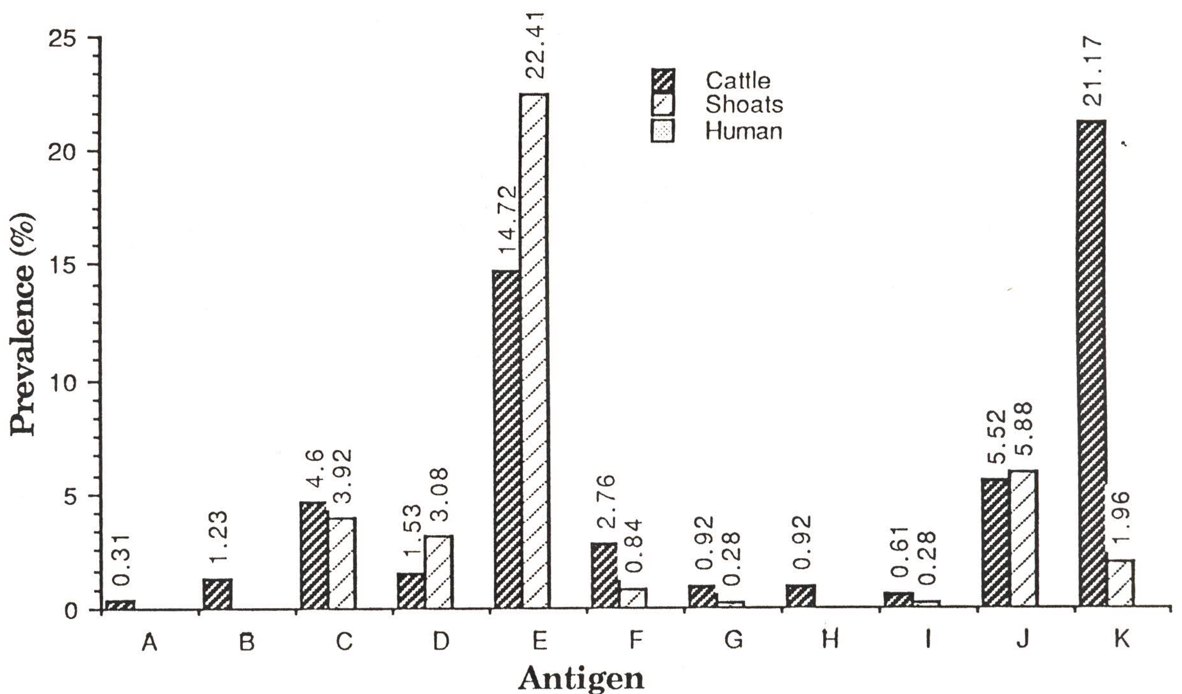

Figure 2: Prevalence rate of antibodies to each of the eleven leptospiral antigens (A - K) between cattle, sheep and goats and human in Nyandarua District.

Key:

A= *L. copenhageni*
B = *L. mankarso*
C = *L. autumnalis*
D = *L. sejroe*
E=*L. hardjo*
F=*L. grippotyphosa*
G=*L. pomona*
H=*L. canicola*
I=*L. australis*
J=*L. patoc*
K=*L. wolffi*
Shoats = Sheep and goats

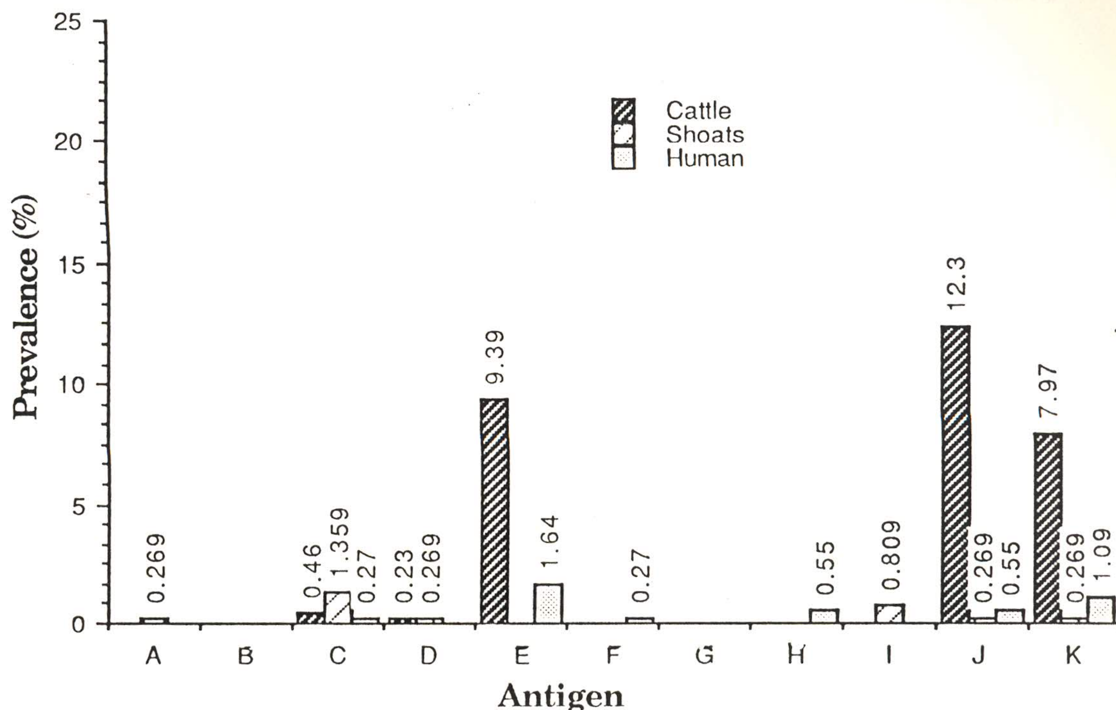

Figure 3: **Prevalence rate of antibodies to each of the eleven leptospiral antigens (A - K) between cattle, sheep and goats and human in Turkana District.**

Key:

A=*L. copenhageni*	E=*L. hardjo*	I=*L. australis*
B= *L. mankarso*	F=*L. grippotyphosa*	J=*L. patoc*
C=*L. autumnalis*	G=*L. pomona*	K=*L. wolffi*
D=*L. sejroe*	H=*L. canicola*	

Shoats = Sheep and goats

Leptospira wolffi (21.2%), *L. hardjo* (14.7%) and *L. patoc* (5.5%) were the most prevalent serovars in cattle from Nyandarua district. The rest of the serovars occurred at prevalence rates of less than 5.0%, the majority having prevalence rates of less than 1%. In sheep and goats, *L. hardjo* (22.4%), *L. patoc* (5.9%) and *L. autumnalis*

(3.9%) appeared to be the most prevalent. There were no positive human sera from this district.

Turkana District showed *L. patoc* (12.3%), *L. hardjo* (9.4%) and *L.wolffi* (8.0%), in that order, as the most prevalent serovars in cattle. *Leptospira autumnalis* (1.4%) and *L. australis* (0.8%) were the most prevalent serovars in sheep and goats, while *L. hardjo* (1.6%) and *L. wolffi* (1.1%) were the most prevalent in human sera from this district.

4.5 Comparison of the number and the prevalence rates at three different levels of reactivity for each animal species to each leptospiral antigen in Nyandarua and Turkana districts.

Out of the total sera sampled from each animal species in each of the two districts, the positive sera to leptospiral antibodies as determined with the MAT were used to calculate the percentage rates of antibodies to each of the eleven leptospiral antigens and the results tabulated as indicated in tables 13, 14, 15, 16, 17 and 18 for each of the animal species within each of the two districts.

Tables 13: Number and prevalence rate at three different levels of reactivity for cattle sera to each of the eleven leptospiral serovars in Nyandarua District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	326	2 (0.6)	1 (0.3)	1 (0.3)
B	326	5 (1.5)	4 (1.2)	1 (0.3)
C	326	41 (12.6)	15 (4.6)	26 (8.0)
D	326	12 (3.7)	5 (1.5)	7 (2.2)
E	326	70 (21.5)	48 (14.7)	22 (6.8)
F	326	16 (5.0)	9 (2.8)	7 (2.2)
G	326	13 (4.0)	3 (0.9)	10 (3.1)
H	326	3 (0.9)	3 (0.9)	0 (0.0)
I	326	4 (1.2)	2 (0.6)	2 (0.6)
J	326	57 (17.5)	18 (5.5)	39 (12.0)
K	326	109 (33.4)	69 (21.1)	40 (12.3)

Key:

Reactive sera = Total reacting sera at 1:50 dilution.

± = Suspicious sera (at 1:50 to 1:100 MAT titers).

+ve = Positive sera (≥ 1:200 MAT titers).

Parenthesis = percentage for the reactive, suspicious and positive sera

A=*L. copenhageni* E=*L. hardjo* I=*L. australis*

B=*L. mankarso* F=*L. grippotyphosa* J=*L. patoc*

C=*L. autumnalis* G=*L. pomona* K=*L. wolffi*

D=*L. sejroe* H=*L. canicola*

Leptospira wolffi and *L. hardjo*, both in the *Hebdomadis* serogroup were the most prevalent serovars in cattle within Nyandarua District. *Leptospira patoc*, *L. autumnalis*, *L. grippotyphosa*, *L. sejroe* and *L. mankarso* occurred at prevalence rates greater than 1.0% but less than 6.0%, while *L. pomona*, *L. canicola*, *L. australis* and *L. copenhageni* showed prevalence rates below 1.0%.

Table 14: Number and the prevalence rate at three different levels of reactivity for sheep and goat sera to each of the eleven leptospiral serovars in Nyandarua District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	357	0 (0.0)	0 (0.0)	0 (0.0)
B	357	2 (0.6)	0 (0.0)	2 (0.6)
C	357	41 (11.5)	14 (3.9)	27 (7.6)
D	357	20 (5.6)	11 (3.2)	9 (2.5)
E	357	127 (35.6)	80 (22.4)	47 (13.2)
F	357	12 (3.4)	3 (0.9)	9 (2.5)
G	357	22 (6.2)	1 (0.3)	21 (5.9)
H	357	2 (0.6)	0 (0.0)	2 (0.6)
I	357	3 (0.8)	1 (0.3)	2 (0.6)
J	357	49 (13.7)	21 (5.9)	28 (7.8)
K	357	30 (8.4)	7 (2.0)	23 (6.4)

Key:

Reactive sera = Reacting sera (at 1:50 dilution).

± = Suspicious sera (at 1:50 to 1:100 MAT titers).

+ve= Positive sera (≥ 1:200 MAT titers).

Parenthesis= percentage for the reactive, suspicious and positive sera

A=*L. copenhageni* E=*L. hardjo* I=*L. australis*

B = *L. mankarso* F=*L. grippotyphosa* J=*L. patoc*

C = *L. autumnalis* G=*L. pomona* K=*L. wolffi*

D =*L. sejroe* H=*L. canicola*

Leptospira hardjo (22.4%) occurred at a higher level than others while *L. patoc*, *L. autumnalis* and *L. sejroe*, respectively, followed

but at prevalences of 3.0-6.0%. All the other serovars had prevalence rates below 3.0%.

Table 15: Number and prevalence at three different levels of reactivity for human sera to each of the eleven leptospiral serovars in Nyandarua District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	315	0 (0.0)	0 (0.0)	0 (0.0)
B	315	2 (0.6)	0 (0.0)	2 (0.6)
C	315	0 (0.0)	0 (0.0)	0 (0.0)
D	315	0 (0.0)	0 (0.0)	0 (0.0)
E	315	20 (6.4)	0 (0.0)	20 (6.4)
F	315	0 (0.0)	0 (0.0)	0 (0.0)
G	315	0 (0.0)	0 (0.0)	0 (0.0)
H	315	0 (0.0)	0 (0.0)	0 (0.0)
I	315	2 (0.6)	0 (0.0)	2 (0.6)
J	315	2 (0.6)	0 (0.0)	2 (0.6)
K	315	0 (0.0)	0 (0.0)	0 (0.0)

Key:

Reactive = Reacting sera (at 1:50 dilution).

± = Suspicious sera (at 1:50 to 1:100 MAT titers).

+ve = Positive sera (≥ 1:200 MAT titers).

Parenthesis= percentage for the reactive, suspicious and positive sera

A=*L. copenhagéni* E=*L. hardjo* I=*L. australis*

B =*L. mankarso* F=*L. grippotyphosa* J=*L. patoc*

C =*L. autumnalis* G=*L. pomona* K =*L. wolffi*

D =*L. sejroe* H=*L. canicola*

Although a few human sera from Nyandarua District showed a reaction at 1:50 dilution and had suspicious reactors, none of them showed positive reactions at 1:200 dilution.

Table 16: Number and prevalence rates at three different levels of reactivity for cattle sera to each of the eleven leptospiral serovars in Turkana District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	439	0 (0.0)	0 (0.0)	0 (0.0)
B	439	2 (0.5)	0 (0.0)	2 (0.5)
C	439	8 (1.8)	2 (0.5)	6 (1.4)
D	439	8 (1.8)	1 (0.2)	7 (1.6)
E	439	55 (12.5)	41 (9.3)	14 (3.2)
F	439	5 (1.1)	0 (0.0)	5 (1.1)
G	439	0 (0.0)	0 (0.0)	0 (0.0)
H	439	2 (0.5)	0 (0.0)	2 (0.5)
I	439	1 (0.2)	0 (0.0)	1 (0.2)
J	439	128 (29.2)	54 (12.3)	74 (16.9)
K	439	52 (11.9)	35 (8.0)	17 (3.9)

Key:

Reactive sera = Reacting sera (at 1:50 dilution).

± = Suspicious sera (at 1:50 to 1:100 MAT titers).

+ve= Positive sera (≥ 1:200 MAT titers).

Parenthesis= percentage for the reactive, suspicious and positive sera

A=*L. copenhageni* E=*L. hardjo* I=*L. australis*

B =*L. mankarso* F=*L. grippotyphosa* J=*L. patoc*

C =*L. autumnalis* G=*L. pomona* K =*L. wolffi*

D =*L. sejroe* H=*L. canicola*

Unlike in Nyandarua, cattle from Turkana showed more reactors against *L. patoc* followed by *L. hardjo* and *L. wolffi* in the

Hebdomadis serogroup. All other serovars occurred at rates below one.

Table 17: Number and prevalence rates at three different levels of reactivity for sheep and goat sera to each of the eleven leptospiral serovars in Turkana District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	369	2 (0.5)	1 (0.3)	1 (0.3)
B	369	4 (1.1)	0 (0.0)	4 (1.1)
C	369	39 (10.6)	5 (1.4)	34 (9.2)
D	369	25 (6.9)	1 (0.3)	24 (6.5)
E	369	0 (0.0)	0 (0.0)	0 (0.0)
F	369	0 (0.0)	0 (0.0)	0 (0.0)
G	369	3 (0.8)	0 (0.0)	0 (0.0)
H	369	0 (0.0)	0 (0.0)	3 (0.8)
I	369	13 (3.5)	3 (0.8)	10 (2.7)
J	369	32 (8.7)	1 (0.3)	31 (8.4)
K	369	2 (0.5)	1 (0.3)	1 (0.3)

Key:
Reactive sera= Reacting sera (at 1:50 dilution).
± = Suspicious sera (at 1:50 to 1:100 MAT titers).
+ve= Positive sera (≥ 1:200 MAT titers).
Parenthesis = percentage for the reactive, suspicious and positive sera

A=*L. copenhageni* E=*L. hardjo* I=*L. australis*
B =*L. mankarso* F=*L. grippotyphosa* J=*L. patoc*
C =*L. autumnalis* G=*L. pomona* K =*L. wolffi*
D =*L. sejroe* H=*L. canicola*

Antibodies to all the eleven leptospiral antigens occurred at relatively lower rates in sheep and goats in Turkana. Except for *L.*

autumnalis, which occurred at 1.4%, all the others showed prevalence rates of below one.

Table 18: Number and prevalence rates at three different levels of reactivity for human sera to each of the eleven leptospiral serovars in Turkana District.

Antigen	Sera tested	Reactive sera	Sera +ve	Sera ±
A	366	0 (0.0)	0 (0.0)	0 (0.0)
B	366	1 (0.3)	0 (0.0)	1 (0.3)
C	366	10 (2.7)	1 (0.3)	9 (2.5)
D	366	2 (0.6)	0 (0.0)	2 (0.6)
E	366	21 (5.7)	6 (1.6)	15 (4.1)
F	366	7 (1.9)	1 (0.3)	6 (1.6)
G	366	0 (0.0)	0 (0.0)	0 (0.0)
H	366	6 (1.6)	2 (0.6)	4 (1.1)
I	366	1 (0.3)	0 (0.0)	1 (0.3)
J	366	17 (4.6)	2 (0.6)	15 (4.1)
K	366	14 (3.8)	4 (1.1)	10 (2.7)

Key:
Reactive sera = Reacting sera (at 1:50 dilution).
± = Suspicious sera (at 1:50 to 1:100 MAT titers).
+ve = Positive sera (≥ 1:200 MAT titers).
Parenthesis = percentage for the reactive, suspicious and positive sera.

A=*L. copenhageni*
B =*L. mankarso*
C =*L. autumnalis*
D =*L. sejroe*
E=*L. hardjo*
F=*L. grippotyphosa*
G=*L. pomona*
H=*L. canicola*
I=*L. australis*
J=*L. patoc*
K=*L. wolffi*

Of the reactive and suspicious sera from humans in Turkana, there were a few showing positive reactors. *Leptospira hardjo* and *L. wolffi* in the *Hebdomadis* serogroup had levels of between 1-2.0% while the rest were below one.

4.6 Results of the influence of other factors considered in this study.

The pH of the soil samples collected from Nyandarua and Turkana districts were 6.4 (close to neutral) and 8.7 (alkaline) respectively (Appendices 6a&b and 7a&b)

Over a 6-year period (1982-87), the average rainfall, relative humidity and temperature were; 839.1mm., 69% and 14.1^{o}C respectively in Nyandarua District. In Turkana District, the averages over a 5-year period (1982-86) were: 274.5mm., 61.9% and 28.9^{o}C respectively.

5: DISCUSSION

Leptospirosis is a widespread zoonosis whose epidemiology is complex. Broadly, the ability of the pathogen to survive outside the host and the availability of susceptible hosts, coupled with the ability of the pathogen to survive and multiply in the host, constitute the various factors that determine the prevalence and incidence of leptospirosis. Environmental factors such as the rainfall pattern, soil pH (around neutrality) and a temperature range of 7-34^{0}C will usually influence the prevalence and incidence of leptospirosis. Moisture is particularly important in the survival rate of the organism in the soil. Areas with high rainfall have revealed high prevalence rates.

The economic importance of leptospirosis in veterinary medicine is now recognised and is directly related to public health. This realization calls for proper control measures of the disease.

Attempts to control the disease are made difficult by the wide distribution of the many potentially pathogenic leptospiral serovars and the lack of any pathognomonic features in man and animals which makes diagnosis often difficult to the field clinician.

Diagnosis based on clinical features alone is not reliable in domestic animals, but the examination of the whole herd may yield useful information. Laboratory methods, including both serological and cultural techniques in addition to clinical and epidemiological information, are essential in order to diagnose the disease in both man and animals. The process of diagnosis is expensive as it

requires specialized facilities and personnel and even then, the successful control of the disease depends on a clear understanding of the factors that govern the maintenance of the pathogenic strains within the environment and within the host.

In man, control depends largely on the control of the disease in animal reservoirs, the correct diagnosis at the right time and subsequent adequate chemotherapy. In domestic animals, the control largely depends on vaccination.

Titers evoked by leptospiral antigens in one animal species may vary between species due to the species variation, and comparison based on the serological titers alone may not be significant since the samples could have been collected at different stages of the immune response.

The eleven reference leptospiral cultures used in the test procedure were readily propagated in EMJH and Fletcher's media. Occasionally, the cultures would get contaminated, but such cultures were purified using a combination of filtration and addition of 5-fluorouracil (Johnson and Rogers, 1964). It was of importance to observe safety measures in the laboratory during these subculturing procedures to minimize contamination and to avoid infection of the personnel working in the laboratory.

The MAT which was used in this study is upto now the one used by most workers for the identification of antibodies to leptospires and is recommended as the standard reference test for serological diagnosis of leptospirosis (WHO., 1982). It is highly sensitive and specific and can be used for the detection of recent and past infections, thus making it ideal for epidemiological investigations

which frequently require retrospective antibody determination. Since the test is only relatively serovar-specific due to cross-reactions, definitive diagnosis of leptospirosis can only be achieved by isolating and sero-typing the organism. Lack of serological evidence for *Leptospira* does not rule out the possibility of infection as several workers have been able to isolate leptospires from animals in the absence of serological evidence (Ellis *et al.*, 1982a&b; MacKintosh *et al.*, 1982; Thierman, 1983).

In the MAT the results are difficult to interpret mainly because of the antigenic complexity of leptospires, defining the end-point titer, quality of the antigens and the possibility of infection with more than one serovar in one particular animal species. The test does not differentiate infection titers from vaccine titers (Rubin *et al.*, 1981).

Since it was impractical to use all of the large number of *Leptospira* serovars, eleven serovars, representing various serogroups, were chosen to make up a battery for whose reference antisera were available. Live cultures were used to try and minimize cross-reactivity. It has been shown that when using live antigens, there is little or no cross-reactivity between serovars *hardjo* and *autumnalis* (Palmer *et al.*, 1987). Using the eleven representative serovars it was possible to show the sera from animals that could have been infected with more than one serovar.

The end-point is arbitrary and subjective. Experience is important in the reading of the test and the advice is for the reading to be done by one reader. The end-points used by other workers were used in the recording of the results. All the sera which showed titers of 1:50 to 1:100 were considered "suspicious" and those that showed

titers of 1:200 and above were taken to be serologically "positive" (Carter and Moujen, 1981).

Sera which were reactive to more than one serogroup, may have been indicative of a mixed infection or of several separate incidences of the disease or more likely, of cross-reactions.

The observed serological prevalence of leptospiral antibodies in cattle (34.0%) for Nyandarua District is lower than recorded in other parts of Kenya. In domestic animals a prevalence of 40% has been reported (Ball, 1966; Tabel and Losos, 1979; Stirling and Lhermette, 1980). D'Souza (1983) reported a prevalence of 41% in a country wide survey in cattle. The slight decrease noted in the prevalence may be attributed to increased awareness of disease control measures and improved animal husbandry practices amongst farmers in this district. Being a wet area for most of the year, the prevalences would be expected to be high as leptospires are known to be associated with high rainfall areas. Most farmers observe cleanliness during milking and water used in most farms is relatively clean. These measures may have accounted for the lower levels in the prevalences, compared to those reported in other parts of Kenya in previous studies.

In Nyandarua District sex ($p < 0.05$) appeared to influence the prevalence rate while age ($p > 0.05$) did not. More male animals (47.1%) had serological evidence of leptospirosis than female animals (30.6%). This could be a result of the more care given towards female cattle as these are a source of milk which is mainly for commercial purposes.

The prevalence rate of leptospiral antibodies in cattle (22.3%) from Turkana District can be considered high in this area taking into account that Turkana is a very dry area most of the year and hence, the level of leptospirosis would be expected to be very low since leptospires are favoured by high rainfall and high moisture content of the soil. This apparently high prevalence may be a result of cattle movements across the common Kenya-Uganda border. It is not unusual for cattle from Uganda to cross into Kenya and move into the district. Such cattle may have come from high rainfall areas where the likelihood of having been infected with leptospires are higher, thus giving reactors.

While sex did not appear to be associated with the prevalence rate of leptospiral antibodies in cattle from this district, age did. These animals are mainly kept for milk and meat, principally for subsistence. Unlike in Nyandarua, there does not appear to be any preference for either sex. The quality of the animals is not considered important; rather, the more the number of animals one has, the high his status in the society. Adult animals showed more reactors (30.5%) than the young ones (13.6%). This could be a result of the fact that, while adult animals are herded freely and in close contact, thus providing more chances of infections between each other, the young animals receive better attention near the homesteads, usually away from the adults, which in effect would possibly minimize the chances of the young animals acquiring infection from the adults.

The prevalence observed in sheep and goats from Nyandarua District was high (34.2%) compared to reports from studies by other workers in other parts of Kenya. A prevalence of 18.38% was reported in a survey for leptospirosis in sheep and goats in Kenya (Wanyangu

et al., 1988). The prevalence observed in this study is also far above those reported from other parts of the world. Prevalences of 12.7% have been reported in Argentina (Michna, 1970), 12.1% in India (Pargaonker and Ramakrishna, 1963), 7.23% in Guyana (Moite and Meyers, 1986), 28.0% in sheep and 27.3% in goats in Morocco (Mailloux, 1969) and 11.3-26.3% in sheep and goats in Spain (Lazaro, 1960). Serological evidence that sheep play a role in the maintenance of leptospires, and in particular *L. hardjo* infections for bovine, has been reported (Hathaway *et al.*, 1982). Cross-transmission is likely to explain the high prevalences in this district in both cattle and sheep, each of the animal species acting as a source of infection to the other. The sheep population in Nyandarua is higher than that of goats. Sex and age did not seem to influence the prevalence rate. This could be due to the fact that sheep in this district are reared mainly for wool production, and hence no particular special care is accorded to any one particular sex. Unlike in cattle where female animals get more attention due to milk production, sex may not be an important factor in wool production.

The prevalence of *Leptospira* antibodies in sheep and goats in Turkana was also lower than that reported in previous studies in other parts of Kenya (Wanyangu *et al.*, 1988), and most other parts of the world (Lazaro, 1960; Pargaonker and Ramakrishna, 1963; Mailloux, 1969; Michna, 1970; Moite and Meyers, 1986). Since these animals are mainly confined in the dry district (unlike cattle), and the fact that not much movements occur between other district or across the borders, coupled with the fact that these animals are usually herded separately from cattle, the low serological prevalence would be expected in this semi-arid to arid district.

Man is susceptible to all pathogenic leptospires and infected animals act as a source of infection to man. The fact that no human sera showed any reactors in Nyandarua was of significance. Better management practices and animal husbandry, coupled with increased awareness in the hygienic practices necessary for the general welfare of the farmer as well as that of his animals, may explain the negative results. The farmers in this district maintain clean milking environments, some use disinfectants, and milk is boiled before consumption. Use of gumboots is not uncommon and most of the people will promptly seek medical attention, both for themselves and their animals where need for this arises. Drainage in most farms is good and water for consumption by both animals and the people is usually clean. All these factors further minimize the chances of man getting infected, which further may explain the failure to detect reactors in human sera from this district.

A low prevalence (4.6%) was observed in people in Turkana. The close association between the people and their livestock, and the fact that these people rarely seek medical attention for themselves and their animals, may have resulted in acquisition of infections from infected animals to the people, thus resulting in reactors in the people. It is not common to boil milk before consumption. These people get their water for consumption from the same sources as animals.

A significant statistical difference was observed in the prevalence rate of *Leptospira* antibodies between cattle, sheep and goats, and humans within both districts. A higher prevalence was observed in cattle, sheep and goats in Nyandarua District than in Turkana District. While no human sera from Nyandarua District

were positive, 4.6% of sera from Turkana District were positive against one or more leptospiral serovars. This could be explained by the differences in animal husbandry, man-animal relationships and the eating habits between the two districts as discussed earlier.

The prevalence rates in cattle, sheep and goats were higher in Nyandarua than in Turkana district. The fact that Nyandarua has a higher annual average rainfall and relative humidity (839.08 mm; 69%) than Turkana (274.5 mm; 61%), may serve to explain the difference in the prevalences between the two districts. Leptospires survive better in high rainfall areas with soils that have a high moisture content. Nyandarua happens to fall in ecological zone II, which is characterized by high rainfall and a high moisture index. This would favour the survival of leptospires considerably. In contrast, Turkana district falls in ecological zones V and VI, with relatively little rainfall and comparatively lower moisture index. Other factors that may explain the observed higher reactors in Nyandarua as compared to those in Turkana, are the temperatures and the soil pH. Leptospires survive better under temperatures between 7-34^{0}C and soil pHs around neutral. Nyandarua had an average annual temperature of 14.1^{0}C, while that of Turkana district was 28.9^{0}C. Soil pH in the study area of Nyandarua was 6.4, while that of Turkana was 8.7.

The most prevalent serovars in all the animal species considered in this study were those of the *Hebdomadis* serogroup, which included *L. hardjo* (which occurred at prevalence rates of 14.7% and 9.4% in cattle from Nyandarua and Turkana districts respectively; 22.4% sheep and goats from Nyandarua and 1.6% in human from Turkana) and *L. wolffi* (which occurred at prevalences

of 27.2% and 8.0% in cattle from Nyandarua and Turkana districts, respectively, and 1.1% in humans from Turkana district). This observation agrees with previous findings by other researchers. D'Souza (1983) indicated *L. hardjo* to have been the most prevalent serovar in cattle and a prevalence of 18.83% was recorded. Reports from other parts of the world have shown infections with *L. hardjo* to be widespread, and consequently, infections with this serovar have often been referred to as cattle associated leptospirosis.

Leptospira autumnalis , though at low levels, appeared to be a common pathogen in sheep and goats from both districts, occurring at 3.9% and 1.4% in Nyandarua and Turkana districts, respectively. This is an infection mainly associated with rodents (Kranedonk *et al.*, 1968). It is likely, therefore, that this infection could have been acquired from carrier rodents in the districts. In the late 1950s, outbreaks of leptospirosis in sheep and goats in Kenya were mainly associated with *L. grippotyphosa* (Burdin *et al.*, 1958) and in the 1960s, serological evidences of the *Hebdomadis* serogroup and the *Icterohaemorrhagiae* serogroup were reported (Ball, 1966). There appears to be an increase in the incidences of the *Hebdomadis* serogroup serovar *hardjo* in sheep and goats, considering the high prevalence of antibodies observed in Nyandarua (22.4%). *Leptospira autumnalis* appears to be an emerging hazard in sheep and goats in Kenya.

Leptospira hardjo and *L. wolffi* (*Hebdomadis* serogroup) which were the most prevalent serovars in cattle, sheep and goats were the same serovars detected in man. Man is susceptible to all pathogenic leptospires. This would mean that serovars found in animals from a particular area are the most likely ones to infect people in such an

area. This may then explain why the most prevalent serovars found in Turkana District were the same serovars detected in people from this area.

The attempts in the present study to investigate what role species, sex and age played in the epidemiology of leptospirosis in cattle, sheep and goats, and man, and the comparison of the disease levels between these animals, appears to be the first such work in Kenya. A wider and more comprehensive study in the whole country is recommended to determine what group of animals are more prone to leptospirosis, especially in the high potential areas, where leptospires are more likely to survive.

6: CONCLUSIONS

From the present study, the following observations and conclusions were made;

1. *Leptospira* antibodies appear to be more prevalent in the wetter, high potential areas compared to drier, low potential areas. High rainfall, high relative humidity, moderate temperature range, a high moisture index and soil pH close to neutrality were recorded in Nyandarua District, which had higher prevalence rates of *Leptospira* antibodies in cattle, sheep and goats.

2. Sex appeared to be associated with the prevalence of *Leptospira* antibodies in cattle in Nyandarua District; male cattle showed a higher prevalence than females in Nyandarua where milk is mainly produced for commercial purposes. This did not appear to be the case in sheep and goats from either the high potential Nyandarua District or the low potential Turkana District, nor in cattle in low potential areas. There is need for more investigations in the high and low potential areas to confirm this observation.

3. Age did not seem to influence the level of *Leptospira* antibodies in Nyandarua, but did so in Turkana.

4. Sex and age did not appear to be associated with the prevalence of *Leptospira* antibodies in sheep and goats in both the wetter Nyandarua District and the drier Turkana District.

5. There appears to be a statistically significant difference in the prevalence rates of *Leptospira* antibodies between cattle, sheep and goats, and humans within Nyandarua and Turkana districts. More cattle, sheep and goat sera were positive against one or more leptospiral serovars in Nyandarua District compared to Turkana District, and while some reactors were detected in human sera from Turkana District, none were positive from Nyandarua District.

6. Antibodies to the serovars of the *Hebdomadis* serogroup (*L. hardjo* and *L. wolffi*) and *L. patoc* in Turkana were the most prevalent in all the three animal species considered in this study.

Based on serological evidence, *L. autumnalis* and *L. australis* appear to be an emerging hazard in sheep and goats in Kenya. The epidemiology of *L. autumnalis*, which is mainly an infection associated with rodents, seems to be changing and appears to be important in Kenyan sheep and goats considering that reactor rates were detected in these animals.

7. Antibodies to serovars of leptospirae detected in man were the same as those detected with the highest prevalence rates in the domestic animals (*L.hardjo* and *L. wolffi* of the *Hebdomadis* serogroup). This seems to imply that man, who is susceptible to all pathogenic leptospires is at a higher risk of infection with serovars associated with the animals close to him.

From the above observations, there appears a need for further detailed investigations coupled with an attempt to isolate the actual causative agents especially in Nyandarua District, where antibodies to *Leptospira* were found to be more prevalent, in order to determine which groups of the animals reared in this place are more prone to infections with leptospirae, and to enable determination of the most applicable control measures.

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APPENDICES

Appendix 1: List of serovars of *Leptospira* reported to have been isolated from man and animals. The list is arranged alphabetically and not all serovars or serogroups have been published or accepted by the Taxonomic Subcommittee on *Leptospira* (TSCL), (WHO, 1982).

Serogroup	Serovar		
Australis	*australis*	*hawain*	*nicaragua*
	bangkok	*jalna*	*peruviana*
	bratislava	*lora*	*pina*
	fugis	*muenchen*	*ramusi*
Autumnalis	*alice*	*fort-bragg*	*rachmati*
	autumnalis	*lanka*	*srebarna*
	bangkinang	*louisiana*	*sumatrana*
	bulgarica	*mooris*	*tingo maria*
	erinacei-auriti	*orleans*	
Ballum	*arboreae*	*ballum*	*castellonis*
Bataviae	*argentiniensis*	*brasiliensis*	*kobbe*
	balboa	*claytoni*	*paidjan*
	bataviae	*djatzi*	
Butembo	*butembo*		
Canicola	*bafani*	*canicola*	*malaya*
	benjamin	*galtoni*	*portland-vere*
	bindjei	*jonsis*	*schueffneri*
	broomi	*kamituga*	*sumneri*

Celledoni	*celledoni*	*whitcombi*	
Cynopteri	*cynopteri*	*tingo mariensis*	
Djasiman	*djasiman*	*gurungi*	*sentot*
Grippotyphosa	*canalzonae*	*muelleri*	*valbuzzi*
	grippotyphosa	*ratnapura*	*vanderhoedeni*
Hebdomadis	*beye*	*kabura*	*nona*
	borincana	*kambale*	*perameles*
	georgia	*kremastos*	*szwajizak*
	goiano	*maru*	*tabaquite*
	hebdomadis	*mini*	*worsifoldi*
	jules		
Icterohaemorrhagiae	*birkini*	*icterohae-morrhagiae*	*ndambari*
	bog-vere	*mankarso*	*sarmin*
	budapest	*monymusk*	*smithi*
	copenhageni	*mwogolo*	*tonkini*
	dakota	*naam*	*weaveri*
	gem	*ndahambukuje*	
Javanica	*an hoa*	*javanica*	*sofia*
	ceylonica	*menoni*	*sorex-jalna*
	coxi	*poi*	*vargonica*
	fluminense	*rio*	*waskurin*
Panama	*cristobali*	*mangus*	*panama*
Pomona	*monjakov*	*pomona*	*tropica*
	mosdok	*proechimys*	
Pyrogenes	*abramis*	*hamptoni*	*pyrogenes*
	alexi	*kawali*	*robinsoni*
	biggis	*manilae*	*varela*
	camlo	*myocastoris*	*zanoni*
	guaratuba	*princestown*	

Sejroe	*balcanica*	*hardjo*	*roumanica*
	caribe	*istrica*	*rupa rupae*
	dikkeni	*medanensis*	*saxkoebing*
	geyaweera	*nyanza*	*sejroe*
	gorgas	*polonica*	*trinidad*
	guaricurus	*recreo*	*wolffi*
	haemolytica	*ricardi*	
Shermani	*babudieri*	*shermani*	
Tarassovi	*atchafalaya*	*gatuni*	*moldaviae*
	atlantae	*guidae*	*navet*
	bakeri	*kanana*	*osetica*
	bravo	*kaup*	*rama*
	carimagua	*kisuba*	*tarassovi*
	hagres	*langati*	*tunis*
	darien	*luis*	*vughia*
Semaranga	*patoc*	*sao paulo*	*semaranga*

Appendix 2: List of representative strains of leptospires whose antisera are useful in the identification of isolates, (WHO, 1982)

Serogroup (or subgroup)	Representative antigens found in antisera to leptospires of	
	Serovar	strain
Australis	*bratislava*	Jez-Bratislava
	lora	Lora
Autumnalis	*autumnalis*	Autumnalis
	bangkinang	Bangkinang 1
	rachmati	Rachmati
Ballum	*ballum*	Mus 127
	castellonis	Castellon 3
Bataviae	*bataviae*	van Tienen
Canicola	*canicola*	Hond Utrecht IV
	schueffneri	Vleermuis 90 C
Celledoni	*celledoni*	Celledoni
Cynopteri	*cynopteri*	3522 C
Djasiman	*djasiman*	Djasiman

Grippotyphosa	*grippotyphosa*	Moskva V
	valbuzzi	Valbuzzi
Hebdomadis	*hebdomadis*	Hebdomadis
Icterohaemorrhagiae	*icterohaemorrhagiae*	RGA
Javanica	*poi*	Poi
Lousiana	*louisiana*	LSU 1945
Mini	*szwjizak*	Szwajizak
	tabaquite	TVRL 3214
Panama	*panama*	CZ 214K
Pomona	*pomona*	Pomona
Pyrogenes	*pyrogenes*	Salinem
	robinson	Robinson
Sejroe	*hardjo*	Hardjoprajitno
Wolffi	*medanensis*	Hond H. C.
	wolffi	3055
Shermani	*shermani*	LT 821
Tarassovi	*tarassovi*	Mitis Johnson

Appendix 3: List of serovars (with serogroup) for use as antigens in a battery of strains in MAT for detecting infections by an unknown serovar, (WHO, 1982).

Serogroup (or subgroup)	serovar	strain
Andamana	*andamana*	CH 11
Australis	*australis*	Ballico
	bratislava	Jez Bratislava
Autumnalis	*autumnalis*	Akiyami A
	butembo	Butembo
	rachmati	Rachmati
Ballum	*ballum*	S 102
	castellonis	Castellon 3
Bataviae	*bataviae*	v. Tienen
Canicola	*canicola*	Hond Utrecht IV
Celledoni	*celledoni*	Celledoni
Cynopteri	*cynopteri*	3522 C
Grippotyphosa	*grippotyphosa*	Moskva V
	valbuzzi	Valbuzzi

Hebdomadis	*borincana*	HS 622
	hebdomadis	Hebdomadis
Icterohaemorrhagiae	*icterohaemorrhagiae*	RGA
	copenhageni	Wijnberg
Javanica	*javanica*	
	Veldrat Bataviae 46	*poi*
Panama	*panama*	CZ 214K
Pomona	*pomona*	Pomona
Pyrogenes	*pyrogenes*	Salinem
Sejroe	*sejroe*	Mus 24
	wolffi	3705
Semaranga	*patoc*	Patoc 1
	sao paulo	Sao Paulo
	semaranga	Semaranga
Shermani	*shermani*	LT 821
Tarassovi	*tarassovi*	Mitis Johnson; Perepelicin

Appendix 4: Preparation of Ellinghausen, McCullough, Johnson and Harris (EMJH) Medium for the purpose of antigen propagation, (Difco Laboratories, Detroit, Michigan, USA)

BACTO-LEPTOSPIRA BASE-EMJH (code 0794).

Ingredient	g/l
Sodium Phosphate Dibasic	1.0
Potassium Phosphate Monobasic	0.3
Sodium Chloride	1.0
Ammonium Chloride	0.25
Thiamine	0.005

To rehydrate;

1. Suspend 2.3g in 900ml of distilled water and agitate to dissolve completely.
2. Sterilize in an autoclave for 15 minutes at 15 pounds pressure (121°C).
3. Allow the sterile medium to cool to room temperature.
4. Aseptically add 100ml of BACTO-LEPTOSPIRA ENRICHMENT-EMJH (code 0795)-(Lyophilized rabbit serum containing native haemoglobin).

5. Mix uniformly and dispense aseptically into sterile tubes or bottles as desired for use.

Final pH of the complete medium is 7.5.

Appendix 5: Preparation of Fletcher's Semi-solid Medium for the purpose of leptospiral antigens, (Fletcher, 1928).

Composition:

Peptone	0.3g
Beef Extract	0.2g
Sodium Chloride	0.5g
Agar	1.5g

1. Add the above indicated weights of ingredients to 920ml of distilled water.
2. Heat to boiling in order to dissolve.
3. Autoclave for 20 minutes at 15 pounds pressure (121°C).
4. Add heat inactivated rabbit serum (56°C, 30-60 minutes) which has been warmed to 50°C, to a final dilution of 10% v/v.

The pH of the medium should be in the range of 7.2-7.6.

Appendix 6a: Soil pH for Nyandarua District.

Soil sample No.	pH
246	6.7
247	7.8
248	5.8
249	5.5
250	6.3
251	6.2
252	6.1
253	7.3
254	5.9
255	5.9
256	6.8
Average	6.4

Appendix 6b: Soil pH for Turkana District.

Soil sample No.	pH
TS1	8.7
L1	8.7
KA2	8.8
2L2	8.6
KT2	8.7
Average	8.7

Appendix 7a: Annual Rainfall (mm), Relative Humidity (%) and Temperature (°C).1982-1987 for Nyandarua District.

Year	Rainfall	R/Humidity	Temperature
1982	1188.7	69.5	14.0
1983	1006.0	63.0	14.0
1984	420.75	66.0	15.0
1985	901.0	78.5	13.5
1986	851.0	81.0	13.0
1987	667.0	56.0	15.0
Average	839.08	69.0	14.1

Appendix 7b: Annual Rainfall (mm), Relative Humidity (%) and Temperature (°C).1982-1986 for Turkana District.

Year	Rainfall	R/Humidity	Temperature
1982	-	55.5	28.5
1983	283.5	55.0	28.0
1984	261.0	57.0	30.0
1985	260.5	72.5	27.5
1986	293.0	69.5	30.5
Average	274.5	61.9	28.9

Appendix 8: Distribution of total number of positives (titre ≥1/200) by each serovar for each animal species in Nyandarua district and the respective % +ve of the total samples tested

ANTIGEN	ANIMAL SPECIES		
	Cattle	Shoats	Human
L. copenhageni	1 (0.3%)	0	0
L. mankarso	4 (1.2%)	0	0
L. autumnalis	15 (4.6%)	14 (3.9%)	0
L. sejroe	5 (1.5%)	11 (3.1%)	0
L. hardjo	48 (14.7%)	80 (22.4%)	0
L. grippotyphosa	9 (2.8%)	3 (0.8%)	0
L. pomona	3 (0.9%)	1 (0.3%)	0
L. canicola	3 (0.9%)	0	0
L. australis	2 (0.6%)	1 (0.3%)	0
L. patoc	18 (5.5%)	21 (5.9%)	0
L. wolffi	69 (21.2%)	7 (2.0%)	0

Total samples tested
Cattle = 326
Shoats = 357
Human = 315

Total reactive(1:50 screening dilution)
Cattle = 161
Shoats = 196
Human = 24

Total suspicious (1:50 to 1:100MAT titers +ves)
Cattle = 50
Shoats = 74
Human = 24

Total positives (≥1:200 MAT titers)
Cattle = 111
Shoats = 122
Human = 0

Shoats = Sheep and goats

Appendix 9: Distribution of total positives (≥1/200) by each serovar for each animal species in Turkana district and the respective % +ve of the total samples tested

ANTIGEN	ANIMAL SPECIES		
	Cattle	Shoats	Human
L. copenhageni	0	1 (0.3%)	0
L. mankarso	0	0	0
L. autumnalis	2 (0.5%)	5 (1.4%)	1 (0.3%)
L. serjroe	1 (0.2%)	1 (0.3%)	0
L. hardjo	41 (9.4%)	0	6 (1.6%)
L. grippotyphosa	0	0	1 (0.3%)
L. pomona	0	0	0
L. canicola	0	0	0
L. australis	0	3 (0.8)	0
L. patoc	54 (12.3%)	1 (0.3%)	2 (0.6%)
L. wolffi	35 (7.8%)	1 (0.3%)	4 (1.1%)

Total samples tested: Cattle = 439; Shoats = 369; Human = 366

Total reactive 1:50 dilution screening: Cattle = 194; Shoats = 88; Human = 51

Total suspicious 1:50 to 1:100 MAT titres +ves: Cattle = 96; Shoats = 74; Human = 34

Total positives (≥1:200 MAT titers): Cattle = 98; Shoats = 14; Human = 17

Shoats = Sheep and goats

Appendix 10: Distribution of reactor rates to any one of the eleven leptospiral serovars for each animal species by sex in the two districts

District	Species	Sex	No.of sera	%React. sera	%Positive sera
Nyandarua	Cattle	M	68	58.8	47.1
		F	258	43.4	27.7
	Sheep & goat	M	72	55.6	36.1
		F	285	54.7	33.7
	Human	M	123	19.5	0
		F	192	7.3	0
Turkana	Cattle	M	136	39.7	213
		F	303	42.9	22.8
	Sheep & goat	M	367	23.7	3.5
		F	2	50.0	50.0

% React. sera = All the sera that showed positive reaction at a screening dilution of 1:50 to one or more antigens

% Positive sera =All the sera with MAT titers of ≥ 1:200 to one or more serovars.

F = Female

M = Male

Appendix 11: Distribution of reactor rates to any one of the eleven leptospiral serovars for each animal species by age-group in the two districts

District	Species	Age group	No. of sera tested	% React. sera	%Positve sera
Nyandarua	Cattle	A	241	46.9	34.0
		Y	85	45.9	34.1
	Sheep&Goat	A	283	57.2	35.3
		Y	74	45.9	29.7
	Human	A	205	9.8	0
		Y	110	16.7	0
Turkana	Cattle	A	226	52.2	30.5
		Y	213	31.0	13.6
	Sheep&Goat	A	369	23.8	3.8
		Y	0	0	0

%React. sera = All the sera that showed positive reaction at a screening dilution of 1:50 to one or more antigens

% Positive sera =All the sera with MAT titers of ≥ 1:200 to one or more serovars.

A = Adult animals

Y = Young animals

Appendix 12: Distribution of the prevalence rates against one or more of the eleven leptospiral antigens in both animals and human beings in Nyandarua and Turkana districts.

District	Species	Sex	Total by sex	Age group	Total +ve by sex and age	%positive
Nyandarua	Cattle	M	68	Adult	18	26.5
				Young	14	20.6
		F	258	Adult	65	24.8
				Young	15	5.8
	Sheep& goat	M	72	Adult	18	25.0
				Young	8	11.1
		F	285	Adult	82	28.8
				Young	14	4.9
	Human	M	123	Adult	0	0
				Young	0	0
		F	192	Adult	0	0
				Young	0	0
Turkana	Cattle	M	136	Adult	20	14.7
				Young	9	6.6
		F	303	Adult	49	16.2
				Young	20	6.6
	Sheep & goat	M	367	Adult	13	3.5
				Young	0	0
		F	2	Adult	1	50.0
				Young	0	
	Human	N/A	-	N/A	17	4.6

% Sera Positive = All the sera with MAT titers of ≥ 1:200 to one or more serovars.

A = Adult animals

Y = Young animals

N/A = Information not available

Appendix 13: Distribution of the positive reactors (%) to each of the eleven leptospiral antigens by animal species in Nyandarua and Turkana.

		ANTIGEN										
District	Animal species	A	B	C	D	E	F	G	H	I	J	K
Nyandarua	Cattle	0.3	1.2	4.6	1.5	14.7	2.8	0.9	0.9	0.6	5.5	21.2
	Sheep& Goats	0	0	3.9	3.1	22.4	0.8	0.3	0	0.3	5.9	2.0
	Human	0	0	0	0	0	0	0	0	0	0	0
Turkana	Cattle	0	0	0.5	0.2	9.3	0	0	0	0	12.3	8.0
	Sheep& Goats	0.3	0	1.4	0.3	0	0	0	0	0.8	0.3	0.3
	Human	0	0	0.3	0	1.6	0.3	0	0.6	0	0.6	1.1

Key:

A-*L. copenhageni* B-*L. mankarso* C-*L. autumnalis* D-*L. sejroe* E-*L. hardjo* F-*L. grippotyphosa*

G-*L. pomona* H-*L. canicola* I-*L. australis* J-*L. patoc* K-*L. wolffi*

Appendix 14: Total number of sera with suspicious reactions to the eleven leptospiral antigens in each district for each animal species.

District	Animal species	No. of sera collected	Total suspicious	ANTIGENS										
				A	B	C	D	E	F	G	H	I	J	K
	Cattle	326	141	1	1	17	7	20	7	10	0	1	38	39
Nyandarua	Sheep & goats	357	157	0	2	24	9	36	10	21	2	2	28	23
	Human	315	26	0	2	0	0	20	0	0	0	2	2	0
	Cattle	439	127	0	2	6	7	12	5	0	2	1	74	18
Turkana	Sheep & goats	369	108	1	4	34	23	0	0	3	0	10	32	1
	Human	366	62	0	1	9	2	15	6	0	4	1	15	9

Suspicious reactors-- All sera showing a microscopic agglutination titers of 1:50 to 1:100 to any one of the eleven leptospiral antigens.

A-*L. copenhageni* B-*L. mankarso* C-*L. autumnalis* D-*L. sejroe* E-*L. hardjo* F-*L. grippotyphosa*

G-*L. pomona* H-*L. canicola* I-*L. australis* J-*L. patoc* K-*L. wolffi*

Appendix 15: Total number of sera with positive reactions to the eleven leptospiral antigens in each district for each animal species.

District	Animal species	No. of sera collected	Total positive	A	B	C	D	E	F	G	H	I	J	K
				ANTIGENS										
	Cattle	326	177	1	4	15	5	48	9	3	3	2	18	69
Nyandarua	Sheep & goats	357	138	0	0	14	11	80	3	1	0	1	21	7
	Human	315	0	0	0	0	0	0	0	0	0	0	0	0
	Cattle	439	133	0	0	2	1	41	0	0	0	0	54	35
Turkana	Sheep & goats	369	12	1	0	5	1	0	0	0	0	3	1	1
	Human	366	16	0	0	1	0	6	1	0	2	0	2	4

positive reactors-- All sera showing a microscopic agglutination titers of 1:200 and above to any of the eleven leptospiral antigens.

A-*L. copenhageni* B-*L. mankarso* C-*L. autumnalis* D-*L. sejroe* E-*L. hardjo* F-*L. grippotyphosa*

G-*L. pomona* H-*L. canicola* I-*L. australis* J-*L. patoc* K-*L. wolffi*

Appendex 16: Distribution of MAT titers to each serovar for the total sera of each animal species in Nyandarua district.

Serovar	Animal species	Total no.	RECIPROCAL TITER								
			0	50	100	200	400	800	1600	3200	≥ 6400
A	Cattle	326	205	1	0	0	1	0	0	0	0
	Sheep & goats	357	209	0	0	0	0	0	0	0	0
	Human	315	299	0	0	0	0	0	0	0	0
B	Cattle	326	204	0	1	2	2	0	0	0	0
	Sheep & goats	357	207	2	0	0	0	0	0	0	0
	Human	315	294	2	0	0	0	0	0	0	0
C	Cattle	326	193	11	5	5	10	7	3	0	0
	Sheep & goats	357	198	11	16	6	7	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0
D	Cattle	326	198	6	1	4	1	7	3	0	0
	Sheep & goats	357	201	8	1	6	5	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0

	Cattle	326	194	10	10	11	14	14	7	0	0
E	Sheep & goats	357	184	25	12	17	23	13	37	0	0
	Human	315	291	5	15	0	0	0	0	0	0
	Cattle	326	198	6	1	2	1	5	0	1	0
F	Sheep & goats	357	202	7	3	3	0	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0
	Cattle	326	195	9	1	2	1	0	0	0	0
G	Sheep & goats	357	195	14	7	1	0	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0
	Cattle	326	202	2	0	0	3	0	0	0	0
H	Sheep & goats	357	207	2	0	0	0	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0
	Cattle	326	203	1	1	1	0	1	0	0	0
I	Sheep & goats	357	207	2	0	1	0	0	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0

	Cattle	326	189	15	19	4	15	4	0	0	0
J	Sheep & goats	357	190	19	11	12	8	1	0	0	0
	Human	315	296	0	2	0	0	0	0	0	0
	Cattle	326	183	21	20	26	21	12	10	0	0
K	Sheep & goats	357	192	17	6	3	3	1	0	0	0
	Human	315	296	0	0	0	0	0	0	0	0

A-*L. copenhageni* B-*L. mankarso* C-*L. autumnalis* D-*L. sejroe* E-*L. hardjo* F-*L. grippotyphosa*

G-*L. pomona* H-*L. canicola* I-*L. australis* J-*L. patoc* K-*L. wolffi*

Appendex 17: Distribution of MAT titers to each serovar for the total sera of each animal species in Turkana district.

			RECIPROCAL TITER								
Serovar	Animal species	Total no. of	0	50	100	200	400	800	1600	3200	≥ 6400
A	Cattle	439	290	0	0	0	0	0	0	0	0
	Sheep & goats	369	326	1	0	1	0	0	0	0	0
	Human	366	336	0	0	0	0	0	0	0	0
B	Cattle	439	288	2	0	0	0	0	0	0	0
	Sheep & goats	369	324	3	1	0	0	0	0	0	0
	Human	366	336	0	1	0	0	0	0	0	0
C	Cattle	439	285	5	4	1	1	0	0	0	0
	Sheep & goats	369	205	22	11	3	2	0	0	0	0
	Human	366	328	8	1	1	0	0	0	0	0
D	Cattle	439	386	4	3	1	0	0	0	0	0
	Sheep & goats	369	312	15	9	1	0	0	0	0	0
	Human	366	335	1	1	0	0	0	0	0	0

	Cattle	439	288	2	11	17	6	16	3	0	0
E	Sheep & goats	369	327	0	0	0	0	0	0	0	0
	Human	366	328	8	7	5	1	0	0	0	0
	Cattle	439	287	3	2	0	0	0	0	0	0
F	Sheep & goats	369	327	0	0	0	0	0	0	0	0
	Human	366	333	3	0	1	0	0	0	0	0
	Cattle	439	290	0	0	0	0	0	0	0	0
G	Sheep & goats	369	325	2	1	0	0	0	0	0	0
	Human	366	336	0	0	0	0	0	0	0	0
	Cattle	439	290	0	2	0	0	0	0	0	0
H	Sheep & goats	369	327	0	0	0	0	0	0	0	0
	Human	366	333	3	1	2	0	0	0	0	0
	Cattle	439	290	0	1	0	0	0	0	0	0
I	Sheep & goats	369	322	5	5	2	1	0	0	0	0
	Human	366	335	1	0	0	0	0	0	0	0

	Cattle	439	265	25	49	30	17	2	5	0	0
J	Sheep & goats	369	302	25	6	1	0	0	0	0	0
	Human	366	331	5	9	1	0	1	0	0	0
	Cattle	439	285	5	13	17	4	11	3	0	0
K	Sheep & goats	369	326	1	0	1	0	0	0	0	0
	Human	366	332	4	5	3	2	0	0	0	0

A-*L. copenhageni* B-*L. mankarso* C-*L. autumnalis* D-*L. sejroe* E-*L. hardjo* F-*L. grippotyphosa*

G-*L. pomona* H-*L. canicola* I-*L. australis* J-*L. patoc* K-*L. wolffi*

Appendix 18: Distribution of MAT titers to the eleven leptospiral antigens for cattle sera from Nyandarua district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	203	1	0	0	1	0	0	0	0
Icterohaemorrhagiae	*mankarso*	204	0	1	2	2	0	0	0	0
Autumnalis	*autumnalis*	193	11	5	5	10	7	3	0	0
Hebdomadis	*sejroe*	198	6	1	4	1	0	0	0	0
Hebdomadis	*hardjo*	194	10	10	11	14	14	7	0	0
Grippotyphosa	*grippotyphosa*	198	6	1	2	1	5	0	1	0
Pomona	*pomona*	195	9	1	2	1	0	0	0	0
Canicola	*canicola*	202	2	0	0	3	0	0	0	0
Australis	*australis*	203	1	1	1	0	1	0	0	0
Semaranga	*patoc 1*	189	15	19	4	15	4	0	0	0
Hebdomadis	*wolffi*	183	21	20	26	21	12	10	0	0

Appendix 19: Distribution of MAT titers to the eleven leptospiral antigens for sheep and goats sera from Nyandarua district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	209	0	0	0	0	0	0	0	0
Icterohaemorrhagiae	*mankarso*	207	2	0	0	0	0	0	0	0
Autumnalis	*autumnalis*	198	11	16	6	7	0	0	0	0
Hebdomadis	*sejroe*	201	8	1	6	5	0	0	0	0
Hebdomadis	*hardjo*	184	25	12	17	23	13	37	0	0
Grippotyphosa	*grippotyphosa*	202	7	3	3	0	0	0	0	0
Pomona	*pomona*	195	14	7	1	0	0	0	0	0
Canicola	*canicola*	207	2	0	0	0	0	0	0	0
Australis	*australis*	207	2	0	1	0	0	0	0	0
Semaranga	*patoc 1*	190	19	11	12	8	1	0	0	0
Hebdomadis	*wolffi*	192	17	6	3	3	1	0	0	0

Appendix 20: Distribution of MAT titers to the eleven leptospiral antigens for human sera from Nyandarua district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	296	0	0	0	0	0	0	0	0
Icterohaemorrhagiae	*mankarso*	294	2	0	0	0	0	0	0	0
Autumnalis	*autumnalis*	296	0	0	0	0	0	0	0	0
Hebdomadis	*sejroe*	296	0	0	0	0	0	0	0	0
Hebdomadis	*hardjo*	291	5	15	0	0	0	0	0	0
Grippotyphosa	*grippotyphosa*	296	0	0	0	0	0	0	0	0
Pomona	*pomona*	296	0	0	0	0	0	0	0	0
Canicola	*canicola*	296	0	0	0	0	0	0	0	0
Australis	*australis*	296	0	2	0	0	0	0	0	0
Semaranga	*patoc 1*	296	0	2	0	0	0	0	0	0
Hebdomadis	*wolffi*	296	0	0	0	0	0	0	0	0

Appendix 21: Distribution of MAT titers to the eleven leptospiral antigens for cattle sera from Turkana district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	290	0	0	0	0	0	0	0	0
Icterohaemorrhagiae	*mankarso*	288	2	0	0	0	0	0	0	0
Autumnalis	*autumnalis*	285	5	1	1	1	0	0	0	0
Hebdomadis	*sejroe*	286	4	3	1	0	0	0	0	0
Hebdomadis	*hardjo*	288	2	11	17	6	16	3	0	0
Grippotyphosa	*grippotyphosa*	287	3	2	0	0	0	0	0	0
Pomona	*pomona*	290	0	0	0	0	0	0	0	0
Canicola	*canicola*	290	0	2	0	0	0	0	0	0
Australis	*australis*	290	0	1	0	0	0	0	0	0
Semaranga	*patoc 1*	265	25	49	30	17	2	5	0	0
Hebdomadis	*wolffi*	285	5	13	17	4	11	3	0	0

Appendix 22: Distribution of MAT titers to the eleven leptospiral antigens for sheep and goats sera from Turkana district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	326	1	0	1	0	0	0	0	0
Icterohaemorrhagiae	*mankarso*	324	3	1	0	0	0	0	0	0
Autumnalis	*autumnalis*	205	22	11	3	2	0	0	0	0
Hebdomadis	*sejroe*	312	15	9	1	0	0	0	0	0
Hebdomadis	*hardjo*	327	0	0	0	0	0	0	0	0
Grippotyphosa	*grippotyphosa*	327	0	0	0	0	0	0	0	0
Pomona	*pomona*	325	2	1	0	0	0	0	0	0
Canicola	*canicola*	327	0	0	0	0	0	0	0	0
Australis	*australis*	322	5	5	2	1	0	0	0	0
Semaranga	*patoc 1*	302	25	6	1	0	0	0	0	0
Hebdomadis	*wolffi*	326	1	0	1	0	0	0	0	0

Appendix 23: Distribution of MAT titers to the eleven leptospiral antigens for human sera from Turkana district.

ANTIGEN		RECIPROCAL TITER								
Serogroup	Serovar	0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	*copenhageni*	336	0	0	0	0	0	0	0	0
Icterohaemorrhagiae	*mankarso*	336	0	1	0	0	0	0	0	0
Autumnalis	*autumnalis*	328	8	1	1	0	0	0	0	0
Hebdomadis	*sejroe*	335	1	1	0	0	0	0	0	0
Hebdomadis	*hardjo*	328	8	7	5	1	0	0	0	0
Grippotyphosa	*grippotyphosa*	333	3	0	1	0	0	0	0	0
Pomona	*pomona*	336	0	0	0	0	0	0	0	0
Canicola	*canicola*	333	3	1	2	0	0	0	0	0
Australis	*australis*	335	1	0	0	0	0	0	0	0
Semaranga	*patoc 1*	331	5	9	1	0	1	0	0	0
Hebdomadis	*wolffi*	332	4	5	3	2	0	0	0	0

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