OCCURRENCE OF BOVINE LEPTOSPIROSIS IN KENYA

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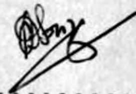
A thesis submitted in part fulfilment for the Degree of Master of Science in the University of Nairobi, Department of Veterinary Pathology and Microbiology.

April, 1983.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.



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This thesis has been submitted for examination with our approval as University supervisors.

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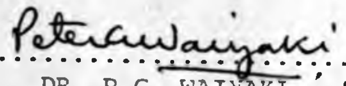

DR. P.G. WAIYAKI.

DEDICATION

To Mum and Dad.

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SUMMARY

Bovine leptospirosis is a worldwide problem and is of economic importance. The losses are due to abortions, stillbirths, infertility, reduced weight gains, decreased milk production and death. The disease in cattle may also be a source of infection to man.

Most detailed investigations of the disease have been carried out in Europe, Australia, New Zealand, North and South America and South East Asia. Relatively little is known of Leptospira infections in African livestock. No extensive countrywide surveys have been carried out to determine the occurrence of leptospiral serovars in Kenya.

Two thousand eight hundred and sixty four bovine serum samples were collected from different ecological zones of Kenya, during the period 1980 to 1982). Each serum sample was screened against the following leptospiral serovars: L. copenhageni, L. mankarso, L. sejroe, L. autumnalis, L. wolffi, L. georgia, L. grippotyphosa, L. pomona, L. hardjo and L. canicola using the microscopic agglutination technique. Positive sera were then titrated to find the end point titre.

Leptospiral antibodies were detected in 41% of the bovine sera tested, 25% of the sera showing microscopic agglutination (MA) titres of 1:200 or greater.

High antibody titres against L. hardjo (14.7%), L. wolffi (10.8%) and L. grippotyphosa (9.2%), were observed, respectively. Titres to the other leptospiral serovars were infrequent and in

general did not exceed 1:200.

L. grippotyphosa infections were found to be more prevalent in the wetter areas (zone II and zone III) while L. hardjo infections were more widespread throughout the country with a higher incidence in the drier areas (zone IV and zones V and VI).

It was concluded that bovine leptospirosis has a high incidence in Kenya and control measures must, therefore, be instituted to control the disease.

SECTION I

INTRODUCTION

Leptospirosis is believed to be one of the world's most widespread zoonoses affecting man and a wide variety of domesticated and wild animals (Van der Hoeden, 1964).

Bovine leptospirosis is of economic importance due to losses from abortions, stillbirths, infertility, reduced weight gains, decreased milk production and deaths (Hanson, 1976). The disease in cattle may also be a source of infection to man. The control and the prevention of the disease are therefore of paramount importance. Effective control depends on accurate diagnosis, treatment and the institution of adequate preventive measures.

The most important serovars (the term "serovar" has replaced the previously used term "serotype" in reference to *Leptospira*) which infect cattle and often lead to disease include *Leptospira pomona*, *L. grippotyphosa*, *L. hardjo*, *L. canicola* and *L. icterohaemorrhagiae*. Some of the other less commonly isolated serovars are *L. autumnalis*, *L. australis*, *L. tarassovi*, *L. sejroe*, *L. ballum* and *L. bataviae* (Amatredjo and Campbell, 1975).

The more significant features in the epidemiology of bovine leptospirosis consist of excretion of *Leptospira* in the urine of healthy or diseased carriers, their survival in wet places and the spread of infection to other animals (Amatredjo and Campbell, 1975).

The portal of entry varies considerably. *Leptospira* usually invade the host through the skin, especially if it is diseased, injured or softened as a result of prolonged contact with water

or mud. Leptospira also readily penetrate the mucosae of the conjunctivae, nasopharynx, muzzle and even the genital tract during breeding (Sleight and Williams, 1961; Sleight et al., 1964).

Invasion of the fully susceptible host leads to leptospiraemia, usually six to eight days after infection. The leptospiraemia phase which lasts four to five days and rarely up to seven days, terminates as a result of the appearance of specific antibody and subsequent phagocytosis which leads to clearance of Leptospira from circulation and their localisation in the kidneys with subsequent leptospiruria (Burnstein and Baker, 1954; Michna, 1970). Leptospiruria, which is often intermittent, begins two to three weeks after infection and may persist for weeks, months or even for the duration of the host's life. The latter state is believed to be the means by which infection is maintained in a wide variety of species (Turner, 1967).

Bovine leptospiral infections range from subclinical to fatal. Affected animals show acute, subacute or chronic signs. Clinical findings encountered with varying degrees of frequency include fever, depression, anorexia, haemolytic anaemia, jaundice, haemoglobinuria, photosensitization, meningitis, reduction in milk production, abortion and death (Hanson, 1977).

Isolation and identification of the causal organisms from urine, blood or tissues of affected animals provides the most convincing confirmation of the disease. Isolation of Leptospira from suspected field cases is, however, limited by the difficulties encountered in growing these fastidious organisms in the laboratory. This situation is aggravated by the veterinarian being called in a few days after the animals

have been sick by which time the *Leptospira* have disappeared from the blood. Urine provides the most reliable source for isolation purposes. A negative result does not, however, rule out the possibility of infection because of the intermittent shedding of *Leptospira* in urine (Turner, 1967).

The history and the clinical picture suggest the presence of leptospirosis and serological examination usually confirms the diagnosis of the disease. Several serological tests are used in the diagnosis of leptospirosis (Turner, 1968; Alexander, 1976). These include the macroscopic agglutination test (slide or plate test), the microscopic agglutination test, indirect haemagglutination test, fluorescent antibody technique and the complement fixation test. More recently the enzyme linked immunosorbent assay (ELISA) technique has also proved useful in the detection of bovine leptospiral antibodies (Azouaou *et al*., 1980). The microscopic agglutination test is often used and is generally accepted as the standard reference test for the detection and quantitation of leptospiral antibodies (Abdussalam *et al*., 1972).

Other diagnostic tests include darkfield microscopic examination of blood, urine and fluid from the body cavities of aborted foetuses and inoculation of the same specimens into artificial culture media or laboratory animals such as guinea pigs and hamsters (Torten, 1979).

There are no pathognomonic lesions on gross pathological examination. The lesions described usually pertain to the affected organs and are extremely variable. Postmortem findings include anaemia, jaundice, haemoglobinuria and subserous and

submucosal haemorrhages. In the later stages there is progressive interstitial nephritis manifested by small, whitish raised areas scattered throughout the renal cortex. The kidneys are usually enlarged. Hepatomegaly and splenomegaly may also be present. These organs sometimes show a brown-black discolouration. Aborted bovine foetuses are usually autolysed making differentiation of lesions very difficult (Hanson, 1976).

Histologically, there is focal or diffuse interstitial nephritis and centrilobular hepatic necrosis. Occasionally there may be vascular lesions in the meninges and brain. The histological demonstration of *Leptospira* requires fixation of tissue soon after death and use of a silver impregnation technique thereafter (Amatredjo and Campbell, 1975).

The effectiveness of antibiotics in the treatment and the control of leptospirosis is controversial (Hanson, 1976; Stoenner, 1976). The disease can, however, be successfully treated with antibiotics especially dihydrostreptomycin. A single injection of dihydrostreptomycin at a dosage level of 25 mg/kg of body weight cures the disease and eliminates the renal carrier-shedder condition (Stalheim, 1969).

The control of bovine leptospirosis is best accomplished by good management practices coupled with vaccination of livestock using *Leptospira* bacterins containing either a single serovar or multiple serovars. The type of bacterin to be used depends on the *Leptospira* serovars prevalent in the area (Hanson *et al.*, 1972).

Most detailed investigations of the disease have been carried out

in Europe, Australia, New Zealand, the Americas and South-East Asia whilst relatively little is known of the *Leptospira* in African livestock (Amatredjo and Campbell, 1975).

Piercy (1951) reported the first confirmed case of leptospirosis in Kenya, which occurred in a dog in 1948. In early 1956 there was a serious outbreak of a disease affecting cattle, sheep and goats in the Nanyuki (Laikipia) district in which over forty per cent of affected animals died. The disease was confirmed to be leptospirosis due to *L. grippotyphosa* and was first described in a preliminary note by Burdin and Froyd (1957) and later in more detail by Burdin *et al*. (1958). More cases of bovine leptospirosis were reported from various parts of Kenya between 1957 to 1968. The 1968 to 1976 Annual Reports of the Kenya Government Veterinary Department do not mention the occurrence of bovine leptospirosis. Some reports vaguely mention haemoglobinuria. In retrospect one wonders if these cases could not have been referring to leptospirosis. Between 1976 and 1982, several outbreaks involving hundreds of cattle have occurred in various parts of the country every year.

Burdin (1963) described the renal histopathology of *L. grippotyphosa* infections in farm animals in Kenya. Ball (1966) reported positive serological and cultural results from several wild and domesticated animals in Kenya and Uganda. Tabel and Losos (1979) reported a localised outbreak of bovine leptospirosis due to *L. grippotyphosa* in the Kiambu area. A serological survey carried out by Stirling and Lhermette (1980) showed a high incidence of bovine leptospirosis in Kenya. The presence of human leptospirosis in Kenya due to

serovars other than *L. grippotyphosa* has been reported by Forrester *et al*. (1969) and De Geus *et al*. (1969).

The purpose of the present investigation was to study the occurrence of bovine leptospirosis in Kenya. The knowledge gained from this study will help determine which leptospiral serovars should be incorporated in vaccines used for protecting susceptible livestock against leptospirosis in Kenya.

SECTION II

LITERATURE REVIEW

1. Classification and nomenclature.

Leptospirosis is a disease caused by infection with spirochaetes belonging to the genus *Leptospira*. These spirochaetes were first isolated in Japan in 1914 (Inada *et al*., 1916). While studying the Japanese isolate, Noguchi (1918) found it morphologically different from all known genera of spirochaetes and decided in 1917 to create a new genus which he named *Leptospira*. *Leptospira* is one of five genera (*Spirochaeta*, *Critispira*, *Treponema*, *Borrelia* and *Leptospira*) belonging to the family Spirochaetaceae and the order Spirochaetalis (Turner, 1974).

The genus *Leptospira* is subdivided into two species:

(1) *L. interrogans*, which includes all parasitic and pathogenic serovars and

(2) *L. biflexa*, which includes all the saprophytic serovars (Turner, 1974).

The basic taxon is the serovar. Serovars having partially common antigenic structures as determined by cross-agglutination tests are combined into serogroups (not a taxonomic subdivision) (Alexander, 1974).

The differences between parasitic *L. interrogans* and saprophytic *L. biflexa* are based mainly on:

(1) the ability or inability to parasitize vertebrate hosts (Turner, 1974),

(2) variation in the guanine-cytosine contents of their deoxyribonucleic acids (Haapala et al., 1969; Brendle et al., 1974),

(3) presence of lipase activity in all strains of L. biflexa and lack of it in some strains of L. interrogans (Faine and Stallman, 1982) and

(4) sensitivity to purine analogues by L. interrogans and lack of it in L. biflexa (Johnson and Harris, 1968; Johnson and Rogers, 1964).

Recent work by Banfi et al. (1981) indicates that saprophytic Leptospira have little or no catalase activity. Unfortunately, the division between the two species is still not absolutely clear and several of the serovars classified as L. biflexa are known to be parasitic. The up-to-date list of classified Leptospira isolated from animals includes a total of 22 serogroups and more than 167 serovars (two of which belong to the L. biflexa species) (Torten, 1979; Appendix 1).

These slender, spiral shaped bacteria are morphologically indistinguishable and serologically heterogeneous. Leptospira within species are not distinguishable on the basis of biochemical characteristics (Alexander, 1974). Many Leptospira will, however, cross-react serologically due to common antigens of various cell components and some soluble extracts obtained by various procedures (Chang et al., 1974; Baker and Cox, 1973; Cox et al., 1957). Cross reactivity can be observed within each species as well as between the two species (Wolff and Broom, 1954; Czekalowski et al., 1953; Baker and Cox, 1973; Cox et al., 1957; Sturdza and Elian, 1961). Serological identification of serovars is carried out by

microscopic agglutination and agglutinin - absorption tests with serovar-specific rabbit antisera.

2. Morphological and cultural characteristics.

Leptospira are flexuous, helical microorganisms 6-20 microns in length and 0.1 - 0.2 microns in diameter. Therefore, they pass through filters that retain most other bacteria. They are hooked at one or both ends. However, in culture media, some strains may become straight. They are motile by means of an axistyle that is regarded as a flagellar analogue by its function and chemical composition (Turner, 1974). Electron microscopy reveals it to be composed of two axial filaments inserted subterminally at both ends. A common external sheath covers both structures but there are no external flagella. Movements are of three basic sorts. These include shunting in either direction of the long axis, rapid rotation or spinning about the long axis and flexion or serpentine, undulating motion (Turner, 1970).

Leptospira are faintly coloured after staining with aniline dyes but are revealed, often distorted, by silver impregnation methods. *Leptospira* are Gram-positive but stain with difficulty and therefore are not classified as such (Turner, 1974). They cannot be seen by ordinary illumination, due to their refraction of light, which is similar to that of glass slides. However, they are readily visible by darkfield microscopy at magnifications of X 100 or greater. Under phase contrast microscopy they are less clear (Torten, 1979).

Unlike most other bacteria, *Leptospira* require specific fatty

acids as a source of carbon and energy and they also utilise glucose (Johnson and Walby, 1972; Stern _et al._, 1969; Ellinghausen, 1968). Parasitic strains require unsaturated fatty acids (containing 15 to 16 carbon atoms or more) or a corresponding polysorbate (Tween). This requirement is increased at temperatures above or below the optimum. Saturated fatty acids are not used unless suitable unsaturated fatty acids are also present (Johnson _et al._, 1969). Ammonium salts can be used as the sole nitrogen source and there are absolute minimal requirements for Vitamin B_{12}, thiamine and various metallic salts (Shenberg, 1967; Burger and Fuchs, 1970; Stalheim and Wilson, 1964; Wooley and Van Eseltine, 1968; Ellinghausen and McCullough, 1965).

Leptospira are aerobic microorganisms with a preference for microaerophilic conditions (Czekalowski _et al._, 1953). When grown in semisolid medium, they flourish best a few millimetres under the top surface, where they form one or more distinct discs of turbidity termed Dinger's discs. The Dinger's disc or ring represents zones of dense leptospiral growth (Czekalowski _et al._, 1953). They can be cultured on various media which are based on a solution of inorganic salts and buffered with phosphates. Some media are supplemented with pooled rabbit serum (Fletcher, 1928; Korthof, 1932; Stuart, 1946) or with bovine serum albumin fraction V and Tween 80 (Ellinghausen and McCullough, 1965; Johnson and Harris, 1967). Most serovars will also grow on synthetic media comprised of chemically defined ingredients (Shenberg, 1967). They grow best on liquid or

semisolid medium but can form colonies on solid medium, especially Cox's agar (Cox and Larson, 1957; Armstrong and Goldberg, 1960; Faine and Van der Hoeden, 1964).

Optimal temperature for *in vitro* propagation of *Leptospira* is 28-30°C although rapid multiplication for 1-2 days may be observed at 37°C. Exposure to 40°C is deleterious and at 56°C it is lethal for pathogenic strains (Turner, 1966). Strains of free living, presumed saprophytic *Leptospira* will multiply at temperatures lower (for example, 13°C) than parasitic and pathogenic strains (Johnson and Harris, 1967). The generation time is about 12 hours. Therefore contamination of cultures by most other bacteria that have shorter generation times will lead to leptospiral death (Torten, 1979). Optimal pH requirements are 7.2 to 7.4 (Turner, 1974). Incubation time for optimal growth ranges from a few days to four weeks or longer (usually 6 - 14 days) (Alexander, 1974).

Leptospira are very sensitive to dehydration and will die within minutes of exposure to dry conditions. Under laboratory conditions, they can be stored in liquid nitrogen and still maintain reasonable virulence on proper thawing (Torney and Bordt, 1969). Stock cultures are usually maintained in liquid or semisolid media with protein supplementation and can be stored without transfer for periods of 3 to 4 months or longer, when kept at room temperature in a dark place.

3. Animal pathogenicity.

Pathogenic *Leptospira* produce subclinical to lethal infections in hamsters, guinea pigs, gerbils and weanling rabbits on intraperitoneal inoculation (Alexander, 1974). Death in hamsters and guinea pigs occurs 4 to 7 days after injection of virulent *Leptospira* and is preceded by severe icterohaemorrhagic and nephrologic syndrome. Hence, these two laboratory animals are used as indicators of virulence and pathogenicity of these microorganisms (Torten, 1979). Adult rabbits are completely resistant and even large doses of highly virulent microorganisms injected intravenously cause only antibody response without clinical disease. For this reason and because they do not become carriers of *Leptospira*, rabbits are the animal of choice for antibody production for serological classification of *Leptospira* (Torten, 1979). Mice on the other hand, do not become clinically ill but become active carriers and shedders of live virulent microorganisms.

4. Bovine leptospirosis.

4.1 History and aetiology.

The first report of leptospirosis in cattle was published in Russia by Mikhin and Azhinov (1935). The causal organism was isolated from calves with acute infectious haemoglobinuria and termed *Leptospira icterohaemoglobinuria vitulorum*. It was later described as *Leptospira grippotyphosa* (Nikolajev, 1946). Freund (1947) recognised the disease in Palestine in 1941 and considered it similar to the condition described by Jungherr (1944) in the

United States.

Following these first descriptions of the disease, leptospirosis has been reported from most parts of the world and many other *Leptospira* serovars have been incriminated as causing bovine leptospirosis. A list of the main groups and serovars isolated from cattle is presented in appendix 2.

The principal serovars that infect cattle include *L. pomona* *L. grippotyphosa*, *L. hardjo*, *L. canicola*, and *L. icterohaemorrhagiae*.

L. pomona was first isolated in the United States by Baker and Little (1948), and in Australia by Sutherland *et al.* (1949). Subsequently *L. pomona* infections in cattle were recorded in Italy (Babudieri, 1949), Denmark (Borg-Petersen and Fennestad, 1956 a,b), Argentina (Savino and Rennella, 1948), New Zealand (Kirschner *et al.*, 1952; Te Punga and Bishop, 1953), Canada (Boulanger and Smith, 1957), Zaire (Van Riel and Van Riel, 1955) and South Africa (Botes and Garifallou, 1967).

Bovine leptospirosis due to *L. grippotyphosa* was described in Russia by Mikhin and Azhinov (1935) and Nikolajev (1946). Bernkopf (1946) and Bernkopf *et al.* (1948 a,b) isolated *L. grippotyphosa* in Israel and later detected significant serological reactions in bovine sera from several other Middle Eastern countries. Serological evidence for the presence of *L. grippotyphosa* infections in cattle was found in Tunisia by Cordier (1953), France (Rossi and Kolochine -Erber, 1955), Belgium (Van Riel and Bienfet, 1953), Germany (Rimpau, 1950), Switzerland (Gsell, 1952), the Netherlands (Wolff and Bohlander, 1952), Japan (Yanagawa *et al.*, 1955), Australia (Forbes *et al.*, 1955) Denmark (Borg-Petersen and Fennestad, 1956 b), Central Africa

(Van Riel and Van Riel, 1955), Kenya (Burdin *et al.*, 1958) and Hungary (Bodi *et al.*, 1964).

Since 1960, *L. hardjo* has received increased attention and is now recognised as an important bovine pathogen as well as an an important zoonosis. The original isolation was made by Wolff in 1938 from a sick man named Hardjoprajitno in Indonesia (Wolff 1953; Wolff, 1969). The serovar is now designated by the abbreviation hardjo (Anon, 1967).

The first isolation of *L. hardjo* from cattle was made by Roth and Galton (1960) in Louisiana, USA, later in Canada by Robertson *et al.* (1964) and in Australia by Sullivan and Stallman (1969). The infection is widespread in New Zealand (Lake, 1973). Michna *et al.*(1974) isolated *L. hardjo* from the kidney of an aborting cow and suggested that this infection was widespread in Scotland.

L. hardjo is closely related to other members of the Hebdomadis serogroup and in particular to *L. sejroe*. Antibodies to *L. sejroe* have been detected in a high percentage of American cattle since 1952 and also in parts of Europe. It has been suggested that many of these cattle were, in fact, infected with *L. hardjo* (Roth and Galton, 1960; Michna *et al.*, 1974).

Van der Hoeden (1955) reported epidemic as well as sporadic outbreaks of *L. canicola* infection in Israel. Heavy losses were reported in calves. Turner *et al.* (1958) isolated *L. canicola* from the urine of a sick two-day-old calf.

Field (1949) and Field and Sellers (1950) reported the isolation and identification of *L. icterohaemorrhagiae* from calves.

Ingram et al. (1952) described an outbreak of the disease in calves. Following recognition of L. icterohaemorrhagiae infection in cattle further reports appeared at frequent intervals(Amatredjo and Campbell, 1975).

4.2 Epidemiology.

Leptospirosis has a world-wide distribution. Some serovars such as L. icterohaemorrhagiae, L. pomona, L. canicola, L. grippotyphosa and L. hardjo are cosmopolitan, but others are more localised(Michna, 1970).

There are well recognised environmental factors which favour the survival of Leptospira outside their hosts leading to an increased incidence of the disease. These are moisture, pH values of soil and water around neutrality and temperature around or below 25°C. In particular there is a strong association between periods of high rainfall and the incidence of leptospirosis. Under favourable conditions Leptospira may persist in the external environment for several weeks (Michna, 1970).

Leptospira are dependent for their continued survival in nature on the presence of suitable host animals. Urinary excretion of Leptospira is the most significant factor in the epidemiology of leptospirosis and the disease is transmitted by contact either directly with infected urine or with a contaminated environment (Turner, 1967). Leptospira are not particularly host specific. Generally each serovar has a reservoir or carrier host in which subclinical infection is associated with prolonged leptospiruria. Small animals, especially rodents are the principal

fresh water the microorganisms survived for 60 days.

Adverse environmental conditions include dessication, excessive sunlight and chemical pollutants such as detergents, soaps and disinfectants. Other factors detrimental to Leptospira include high temperatures (about 50°C), salinity and pH values outside the range of about 6.2 to 8.0 (Torten, 1979).

Leptospira are harboured by many kinds of domesticated and wild animals. The natural reservoirs of Leptospira have been reviewed by Turner (1967) and Michna (1970) who found the order Rodentia (rats, mice, voles, gerbils, coypu) to be the most important. Other important orders in this regard include Insectivora (hedgehogs, shrews), Carnivora (dogs, foxes, jackals, mongooses, skunks, racoons, cats), Marsupialia (bandicoots, opossums), Chiroptera (bats), Artiodactyla (deer) and Lagomorpha (hares and rabbits). Domesticated animals are also important reservoirs and transmission can involve other animals, wild or domesticated, and man.

Leptospiral infection is not restricted to mammals. These organisms have also been isolated from birds, reptiles, fish, amphibians and arthropods. At present they are considered to be of minor importance in leptospiral epidemiology (Turner, 1967).

Prolonged excretion of Leptospira in bovine urine is significant in promoting the spread of the disease. Sutherland et al. (1949) reported an outbreak of leptospirosis in calves in which 24 out of 54 animals died during the period of leptospiruria in the first infected cattle. Lyubashenko et al. (1966) reported that up to five per cent of animals in one herd of cattle were found to be carriers of L. canicola, L. grippotyphosa, L. hebdomadis

L. icterohaemorrhagiae, *L. pomona* and *L. tarassovi*, with leptospiruria persisting from 120 to 700 days. Hodges and Ris (1974) inoculated calves with *L. hardjo*, *L. pomona*, *L. ballum* or *L. copenhageni* and found that leptospiruria lasted for up to 26, 37, 45 and 50 days respectively for *L. hardjo*, *L. pomona*, *L. ballum* and *L. copenhageni* infections. Ellis and Michna (1977) reported urinary excretion of *L. hardjo* for up to 174 days following experimental inoculation.

Sullivan and Stallman (1969) collected urine from a clinically normal heifer for 44 days, and up to day 38 the animal was found to be excreting large numbers of viable *L. hardjo*. Lake (1973) isolated *L. hardjo* from the urine of a healthy cow.

Transmission may also be facilitated among cattle and between cattle and man under intensive management conditions (Christmas *et al.*, 1974). In an epidemiological study the frequency of titres to *L. grippotyphosa* was found to be higher in pastured cattle than in animals in single stalls (Raetz and Herr, 1973).

Spread of *Leptospira* occurs not only among cattle but also from other animals or man. It is of comparative interest that the pig has sometimes been a source of leptospiral infection to cattle and man (Lyubashenko *et al.*, 1966; Alexander *et al.*, 1964; Burdin *et al.*, 1958). Ewy (1949) reported leptospirosis in dogs and suspected that infection could be transmitted by cattle. Fiocre and Dorolle (1966) reported *L. australis* infection in a calf and suggested that the source of infection was a dog on the farm from which they isolated the organisms. The dog was suspected as a source of bovine infection after *L. icterohaemorrhagiae* was isolated on farms according to Ioli *et al.* (1967). Bezdenezhnuikh

and Kashanova (1956) isolated L. grippotyphosa and L. hebdomadis from an outbreak among calves and noted that the source of infection was water contaminated by infected dogs and cats.

Calves with experimental leptospirosis spread the infection to heifers, pigs and goats by contact according to Morter and Morse (1956). Mason et al. (1972) identified feline cases of leptospirosis and suspected that the cats might have had contact with cattle infected with L. pomona. Van der Hoeden (1955) showed that jackals were the primary reservoir in three out of four outbreaks of L. canicola infection in cattle. Andrews et al. (1966) suspected an interaction between cattle and deer in leptospiral infection after studying the incidence in both species for six years.

Rodents are among the chief carriers of Leptospira and they sometimes act as a link in the epidemiological chain in leptospirosis (Messina and Campbell, 1975). Field and Sellers (1950) isolated L. icterohaemorrhagiae from a calf and suspected that rodents acted as reservoirs of the organism. When Clark et al. (1962) investigated L. grippotyphosa infections in cattle, they succeeded in isolating the organism from voles trapped on the farms and from stream water. The work of Emanuel et al. (1964) in Northern Queensland showed that the environmental association between cattle and small feral mammals was close, and it seemed likely that there had been transmission of infection between them. Bodi et al. (1964) reported outbreaks of L. grippotyphosa in cattle and suspected mice, rats and voles to be the reservoirs of of the organism.

Hedgehogs were suspected to be capable of playing an important epidemiological role in the spread of infection to domestic animals

when 36 out of 453 hedgehogs investigated proved to be carriers of *L. canicola* (Van der Hoeden, 1958a; Van der Hoeden *et al*., 1967). The same opinion was expressed by Webster (1957) who found suspicious leptospiral lesions in the kidneys of hedgehogs caught on a dairy farm with a severe outbreak of *L. pomona* infection. Martin *et al*. (1967) investigated an outbreak of bovine leptospirosis and succeeded in isolating *L. grippotyphosa* from racoons and opossums trapped on the farm. Skunks were regarded as an important reservoir host in a bovine outbreak described by Ferris and Andrews (1967).

There is some evidence, however, that wildlife associated with livestock can remain relatively free from *Leptospira* (Gordon-Smith *et al*., 1961; Doherty, 1967a). Cattle are now recognised to be the maintenance host for *L. hardjo* and that there is no wildlife reservoir (Little, 1981).

Leptospirosis in humans is primarily associated with occupational exposure. The association between cattle and man in leptospiral infection is exemplified by the fact that the majority of human cases occur in people who are in close contact with cattle (Amatredjo and Campbell, 1975).

4.3 Modes of transmission.

4.3.1 Indirect Contact.

Transmission occurs mainly through contact with an environment (soil, water, pasture, foodstuff, bedding) which is contaminated with urine containing virulent *Leptospira* (Sutherland *et al*., 1949; Doherty, 1967a; Hanson *et al*., 1964). *Leptospira* usually

enter through abraded or diseased skin and they readily penetrate the mucous membranes of the eye, nose and mouth. Experimental transmission in cattle by subcutaneous inoculation is possible (Sleight and Williams, 1961; Doherty, 1967b). Cattle were infected by spraying or placing infected urine or a leptospiral culture into the nose (Baker and Little, 1948; Ringen and Bracken, 1956). Baker and Little (1948) suggested that inhalation may be an important mode of transmission in cattle since droplets of urine could be dispersed in the air several metres from the urinating animal.

4.3.2 Direct Contact.

Van der Hoeden (1958b) and Sleight *et al.* (1964) observed *Leptospira* in the semen of bulls and suggested the possibility of venereal transmission. Roberts (1958) and Sleight and Williams (1961) showed that transmission of *Leptospira* in cattle occurred through coitus and artificial insemination.

Transplacental infection of the foetus *in utero* has been shown to occur in cattle (Fennestad and Borg-Petersen, 1962).

Leptospira may be excreted in milk and therefore there is a possibility of infecting the calf orally (DeLay *et al.*, 1955; Mitchell, 1959; Sullivan, 1974). Milk has, however, been shown to exhibit leptospirocidal activity and is therefore unlikely to be important in transmission (Kirschner and Maguire, 1955; Kirschner *et al.*, 1957). Nevertheless, DeLay *et al.* (1955) isolated *L. pomona* from milk mixed with urine of affected cattle. Little and Baker (1950) also isolated *Leptospira* from milk mixed with blood. On the other hand, infection through the udder from the

outside was experimentally possible, according to Mitchell *et al*. (1960) who inoculated infected milk into the mammary gland of a healthy cow.

The habit of cattle licking the genitalia, scrotum, teats, and other parts of their neighbours was suspected as a possible mode of transmission by Sutherland (1950). Transmission through the food chain has been demonstrated in carnivorous animals but appears not to be a factor in cattle (Reilly *et al*., 1970).

4.3.3 Ectoparasite vectors.

Callow (1967) suggested that ticks may play a role in the spread of leptospirosis. Krepkogorskaya and Rementsova (1957) isolated *L. grippotyphosa* from ticks taken off cattle and Van der Hoeden (1958a) isolated *L. canicola* from a tick found on a hedgehog. These findings together with the observation that arthropods may maintain for a long time viable *Leptospira* taken in with a blood meal add more weight to the possibility of infection of cattle through ticks (Anon, 1967). Flies may also act as vectors (Turner, 1967).

4.4. Pathogenesis.

The pathogenesis of leptospirosis is broadly divided into two phases. The first phase is characterised by leptospiraemia

while the second phase of immunity and leptospiruria is associated with increasing concentrations of antibodies and the localisation of Leptospira in the renal tubules (Turner, 1967).

Leptospira penetrate the host's epithelium but there is no obvious lesion at the site of entry. Once in the blood stream they migrate to the visceral tissue where they multiply rapidly and are then transported to all tissues of the body during a primary leptospiraemia, usually six to eight days after infection (Hanson, 1976). In this way the Leptospira reach and may affect any tissue or organ.

The basic mechanism of the pathological changes appears in part to be related to increased endothelial permeability but production of endotoxins has been demonstrated in some serovars (Sullivan, 1974). A heat-labile haemolysin which lyses ruminant erythrocytes, causing haemolytic anaemia and haemoglobinuria, has also been detected in affected cattle (Russel, 1956).

Acute nephritis commonly follows penetration of Leptospira through the intracellular spaces into the glomeruli and proximal tubules causing oedema, haemorrhages and cellular necrosis (Hadlow and Stoenner, 1955). Localisation in the kidney may result in extensive interstitial nephritis with marked interstitial cellular infiltration with lymphocytes, plasma cells and connective tissue. The Leptospira usually remain within the lumina of the proximal convoluted tubules where they multiply and are released in the urine. Occasionally, the Leptospira penetrate the epithelial cells where they remain free from antibody effects (Hirschberg and

Vaughn, 1973). Hepatitis follows localisation of *Leptospira* in the liver (Amatredjo and Campbell, 1975). Ocular lesions due to vascular congestion may also occur occasionally (Hoag and Bell, 1954b).

In pregnant animals *Leptospira* penetrate the placental barrier during maternal leptospiraemia with subsequent foetal leptospirosis and death. Abortions and stillbirths are therefore a common sequel of leptospiral infection.

Agglutinins appear in cattle sera a few days following onset of acute signs, increase for one to three weeks, and persist from several weeks to as long as eight to ten years in some animals (Hanson, 1976).

4.5. Clinical features.

Leptospirosis affects cattle of all ages and both sexes. However, some breeds may be more susceptible than others (Van der Hoeden, 1953). The disease has a variety of manifestations which occur in different combinations. Since the rate of infection is fairly variable, the disease could be of minor significance, affecting only a few animals, or it could be a major herd problem (Stoenner, 1976; Yanagawa, 1970). Moreover, a considerable percentage of leptospiral infections are subclinical (Michna, 1970).

Clinically it may not be possible to distinguish the disease produced by the various serovars. However, *L. pomona* and *L. grippotyphosa* are usually associated with the acute form of the infection causing more severe signs and lesions (Hanson, 1977). They usually occur in sudden epizootics with several years between epizootics, while *L. hardjo* is more often associated with the chronic

form of the disease and occurs over periods lasting a number of years (Hanson *et al.*, 1972).

Freund (1947) classified bovine leptospirosis into three clinical forms, namely, peracute, acute and chronic type with relapses.

Acute leptospirosis is characterised by the sudden onset of high fever (40°C or greater) and haemolytic anaemia following an incubation period of 4 to 10 days. In more severe cases there is haemoglobinuria, jaundice, anorexia, depression, pneumonia and the development of uraemia in the terminal stage, seen mainly in calves which die within three to five days of illness (Amatredjo and Campbell, 1975; Hanson, 1977). In adult cows there is fever, marked drop in milk production, haemoglobinuria and pregnant animals often abort.

Occasionally there may be nervous involvement with meningitis. Affected animals show aggressiveness, incoordination, hyperexcitability and convulsions (Hoag and Bell, 1954a; Stoenner *et al.*, 1963). Conjunctivitis, which may be mild to catarrhal and mucopurulent accompanied by bilateral uveitis has also been reported to occur in the acute disease (Hoag and Bell, 1954b).

Bodi *et al.* (1964) reported that in Hungary, 19 per cent of dairy cows affected by *L. grippotyphosa* showed fever, anorexia, weakness, drop in milk production, icterus and skin necrosis.

Fatal infections are more common in calves than in adult cattle. Death is primarily due to renal failure complicated by hepatitis and pneumonia (Hanson, 1977).

In the subacute form, lasting about two weeks in older animals and milking cows, the onset is slow. Lactation is reduced and the

milk resembles colostrum and may contain flakes and blood clots. There is little swelling of mammary tissue and all four quarters are equally affected. The condition is often described as 'flaccid mastitis' or 'flabby udder mastitis' but is really an agalactia and the term 'milk drop syndrome' is perhaps a better description (Little, 1981). Slight jaundice and impaired rumination may occur, while kidneys are enlarged and nephritic. Recovery is often prolonged (Michna, 1970).

Chronic leptospirosis has been associated with abortions, stillbirths, retained placenta and weak calves. There may be no other clinical manifestations (Te Punga and Bishop, 1953). Abortions may occur at any stage of gestation but are more common during the last half of the gestation period (Stoenner, 1967; Hanson, 1976). Retained placenta following abortions may contribute to later infertility problems (Hanson, 1976) and even deaths possibly due to toxaemia resulting from the retained foetal membranes (Kenzy et al., 1961). Infertility has been associated with several serovars but most frequently with L. hardjo. L. hardjo has been isolated from bovine urine in the absence of clinical signs (Roth and Galton, 1960; Michna et al., 1974; Sullivan and Stallman, 1969). However, Robertson et al. (1964) and Sulzer et al. (1964) obtained cultures from the urine in an outbreak of atypical mastitis and abortion. Serological monitoring may provide indirect evidence of an association between L. hardjo and abortion when high antibody titres to the organism are found after the clinical disease (Corbould, 1971; Hoare and Claxton, 1972; Johnson et al., 1974). A causal relationship may also be inferred when the abortion rate declines following long term vaccination (Hanson et al., 1972).

5. Serological techniques.

5.1. Microscopic agglutination test.

The most widely used tool for the laboratory diagnosis of leptospirosis is the microscopic agglutination (MA) test or its modifications. This serologic procedure is highly sensitive and specific, and can be used to test animal as well as human sera for the diagnosis of recent as well as past infections (Alexander, 1976). However, the test has limitations because of its high serological specificity. Consequently, to ensure detection of antibodies which may be provoked by any of the large number of serovars, it is necessary to employ a battery of different serovar antigens which cover most of the known cross-reactions of *Leptospira*. The following 15 serovars have been recommended for use in MA tests: *copenhageni*, *poi*, *canicola*, *castellonis*, *pyrogenes*, *grippotyphosa*, *wolffi*, *borincana*, *szwajizak*, *djatzi*, *autumnalis*, *bratislava*, *pomona*, *tarassovi* and *patoc* (Abdussalam *et al*., 1972). *L. biflexa*, serovar *patoc*, is used because it frequently cross-reacts with leptospiral antibodies in human sera irrespective of the infecting serovar. The proposed list of antigens may be modified according to local experience and needs. Moreover, substitution of local isolates of the same or related type could provide a more sensitive test.

Antigens used in the MA test may be live or formalized whole leptospiral cells (Babudieri, 1961; Sulzer and Jones, 1974; Alexander, 1976). Cells used for antigens are usually grown in a protein - supplemented liquid medium but there is no established

medium for antigen production. The recommended density of cultures in the antigen preparation is 2×10^8 organisms per ml (Anon, 1967) which can be determined nephelometrically or by microscopic counts in a Petroff - Hausser chamber. Density can also be estimated by microscopic examination of 0.01 ml drop under a 22 x 22 mm cover slip. A count of 100 to 200 organisms per high dry field (450 magnification) will provide an antigen of satisfactory density (Alexander, 1976). The MA test requires that the cultures be young (5-7 days old) and free of contaminants. The test can be performed either in microplates or test tubes by placing within them equal volumes of antigen and the proper dilution of the serum to be tested (Cole et al., 1973; Sulzer and Jones, 1974). After incubation at 30°C for 2 to 3 hours, the test is read by examination for agglutination under darkfield microscopy. The MA test end point is defined as the highest final dilution of serum in which 50% or more of the cells are agglutinated (Anon, 1967).

The disadvantages of the MA test is that it is laborious and time consuming. Moreover, it involves handling of live cultures with inherent risk to personnel and maintenance of a large number of serovars.

Adaptation of the MA test for use with microtitration techniques as described by Galton et al. (1965) and modified by Cole et al. (1973) has resulted in considerable savings in time and reagents. Moreover, the test can be read directly on the plate and there is reduced exposure of the investigator to live

antigens.

5.2. Macroscopic agglutination test.

This test is performed on a glass slide or plate by mixing a drop of serum with a drop of formalin killed antigen. The serum antigen mixture is allowed to react for a few minutes and then examined with the naked eye for presence or lack of agglutination. Originally it was hoped that this test, which is simpler to perform would replace the microscopic technique. Large scale testing has, however, shown it to be less accurate and less sensitive, especially for surveillance purposes (Wolff and Bohlander, 1966; Crawford, 1964; Solorzano, 1967). Two types of antigens for macroscopic agglutination tests have been developed, one by Galton *et al*. (1958) and the other by Stoenner and Davis (1967). Properly prepared antigens which are formalin-killed may keep for over 12 months. Recent infections in both man and animals can be detected by the macroscopic technique if paired sera are available. However, for specific identification of the causative serovar, the microscopic agglutination test should be performed (Anon, 1967).

5.3. Haemolytic and indirect haemagglutination tests.

Several procedures for coating red blood cells with soluble antigenic extracts of *Leptospira* have been described (Cox *et al*., 1957; Sulzer *et al*., 1975; Baker and Cox, 1973; Chang *et al*; 1957; Rothstein and Hiatt, 1956). A soluble extract could be an erythrocyte sensitizing substance (ESS) that when coated on red cells, causes lysis in the presence of specific antibodies and

complement (Torten, 1979).

The haemolytic test can detect antibodies in human sera irrespective of the infecting serovar. It lacks sensitivity for detecting antibodies in animal sera and its use for this purpose is not recommended (Sulzer *et al.*, 1975; Baker and Cox, 1973). It has limited usefulness as a serological survey tool (Tan, 1969).

The indirect haemagglutination test is carried out using sensitised erythrocytes which have been fixed with formaldehyde, glutaraldehyde or pyruvic aldehyde (Baker and Cox, 1973; Sulzer *et al.*, 1975; Sulzer and Jones, 1974). These agents preserve the red cells and prevent them from lysing during antigen-antibody reaction. When using treated and antigen coated red cells, a positive reaction is haemagglutination rather than haemolysis. The fixed antigens are used with human O red blood cells in an indirect haemagglutination procedure. Antigen extracts for haemolytic or haemagglutination tests are usually obtained from saprophytic *Biflexa* serovars such as Patoc I.

5.4. Complement fixation test (CFT).

Complement fixation tests for diagnosing leptospirosis using whole leptospiral cells or soluble extracts have been described (Sturdza and Elian, 1961; Nicolescu and Borsai, 1972; Cox, 1957; Schubert *et al.*, 1956). These tests usually employ saprophytic *L. biflexa* antigen and have genus - specific activity (Sturdza *et al.*, 1960, Turner, 1968). The CFT test is useful for detecting current and relatively recent infections in humans (Turner, 1968). However, the test is not suitable for surveillance or for detecting animal infections (Torten, 1979).

SECTION III

MATERIALS AND METHODS

1. Serum samples.

Bovine sera were obtained from various parts of Kenya representing different ecological zones, as shown in figure 1 and table 1. The sera were collected over a three year period from 1980 to 1982.

Blood samples were collected into universal bottles or tubes without any additives and kept at room temperature until a clot formed. The clot was loosened with an applicator stick and serum decanted into tubes. The serum was centrifuged at 350 x g for 10 minutes (Centrifuge model G F-6, Measuring and Scientific Equipment Ltd., London). The serum was decanted into sterile bijou bottles or plastic storage vials and stored at -20°C until used.

2. Leptospira reference cultures and antisera.

The Leptospira reference cultures listed in appendix 4 and the corresponding antisera were kindly supplied by The Director, Leptospira Reference Laboratory, Colindale Avenue, London. The cultures were supplied in Ellinghausen's Modified Johnson Harris (EMJH) liquid media. This polysorbate medium consists of Bacto - Leptospira Medium Base EMJH supplemented with Bacto - Leptospira Enrichment EMJH (Difco Laboratories, Detroit, Michigan, USA).

3. Propagation of Leptospira and antigen production.

The reference cultures were subcultured by transferring one ml from each serovar into 10 ml of Fletcher's semi-solid medium supplemented with ten per cent rabbit serum and one ml was also inoculated into 10 ml of polysorbate medium (EMJH). Fletcher's medium was dispensed into screw cap tubes while the EMJH was placed into universal bottles.

Duplicate sets of each culture were initially made in each medium. One set of each culture was incubated at 29 - 30°C and the other at room temperature. The cultures incubated at 29 - 30°C were kept until a well developed Dinger's ring appeared in the Fletcher's medium at which time they were stored in a dark cupboard at room temperature. Dinger's disc represents zones of dense leptospiral growth (Czekalowski et al., 1953).

The cultures were checked under the darkground microscope to establish that they were pure and free from contaminants. One ml of the culture was then transferred into 10 ml of fresh EMJH medium labelled correspondingly with the serovar name and date of inoculation.

The cultures in Fletcher's medium were used as stock cultures in the laboratory and were subcultured at 6 to 8 week intervals at which time they were checked for purity and viability by darkground examination. Cultures in liquid EMJH medium were subcultured at weekly intervals and used for antigen production.

Any culture developing contaminants was purified or decontaminated in either of the following two ways.

3.1 Use of 5 - fluorouracil.

The problem of contamination was overcome by incorporating 5 - fluorouracil (Sigma Chemical Company, St.Louis, USA) into the culture medium at a concentration of 150-200 micrograms per ml. This selective agent inhibited the growth of contaminating bacteria while at the same time allowing *Leptospira* to grow.

3.2 Use of millipore filters.

Cultures were also purified by filtration through bacteriological filters with average pore size ranging from 0.22-0.45 microns. This was carried out using disposable millipore filters (Millipore S.A. Molsheim - France) mounted on a syringe. The contaminated culture was gently passed through the filter and dispensed into two or three tubes of fresh medium which were incubated as described above.

4. Microscopic agglutination (MA) test (Modified microtechnique).

The performance of the test was based on the original microtechnique described by Galton *et al*. (1965) and modified by Cole *et al*. (1973). Figure 2 shows microtechnique equipment used in carrying out the test.

4.1 Test Procedure.

4.1.1 Antigen production.

Antigens used were 5 to 7 day old cultures grown in EMJH medium at 29 - 30°C. These cultures were grown in 10 ml volumes of medium from inocula that consisted of 1 ml volumes of 7 day old cultures. All cultures were checked for purity, viability and the density estimated. A culture density of approximately 2 x 10^8 organisms per ml as recommended previously (Anon, 1967) was used. Overly dense cultures were diluted with medium to contain approximately 2 x 10^8 organisms per ml before use. Density was estimated by the method of Alexander (1976). Briefly, the number of leptospiral microorganisms was estimated by examination of a 0.01 ml drop under a 22 x 22 mm cover slip. A count of 100-200 organisms per high power field (magnification X 450) gave a satifactory density.

4.1.2 Screening of Sera.

All sera were dispensed into flat bottomed microtitre plates (Linbro Chemical Co. New Haven, Conn. and Dynatech Laboratories, Alexandria, Virginia) and were screened at a dilution of 1:50 against all the ten serovars, namely: *L. copenhageni*, *L. mankarso*, *L. sejroe*, *L. autumnalis*, *L. wolffi*, *L. georgia*, *L. grippotyphosa*, *L. pomona*, *L. hardjo* and *L. canicola*. Each column of the microtitre plate was used for a different serum and a separate row was used for each antigen. Briefly, volumes of 0.05 ml of sera were added to 1.2 ml of phosphate buffered saline (PBS,pH 7.4) containing 0.4 per cent

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formalin, in appropriately labelled plastic screw cap vials and mixed. This gave a serum dilution of 1:25. By means of an Eppendorf dropper pipette (Eppendorf, West Germany) volumes of 0.025 ml of the diluted sera to be tested were dispensed into each of ten wells in the appropriate column of two adjacent plates. Similarly, a volume of 0.025 ml of the appropriate antigen suspension was added to each corresponding well. After addition of the antigen the serum dilution was 1:50 in a volume of 0.05 ml. A separate pipette dropper tip was used for each serum and each antigen.

Each plate was then gently tapped to mix the contents, covered with parafilm (American Can Company, Dixie-Marathon, Greenwich, Ct.) to exclude debris and prevent evaporation and incubated at 30°C for 2 to 3 hours. The tests were then examined for the presence of agglutinated Leptospira as described later.

Hyperimmune control sera prepared against each antigen used were titrated in each batch of tests. Other controls included known negative sera and antigen controls (PBS + antigen), which were also incorporated into each batch of tests.

4.1.3 Titration of positive sera.

Any serum producing agglutination of 50 per cent or more of the Leptospira antigens in the screening test was then titrated. Dilutions from 1:50 to 1:1600 were made for each serum in two fold dilution steps. Any serum positive at a

1:1600 dilution was further titrated to find the end point.

Separate plates were used for respective antigens when carrying out the titrations. Six wells were used for each serum titrated, thereby allowing 16 sera to be examined against a single antigen on each plate. The arrangement is shown in appendix 5.

To the first well of each row in the two halves of the plate was added 0.05 ml of the test serum which was dispensed using a 0.05 ml Eppendorf dropper pipette. To each of the rest of the wells was added 0.025 ml of PBS (with 0.4 per cent formalin) using a 0.025 ml dropping pipette. With the aid of a Titertek multichannel pipette carrying eight tips (Flow Laboratories, Rockville, Maryland, U.S.A.), 0.025 ml of serum was drawn up from each well in the first column and dispensed into succeeding wells to make doubling dilutions of the sera giving a dilution range of 1:50 to 1:1600. In this way eight serial dilutions could be completed at once. Antigen (0.025 ml) was added to each well in the plate, mixed and incubation carried out as described above.

Delivery of accurate volumes by the dropper pipettes was routinely tested before and after each batch of tests was run by measuring a certain volume and counting the number of drops. Alternatively a special blotter material (Microdiluter Delivery Tester, Dynatech Laboratories, Alexandria, Virginia) was used.

4.1.4 Reading the test.

After incubation, the plates were placed on the stage of a

darkfield zoom dissecting microscope (Wild Heerbrugg WILD M8, Switzerland; Figure 3) and the wells examined for agglutination at a magnification of X 120.

The reading on each well was scored as either negative or positive. The end-point in a positive test was taken as the highest final dilution of the serum in which at least 50 per cent of the Leptospira were agglutinated as recommended previously (Anon, 1967).

The degrees of agglutination were recorded as follows:

4+ - 75 per cent or more cells agglutinated.

3+ - 50 - 75 per cent of cells agglutinated; many clumps present in the field.

2+ - 25 - 50 per cent of cells agglutinated; at least one specific clump in each field.

1+ - occasional small clump or small stellate aggregations.

4.1.5 Interpretation of titres in the microscopic agglutination test.

An end-point titre of 1:50 to 1:100 was considered as being suspicious while any titres of 1:200 or above was regarded as positive (Carter and Moojen, 1981).

5. Electron microscopy.

Seven-day old cultures of selected leptospiral organisms were stained with phosphotungstic acid and examined in the electron microscope (EM 9A, Carl Zeiss, West Germany).

SECTION IV

RESULTS

1. Serum samples.

A total of 2,864 bovine sera were collected from various parts of Kenya. The sampling areas were grouped according to ecological zones. A breakdown of the number of sera collected from each zone and each sampling area is shown in table 1.

2. Propagation of Leptospira and antigen production.

Leptospira organisms were grown in culture media containing 150 to 200 micrograms per millilitre of 5-fluorouracil. Cultures in Fletcher's semi-solid medium were used as stock cultures and could be stored at room temperature for eight weeks before subculturing into fresh medium. Leptospiral growth in Fletcher's medium was observed as one or more characteristic zones of growth (Dinger's disc) seen one to three centimetres below the surface. The usual appearance of the Dinger's disc is shown in figure 4.

Cultures in liquid EMJH medium were subcultured at weekly intervals and used for antigen production. Five to seven day old cultures grown at 29-30°C (Figure 5) were found to be most suitable for use as antigen as the density of such cultures was approximately 2×10^8 organisms per millilitre.

Contaminated cultures were successfully decontaminated using 5-fluorouracil and millipore filters.

3. Microscopic agglutination test (modified microtechnique).

Leptospiral agglutination by the microscopic agglutination test (modified microtechnique) was observed as relatively large, irregularly outlined clumps which had a lacy, frayed rope appearance. Figure 6 shows the varying degrees of positive agglutination reactions and negative reaction as seen under the darkground microscope (at X 120 magnification).

4. Serological findings.

Table 1 shows the distribution of reactor rates in bovine sera by ecological zones and sampling areas. Of the 2,864 sera screened for the presence of leptospiral antibodies, 1,168 sera (40.8%) were positive at a screening dilution of 1:50 to one or more of the ten antigens. On titration, microscopic agglutination titres of 1:200 or above (positive titres), were observed in 723 sera (25.2%).

Leptospiral antibodies were detected in 46.7% of the 716 sera tested from ecological zone II, 31.4% of which had positive titres. In zone II, Nyeri and Kericho had the highest reactor rates with 66.7% and 61.2% of the sera being reactive, respectively, for each area. Positive titres were found in 48.6% of the 72 sera tested from Nyeri and in 42.4% of the 389 sera tested from Kericho.

In zone III, 46.9% of the 706 sera tested were reactive while 27.3 % of them showed positive titres. Of the 173 sera tested from Kitale, 82.1% were reactive with 52% of them showing positive titres.

Out of the 685 sera tested from areas in zone IV, 35.5%

were reactive while 25% were positive. Similarly, 34.4% of 757 sera from zone V and VI were reactive while 17.7% were positive. All 48 sera tested from Garissa were negative.

Sera with suspicious reactions to the ten leptospiral antigens and those sera with positive reactions to the same ten leptospiral antigens are shown in tables 2 and 3, respectively. The total number of sera with suspicious reactions recorded was 1,100 while the total number of cattle with suspicious reactions was 445. Similarly, the total number of sera with positive reactions was 1,033 while the total number of cattle with positive reactions was 723. Several of the animals showed reactions to more than one leptospiral antigen.

Table 4 shows the distribution of microscopic agglutination titres to *Leptospira* in 2,864 bovine sera from Kenya by serovars. The Hebdomadis serogroup which includes serovars *hardjo*, *wolffi*, *georgia* and *sejroe* was the most prevalent followed by the Grippotyphosa serogroup.

Table 5 shows the number and percentage of sera that were reactive, suspicious or positive to each of the ten antigens. Antibodies to *L. hardjo* (27.9%), *L. wolffi* (24.2%) and *L. grippotyphosa* (14.8%) were the most prevalent. Positive titres were observed most frequently for *L. hardjo* (14.7%), *L. wolffi* (10.8%) and *L. grippotyphosa* (9.2%) respectively, while positive titres to the other antigens were less than 1%.

The distribution of microscopic agglutination titres using the ten leptospiral antigens by sampling areas is shown in appendix 6, whereas the distribution of microscopic agglutination

titres to each of the ten leptospiral antigens is presented in appendix 7. Agglutination reactions were most commonly observed to L. hardjo, L. wolffi and L. grippotyphosa antigens. There were few positive titres to the other Leptospira antigens. The distribution of titres to L. hardjo, L. wolffi and L. georgia followed a similar pattern although titres to L. georgia were fewer in number and of lower titre (less than 1:1600). Titres to L. hardjo (27.9%) were generally higher (up to 1:6400) and greater in number than titres to L. wolffi (25.2%) and L. georgia (5.1%).

The majority of positive titres to L. hardjo and L. wolffi were seen to occur with sera originating from Kajiado, Marsabit and Trans Mara. Reactions were less commonly seen in sera from Kericho, Kitale, Nyeri, Nakuru, Rumuruti and the coastal area (Malindi, Lamu and Mombasa).

Reactions to L. grippotyphosa were most commonly observed in sera from Kitale (58.4%), Kericho (39.6%), Nyeri (31.9%) and Malindi (29.8%). There were few positive titres in sera from Rumuruti (9.9%), Marsabit (8.6%), Nakuru (7.8%), Kiambu (7.5%) and Eldoret (4.8%). Positive titres to sera from Trans Mara (2.4%) and Kajiado (0.7%) were infrequent.

The distribution of per cent positive reactors to each of the ten leptospiral antigens by ecological zones is presented in table 6. Positive titres to L. grippotyphosa were found in 19.3% and 12.2% of the sera tested from zone II and zone III, respectively. In both zone IV and zones V and VI, 2.8% of the sera tested had positive titres. Positive reactions to L. hardjo

and L. wolffi were found in sera from almost all sampling areas. Positive reactors to L. hardjo were found in zone II (12.2%), zone III (14.6%), zone IV (18.8%) and zones V and VI (13.3%). Positive reactions to L. wolffi were seen in 7.5% of sera from zone II, 9.1% of sera from zone III,17.1% of the sera from zone IV and 9.8% of sera from zones V and VI.

Appendix 8 shows the distribution of microscopic agglutination titres to the ten antigens in 2,864 bovine sera by ecological zones. The results show that reactions to L. hardjo and L. wolffi were present in sera from all the zones. Zone IV had the highest number of positive titres to both L. hardjo and L. wolffi with the highest titres recorded for L. hardjo at a dilution of 1:6400. This was followed by zones V and VI which also had high titres to L. hardjo and L. wolffi. Zone II and zone III had less sera showing positive titres to these antigens.

The highest titre (1:12,800) recorded was to L. grippotyphosa antigen with sera originating from zone III. Positive titres to L. grippotyphosa were frequently observed in sera from zone II and zone III with very few positive titres seen in sera from zone IV and zones V and VI.

Of the 2,684 cattle sera tested, 800 were reactive with L. hardjo antigen, 695 sera were positive to L. wolffi and 441 sera reacted with L. grippotyphosa antigen. Some sera reacted with one or more of these antigens. Sera reactive with both L. wolffi and L. hardjo were seen in 638 sera while only 151 sera reacted with both L. hardjo and L. grippotyphosa. Only 91 sera

reacted with all three antigens (*L. grippotyphosa*, *L. hardjo* and *L. wolffi*). The number of bovine sera reactive with these selected antigens or groups of antigens is presented in table 7.

5. Electron microscopy.

Figure 7 shows electron micrographs of some of the leptospiral organisms used as antigen. These include *L. grippotyphosa*, *L. pomona* and *L. hardjo*.

SECTION V

DISCUSSION

The *Leptospira* reference cultures used as antigen were readily propagated in EMJH and Fletcher's media. Some cultures, however, became contaminated occasionally, despite aseptic technique employed when subculturing the organisms and scrupulous attention paid to cleansing and sterility of all glassware used in culture work. The problem of contamination was solved by the incorporation of 5-fluorouracil, a pyrimidine analogue, into the culture media at a concentration of 150 to 200 micrograms per millilitre (Turner, 1970). This selective agent inhibited the growth of contaminating bacteria while at the same time it allowed *Leptospira* to grow.

The microscopic agglutination test (or its modifications) is the serological procedure most often used for the demonstration of leptospiral antibodies. This test is highly sensitive and specific and can be used to test animal sera for the diagnosis of recent as well as past infections (Alexander, 1976). Therefore, it is a useful tool for epidemiological investigations which frequently entail retrospective determination of antibodies which may have been provoked many months previously.

The microscopic agglutination test is only relatively serovar - specific since cross reactions commonly occur because agglutinins elicited by *Leptospira* of a particular serovar often agglutinate *Leptospira* of related serovars, particularly those in the same serogroup (Anon, 1967). Initially, it was hoped that

antigens belonging to all the serogroups would be available for the study. Since not all the antigens were available, however, it is possible that some of the antigens that were not available could be occurring in the study area. Titres to leptospiral antigens in serological surveys are serogroup indicative only, although the test frequently provides clues on the identity of the infecting serovar. It is stressed, however, that the determination of the infecting serovar can only be definitely established by isolation and typing of the organism (Alexander, 1976).

Difficulties in interpretation of the test are due to several factors including the antigenic complexity of Leptospira, the definition of the end-point, the quality of antigen suspensions and the possibility of infection with more than one serovar (Turner, 1968).

The end-point of the microscopic agglutination test was taken as the highest dilution of serum in which 50% or more of the Leptospira were agglutinated (Anon, 1967). As recommended, previously (Anon, 1967) , the definition was clarified by the preparation of pictorial illustrations showing typical gradation of reactions (Figure 6). Moreover, the scoring of the degrees of agglutination were originally made by examining a number of positive sera by the microscopic agglutination test and making additional readings by transferring samples on to microscope slides and examining for agglutination using darkfield microscopy.

According to Turner (1968), some authors have used an arbitrary "significant" titre when reporting the results of

serological surveys. Only titres equal to or greater than this significant titre were included in their results. The titres used have varied from 1:30 to 1:400, with 1:100 being the most commonly used. The level chosen was quite arbitrary and was often a matter of expedience, mainly the minimum dilution used in the screening of the sera being used.

In the present study, a titre of 1:50 was evaluated as being reactive, that of 1:100 as suspicious, while a titre of 1:200 or above was considered as positive or indicative of present or past infection (Carter and Moojen, 1981; Shotts, 1976).

Young, actively growing cultures in liquid EMJH medium, which were free from contaminants, were used in the test. The antigen density was established as containing approximately 2×10^8 organisms per millilitre prior to use, so that consistent results were obtained (Cole _et al_., 1973; Anon, 1967).

There seems to be a high prevalence of bovine leptospirosis in Kenya as shown in table 1. Out of 2,864 sera examined, 1,168 sera (40.8%) were positive at a screening dilution of 1:50, to one or more of the ten leptospiral serovars used as antigen. Similar reactor rates were observed by earlier workers in Kenya. Stirling and Lhermette (1980) found 39.8% of 1,066 bovine sera from various parts of Kenya positive at a screening dilution of 1:30, to one or more of eighteen leptospiral serovars used as antigen in a modified microscopic agglutination test. In a similar survey, Ball (1966) found 61 bovine sera (41%) out of 150 sera tested from Kombeni area (Mombasa) to be reactive to one or more of seventeen leptospiral serovars, at a screening dilution of

1:100. However, if a titre of 1:300 is considered significant, the incidence was seen to drop to 22%. In the present study, 25% of the 2,864 bovine sera showed titres of 1:200 or above, to one or more of the ten antigens.

The incidence of leptospirosis is linked with environmental factors that favour the survival of Leptospira. These include moisture, warmth and a soil pH around neutrality. In particular there is a strong association between periods of high rainfall and the incidence of leptospirosis (Sullivan, 1974). This would imply that the incidence of leptospirosis is likely to be greater in those places with the highest rainfall.

This association is evident from the results presented in table 1 and table 6 which show that a higher percentage of reactors were present in the wetter areas, comprising of zone II and zone III, when compared to the drier areas (zone IV, and zones V and VI). The highest reactor rates were found in the high rainfall areas of Kitale, Nyeri, Kericho, Lamu and Malindi, whereas the drier areas had markedly lower reactor rates. This is most dramatically seen in the extremely arid area of Garissa where there were no reactors. A similar observation was made by Stirling and Lhermette (1980) who concluded that locality was the most significant factor determining the incidence of the disease.

Many of the sera were positive to more than one serovar which may represent mixed infections, several separate incidences or possibly cross reactions of specific antibodies with several closely related antigens. The Hebdomadis serogroup was the most prevalent followed by the Grippotyphosa serogroup. Cross - reactions were commonly observed within the Hebdomadis serogroup which included

serovars hardjo, wolffi, georgia and sejroe. Antibodies to L. hardjo (29.9%), L. wolffi (24.2%) and L. grippotyphosa (14.8%) were the most prevalent.

Other workers in Kenya have also shown the Hebdomadis serogroup to be most prevalent. Stirling and Lhermette (1980) found about 20 per cent of the 1,066 cattle examined, showing positive titres to the Hebdomadis group. Ball (1966) found that the highest titres obtained in her study were to L. grippotyphosa and L. wolffi. However, since L. wolffi was the only representative serovar used for the Hebdomadis serogroup it was not possible to conclude which serovar in this group the animals had contacted.

Alexander and Evans (1962) found L. wolffi, strain 3705, the most sensitive antigen of 17 serovars in the Hebdomadis serogroup used to detect agglutinins for this group in 137 sera from cattle in the United States. In the present study, it was observed that most sera reacting to L. hardjo also reacted to L. wolffi and L. georgia. Titres to L. georgia were generally lower and fewer in number indicating that it was a less sensitive antigen for detecting agglutinins in the Hebdomadis group. Titres to L. hardjo were generally higher than those to L. wolffi which might incriminate L. hardjo as the main serovar responsible for infection. Sullivan (1974) found that serological evidence in Australia and the United States suggests that the incidence of L. hardjo infection in cattle is increasing and that L. hardjo may replace L. pomona as as the most common serovar infecting cattle in these countries.

These observations indicate that where a serogroup is not known to occur, the selection of a serovar to represent that serogroup may not always be appropriate. For example, cattle

sera were found to react with L. sejroe in the United States. but eventually L. hardjo and not L. sejroe was isolated, and the same happened in Canada (Turner, 1968). An observation that may be pertinent is that L. hardjo infection emerged as a disease of cattle more or less simultaneously in Britain, Italy, Australia and New Zealand a few years after the first observation in the United States (Little, 1981).

Burdin et al. (1958) isolated L. grippotyphosa from an outbreak of leptospirosis involving cattle, sheep and goats in the Nanyuki area of Kenya. They did a serological survey and also showed that L. grippotyphosa was widespread in the area studied. Tabel and Losos (1979) reported a localised outbreak due to L. grippotyphosa in the Kiambu area and suspected that infections due to this serovar occur more frequently in cattle than is actually recognised. Stirling and Lhermette (1980) found only a 4.2 per cent incidence of this serovar. However, the present study revealed a higher incidence of L. grippotyphosa infections (Tables 5 and 6). Antibodies to L. grippotyphosa were found in 14.8% of sera tested with 9.2% of them showing positive titres. The results indicate a higher prevalence of L. grippotyphosa infections in the wetter areas (zone II and zone III) than in the drier areas (zone IV and zones V and VI). It appears that L. hardjo infections are more widespread throughout the country with the highest incidence occurring in zone IV which includes Nakuru, Rumuruti, Dol Dol and Kajiado areas.

Infection with leptospiral serovars other than those belonging to the Hebdomadis and Grippotyphosa serogroups was found to be very infrequent, with less than 1% of positive reactors (Table 6).

Ball (1966) and Stirling and Lhermette (1980) reported similar observations. According to Ball (1966), L. pomona reactors were not present in Kenya and Ugandan cattle.

The purpose of the present study was to ascertain which leptospiral serovars infect Kenyan cattle and their relative importance and distribution in the country. The most prevalent serovars were L. hardjo and L. wolffi (Hebdomadis serogroup) followed by L. grippotyphosa (Grippotyphosa serogroup). Infection with the Hebdomadis serogroup was found to be widespread with L. hardjo being the most commonly occurring serovar. L. grippotyphosa infections were found to be more prevalent in the wetter areas (zone II and zone III).

There is a high prevalence of bovine leptospirosis in Kenya. The most frequent serovar affecting cattle, L. hardjo is a common human pathogen. This study has thus clearly revealed the imminent danger of a zoonosis pertaining to leptospirosis in Kenya which must be attended to urgently.

Vaccination using bacterins containing leptospiral serovars prevalent in the area is an effective control measure. Therefore it is recommended that bacterins for use in Kenyan cattle should contain both L. grippotyphosa and L. hardjo serovars.

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Table 1. Distribution of reactor rates to any of ten leptospiral antigens in bovine sera by ecological zones and sampling areas.

Ecological Zone*	Area	Bovine sera tested	% sera reactive**	% sera positive***
II	Nyahururu	52	25.00	11.54
	Ol Kalou	15	0	0
	Nyeri	72	66.67	48.61
	Kericho	389	61.18	42.42
	Kakamega	28	25.00	7.14
	Kiambu	160	17.50	10.63
	Zone total	716	46.65	31.42
III	Malindi	84	66.67	29.76
	Lamu	44	59.09	34.09
	Mombasa	69	26.09	13.04
	Kitale	173	82.08	52.02
	Eldoret	124	10.48	4.84
	Trans Mara	212	35.85	22.64
	Zone total	706	46.88	27.33
IV	Nakuru	102	31.37	24.50
	Rumuruti	71	46.48	25.35
	Dol Dol	54	24.07	22.22
	Kajiado	458	36.03	25.33
	Zone total	685	35.47	24.96

Table 1. continued: Distribution of reactor rates to any of ten leptospiral antigens in bovine sera by ecological zones and sampling area.

Ecological Zone*	Area	Bovine sera tested	% sera reactive**	% sera positive***
V & VI	Garissa	48	0	0
	Marsabit	709	36.67	18.90
	Zone total	757	34.35	17.70
	Grand total	2,864	40.78	25.24

*See Appendix 3 for details of ecological zones.

**Reactive: all sera positive at a screening dilution of 1:50 to one or more antigens.

***Positive: all sera with microscopic agglutination titres of 1:200 or above to one or more antigens.

Table 2. Number of bovine sera with suspicious reactions to the ten leptospiral antigens in each sampling area.

Ecological zone	Area	Number of bovine sera tested	Antigens										Total number of suspicious reactions	Number of suspicious reactors**
			1*	2	3	4	5	6	7	8	9	10		
II	Nyahururu	52	0	0	0	0	3	0	5	0	6	0	14	7
	Ol Kalou	15	0	0	0	0	0	0	0	0	0	0	0	0
	Nyeri	72	0	0	0	0	10	1	6	0	17	0	34	13
	Kericho	389	0	3	0	0	58	3	44	7	64	0	179	73
	Kakamega	28	1	0	0	0	1	1	0	3	0	1	7	5
	Kiambu	160	0	0	0	3	11	5	2	0	8	0	29	11
	Zone total	716	1	3	0	3	83	10	57	10	95	1	263	109
III	Malindi	84	1	0	0	0	31	0	16	0	17	0	65	31
	Lamu	44	0	0	0	0	18	0	3	0	10	0	31	11
	Mombasa	69	0	0	0	2	10	0	1	0	6	0	19	9
	Kitale	173	0	0	0	0	12	5	33	12	41	2	105	52
	Eldoret	124	0	0	0	0	2	0	0	0	11	0	13	7
	Trans Mara	212	0	0	0	4	19	18	2	1	23	0	67	28
	Zone total	706	1	0	0	6	92	23	55	13	108	2	300	138
IV	Nakuru	102	0	0	0	0	3	2	2	1	6	0	14	7
	Rumuruti	71	1	1	0	1	15	8	3	3	11	0	43	15
	Dol Dol	54	0	0	0	0	3	5	1	0	1	0	10	1
	Kajiado	458	0	0	1	0	70	23	2	1	59	0	156	49
	Zone total	685	1	1	1	1	91	38	8	5	77	0	223	72

Table 2. continued; Number of bovine sera with suspicious reactions to the ten leptospiral antigens in each sampling area.

Ecological zone	Area	Number of bovine sera tested	Antigens										Total number of suspicious reactions	Number of suspicious reactors**
			1*	2	3	4	5	6	7	8	9	10		
V & VI	Garissa	48	0	0	0	0	0	0	0	0	0	0	0	0
	Marsabit	709	0	0	1	3	117	52	40	2	99	0	314	126
	Zone total	757	0	0	1	3	117	52	40	2	99	0	314	126
	Grand total	2,864	3	4	2	13	383	123	160	30	379	3	1,100	445

* 1. L. copenhageni
2. L. mankarso
3. L. sejroe
4. L. autumnalis
5. L. wolffi
6. L. georgia
7. L. grippotyphosa
8. L. pomona
9. L. hardjo
10. L. canicola

**Suspicious reactors refers only to all animals whose sera showed microscopic agglutination titres of 1:50 to 1:100 to any of the ten leptospiral antigens; while the total number of suspicious reactions includes all sera showing reactions at the screening dilution of 1:50 and also at a dilution of 1:100 with any antigen.

Table 3. Number of bovine sera with positive reactions to the ten leptospiral antigens in each sampling area.

Ecological zone	Area	Number of bovine sera tested	Antigens										Total number of positive reactions	Number of positive reactors**
			1*	2	3	4	5	6	7	8	9	10		
II	Nyahururu	52	0	0	0	0	4	0	1	0	1	0	6	6
	Ol Kalou	15	0	0	0	0	0	0	0	0	0	0	0	0
	Nyeri	72	0	0	0	0	16	1	17	0	21	0	55	35
	Kericho	389	0	0	0	0	33	0	110	2	58	1	204	165
	Kakamega	28	0	0	0	0	0	0	0	1	1	0	2	2
	Kiambu	160	0	0	0	1	1	0	10	1	6	0	19	17
	Zone total	716	0	0	0	1	54	1	138	4	87	1	286	225
III	Malindi	84	0	0	0	0	12	0	9	0	17	0	38	25
	Lamu	44	0	0	0	0	2	0	0	0	15	0	17	15
	Mombasa	69	0	0	1	0	3	4	0	1	6	0	15	19
	Kitale	173	0	0	0	0	10	0	68	1	24	0	103	90
	Eldoret	124	0	0	0	0	0	0	6	0	0	0	6	6
	Trans Mara	212	0	0	0	1	37	10	3	0	41	0	92	48
	Zone total	706	0	0	1	1	64	14	86	2	103	0	271	193
IV	Nakuru	102	0	0	0	0	16	0	6	0	15	0	37	25
	Rumuruti	71	0	0	0	0	9	2	4	4	10	0	29	18
	Dol Dol	54	0	0	0	0	3	0	8	0	5	0	16	12
	Kajiado	458	0	0	0	0	89	4	1	1	99	0	194	116
	Zone total	685	0	0	0	0	117	6	19	5	129	0	276	171

Table 3. continued: Number of bovine sera with positive reactions to the ten leptospiral antigens in each sampling area.

Ecological zone	Area	Number of bovine sera tested	Antigens										Total number of positive reactions	Number of positive reactors**
			1*	2	3	4	5	6	7	8	9	10		
V & VI	Garissa	48	0	0	0	0	0	0	0	0	0	0	0	0
	Marsabit	709	0	0	0	0	74	2	21	2	101	0	200	134
	Zone total	757	0	0	0	0	74	2	21	2	101	0	200	134
	Grand total	2,864	0	0	1	2	309	23	264	13	420	1	1,033	723

* 1. L. copenhageni
2. L. mankarso
3. L. sejroe
4. L. autumnalis
5. L. wolffi
6. L. georgia
7. L. grippotyphosa
8. L. pomona
9. L. hardjo
10. L. canicola

**Positive reactors refer to all animals whose sera showed positive reactions (microscopic agglutination titres of 1:200 or above) to any of the ten leptospiral antigens while the total number of positive reactions include positive reactions to each antigen.

Table 4. Distribution of microscopic agglutination titres to ten leptospiral antigens for 2,864 bovine sera from Kenya by serovars.

Serogroup	Serovar	Reciprocal titres								
		0	50	100	200	400	800	1600	3200	≥ 6400
Icterohaemorrhagiae	copenhageni	2,861	1	2						
Icterohaemorrhagiae	mankarso	2,860	2	2						
Hebdomadis	sejroe	2,861	1	1	1					
Autumnalis	autumnalis	2,849	9	4	2					
Hebdomadis	wolffi	2,172	177	206	141	92	54	20	2	
Hebdomadis	georgia	2,718	81	42	17	2	2	2		
Grippotyphosa	grippotyphosa	2,440	86	74	77	58	50	69	5	5
Pomona	pomona	2,821	20	10	8	3	2			
Hebdomadis	hardjo	2,065	148	231	206	127	53	27	6	1
Canicola	canicola	2,860	3		1					

Table 5. Distribution of reactive, suspicious or positive bovine sera to each of the ten leptospiral antigens.

Serogroup	Serovar	Sera tested	Sera reactive*	Sera suspicious	Sera positive	% Sera reactive	% Sera suspicious	% Sera positive
Icterohaemorrhagiae	copenhageni	2,864	3	3	0	0.10	0.10	0
Icterohaemorrhagiae	mankarso	2,864	4	4	0	0.14	0.14	0
Hebdomadis	sejroe	2,864	3	2	1	0.10	0.07	0.03
Autumnalis	autumnalis	2,864	15	13	2	0.52	0.45	0.07
Hebdomadis	wolffi	2,864	692	383	309	24.16	13.37	10.79
Hebdomadis	georgia	2,864	146	123	23	5.10	4.29	0.80
Grippotyphosa	grippotyphosa	2,864	424	160	264	14.80	5.59	9.22
Pomona	pomona	2,864	43	30	13	1.50	1.05	0.45
Hebdomadis	hardjo	2,864	799	379	420	27.90	13.23	14.66
Canicola	canicola	2,864	4	3	1	0.14	0.10	0.03

*Reactive: all sera positive at a screening dilution of 1:50

Suspicious: all sera with microscopic agglutination titres of 1:50 and 1:100

Positive: all sera with microscopic agglutination titres of 1:200 or above

Table 6. Distribution of positive reactors (per cent) to each of the ten leptospiral antigens by ecological zones.

Ecological zone	Antigens									
	1*	2	3	4	5	6	7	8	9	10
II	0	0	0	0.14	7.54	0.14	19.27	0.56	12.15	0.14
III	0	0	0.14	0.14	9.07	1.98	12.18	0.28	14.59	0
IV	0	0	0	0	17.08	0.88	2.77	0.73	18.83	0
V & VI	0	0	0	0	9.78	0.26	2.77	0.26	13.34	0

*1. *L. copenhageni*

2. *L. mankarso*

3. *L. sejroe*

4. *L. autumnalis*

5. *L. wolffi*

6. *L. georgia*

7. *L. grippotyphosa*

8. *L. pomona*

9. *L. hardjo*

10. *L. canicola*

Table 7. Number of bovine sera reactive* to selected leptospiral antigens or groups of antigens.

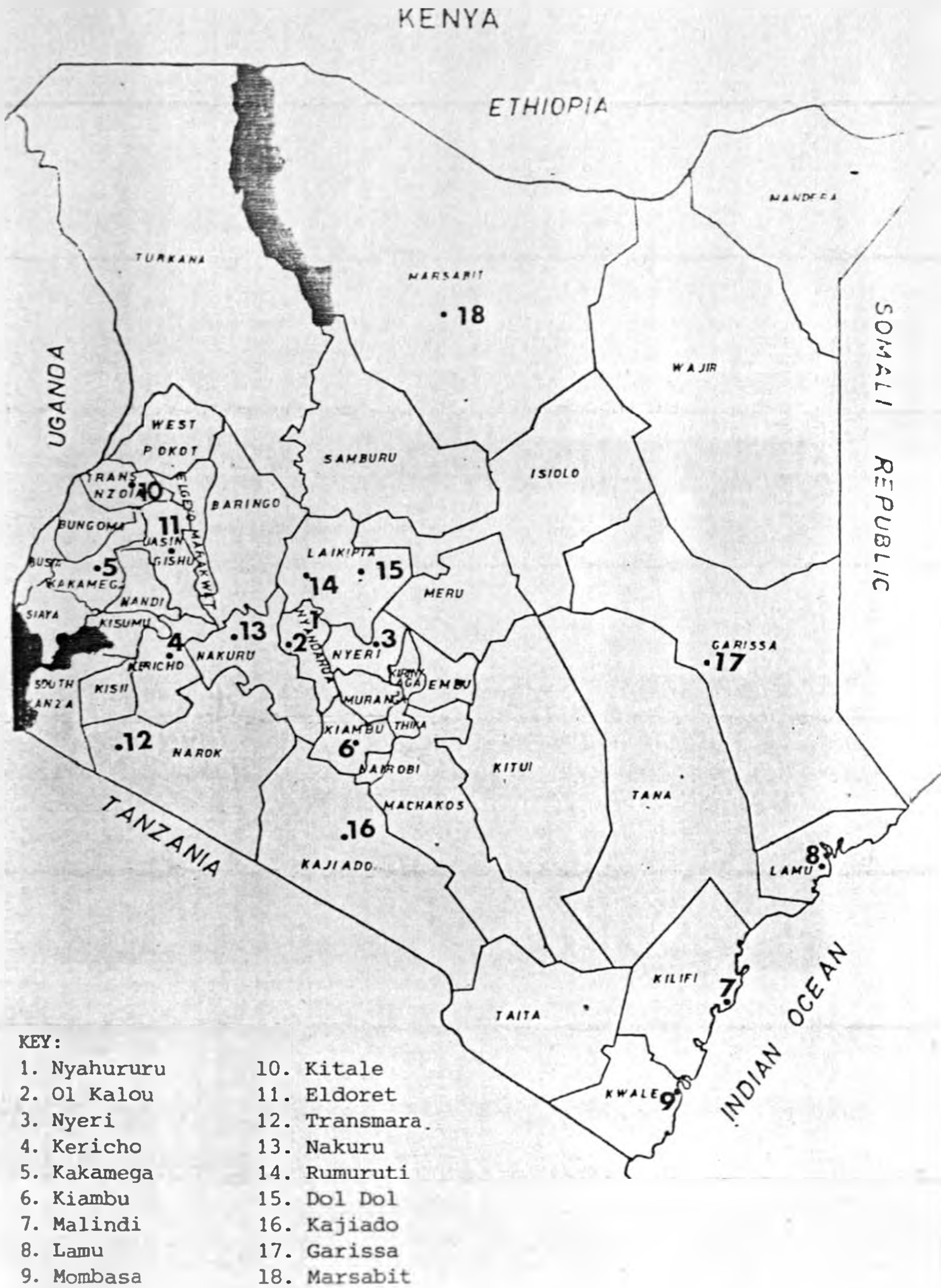
Ecological zone	Area	Bovine sera tested	Antigen or antigen group*					
			a	b	c	d	e	f
II	Nyahururu	52	7	7	6	7	0	0
	Ol Kalou	15	0	0	0	0	0	0
	Nyeri	72	38	24	24	23	15	9
	Kericho	389	123	92	154	72	53	30
	Kakamega	28	1	1	0	1	0	0
	Kiambu	160	14	13	11	13	2	2
	Zone total	716	183	137	195	116	70	41
III	Malindi	84	34	43	26	33	11	11
	Lamu	44	24	20	3	19	2	1
	Mombasa	69	12	13	2	11	0	0
	Kitale	173	63	23	102	23	24	5
	Eldoret	124	12	2	6	2	5	0
	Trans Mara	212	65	55	5	51	1	1
	Zone total	706	210	156	144	139	43	18
IV	Nakuru	102	21	19	8	19	1	0
	Rumuruti	71	21	25	7	21	2	2
	Dol Dol	54	6	6	9	6	2	2
	Kajiado	458	157	161	3	157	1	1
	Zone total	685	205	211	27	203	6	5

Table 7. continued: Number of bovine sera reactive* to selected leptospiral antigens or groups of antigens.

			Antigen or antigen group*					
Ecological zone	Area	Bovine sera tested	a	b	c	d	e	f
V & VI	Garissa	48	0	0	0	0	0	0
	Marsabit	709	202	191	75	180	32	28
	Zone total	757	202	191	75	180	32	28
	Grand total	2,864	800	695	441	638	151	92

*Reactive: all sera positive at a screening dilution of 1:50

**a. *L. hardjo*
b. *L. wolffi*
c. *L. grippotyphosa*
d. *L. hardjo* and *L. wolffi*
e. *L. hardjo* and *L. grippotyphosa*
f. *L. hardjo* , *L. wolffi* and *L. grippotyphosa*



KEY:

1. Nyahururu
2. Ol Kalou
3. Nyeri
4. Kericho
5. Kakamega
6. Kiambu
7. Malindi
8. Lamu
9. Mombasa
10. Kitale
11. Eldoret
12. Transmara
13. Nakuru
14. Rumuruti
15. Dol Dol
16. Kajiado
17. Garissa
18. Marsabit

Figure 1. Map of Kenya showing sampling areas.

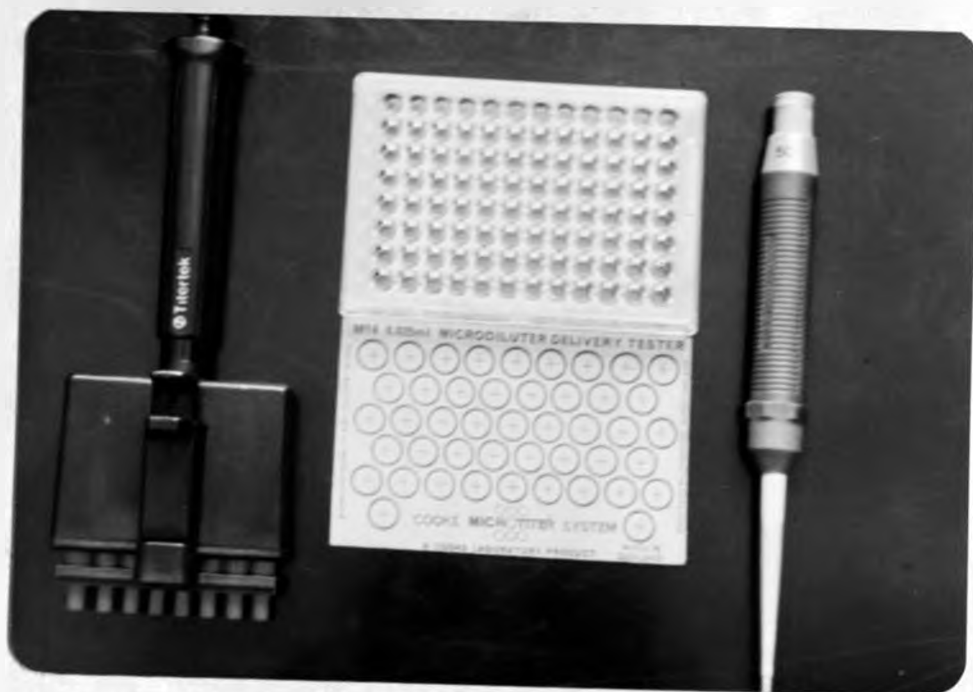


Figure 2. Microtechnique equipment including Titertek multichannel pipette, microtitre plate, microdiluter delivery tester and Eppendorf dropper pipette.

Figure 3. Darkfield zoom dissecting microscope used for examining wells for agglutination.

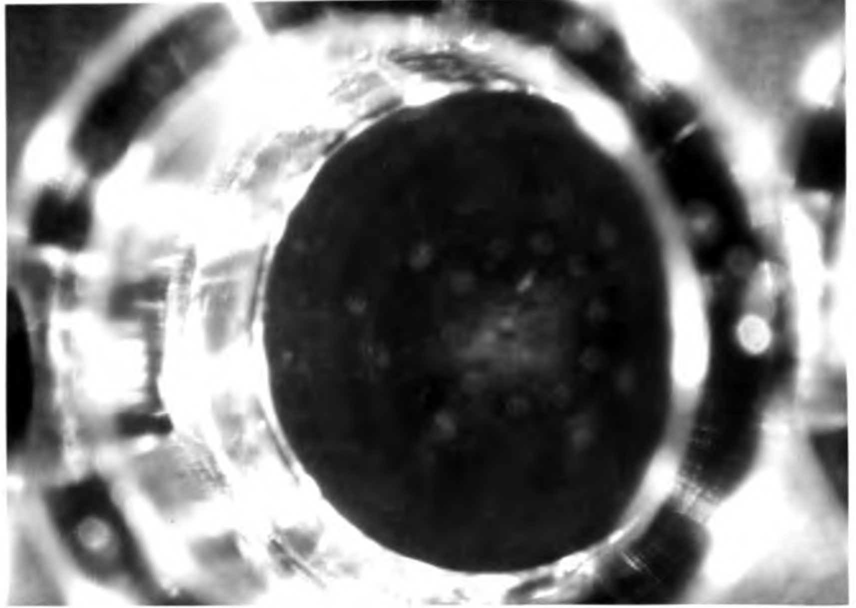
(a) (b) (c) (d)

Figure 4. Zones of dense leptospiral growth (Dinger's discs) observed in cultures in Fletcher's medium after seven days incubation at 29-30°C: (a) is uninoculated Fletcher's medium; (b), (c) and (d) are inoculated cultures.

(a) (b) (c)

Figure 5. Leptospiral growth in EMJH medium observed as marked turbidity after seven days incubation at 29-30°C: (a) and (c) are inoculated cultures; (b) is uninoculated EMJH medium.

(a)



(b)

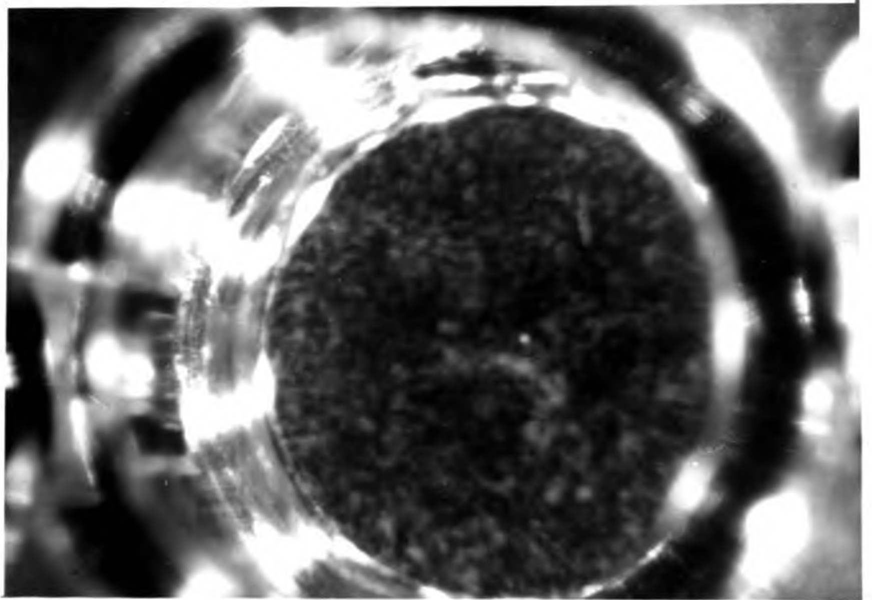
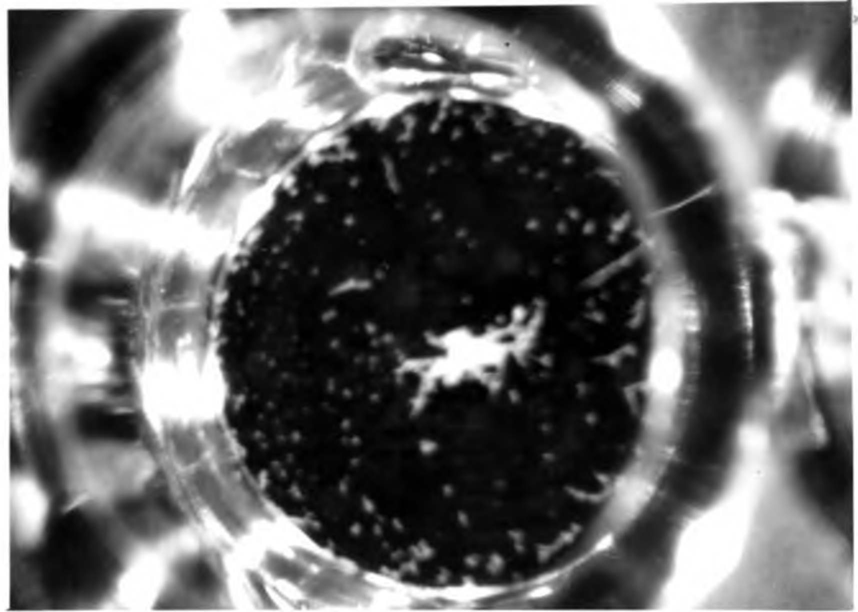


Figure 6. Leptospiral agglutination by microscopic agglutination test (modified microtechnique) showing (a) negative reaction; (b) 1+ reaction.

(c)



(d)

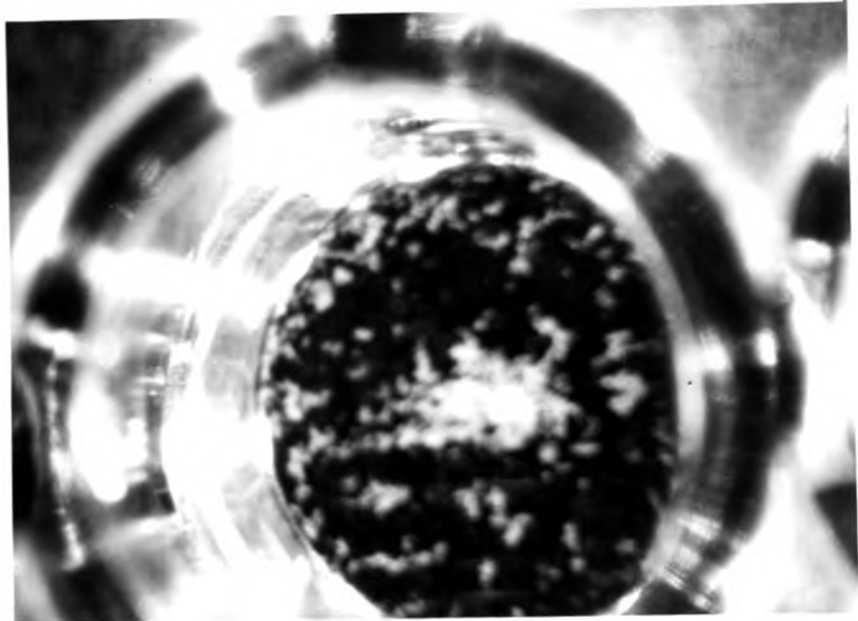


Figure 6. continued: Leptospiral agglutination by microscopic agglutination test (modified microtechnique) showing (c) 2+ reaction; (d) 3+ reaction.

(e)

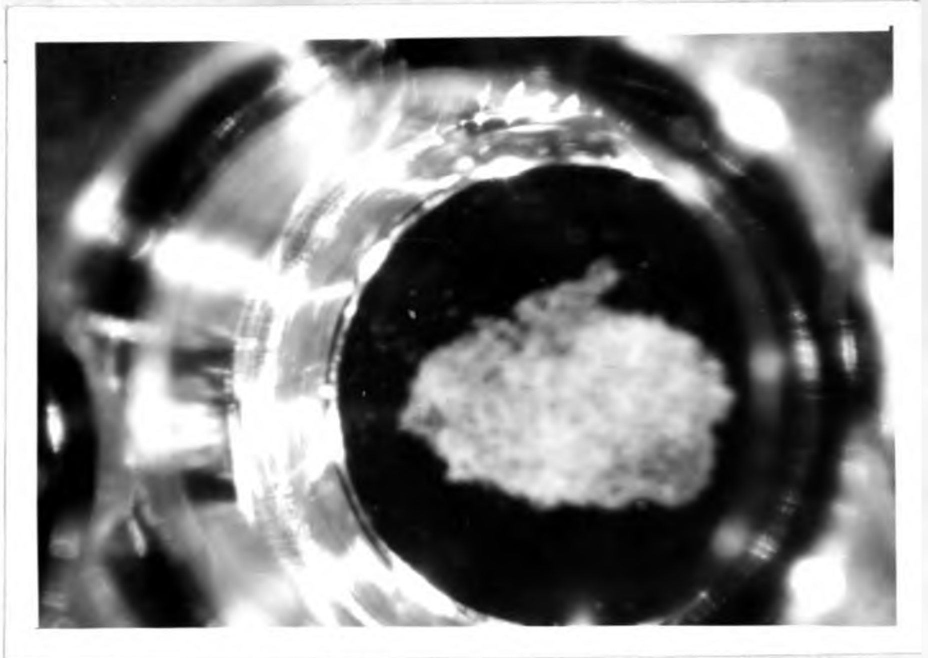


Figure 6. continued: Leptospiral agglutination by microscopic agglutination test (modified microtechnique) showing (e) 4+ reaction.

(a)

(b)

(c)

Figure 7. Electron micrographs (at X 7,000 magnification) of (a) L. grippotyphosa; (b) L. pomona; and (c) L. hardjo.

Appendix 1. Current list of leptospiral serogroups and pathogenic serovars.

Serogroup	Serovars
Andamana*	andamana
Australis	australis, bangkok, bratislava, fugis, hawain, jalna, lora, muenchen, nicaraguan, peruviana, pina
Autumnalis	alice, autumnalis, bangkinang, bulgarica, djasiman, erinacea - auriti, fort - bragg, gurungi, lanka, louisiana, mooris, mujumkumi, orleans, rachmati, sentot, srebrana, sumatrana, weersingha
Ballum	arboreae, ballum, castellonis
Bataviae	argentiniensis, balboa, bataviae, belorussiae, braziliensis, claytoni, djatzi, habaki, kobbe, paidjan
Bufonis	carlos
Canicola	bafani, benjamin, bindjei, broomi, canicola, jonsis, galtoni, kahendo, kamituga, kasherski, malaya, portland-vere, schueffneri, sumneri
Calledoni	calledoni, whitcombi
Cynopteri	butembo, canalzonea, cynopteri
Grippotyphosa	grippotyphosa, ratnapura, rattus, vanderhoedeni

Appendix 1. continued: Current list of leptospiral serogroups and pathogenic serovars.

Serogroup	Serovars
Hebdomadis	balcanica, beye, borincana, georgia, goiano, gorgas, guaicurus, hardjo, hebdomadis, haemolytica, jewaweera, jules, kabura, kambale, kremastos, maru, medanensis, mini, nona, perameles, polanica, recreo, ricardi, saxkoebing, sejroe, szwajizak, tabaquite, trinidad, wolffi, worsfoldi
Icterohaemorrhagiae	birkini, bog-vere, budapest, copenhageni, dakota, gem, hualien, icterohaemorrhagiae, mankarso, monymusk, mwogolo, naam, ndahambukuje, ndambari, sarmin, smithi, tonkini, weaveri
Javanica	anhoa, ceylonica, coxi, javanica, poi, sofia, sorex-jalna, waskurin
Kazachstanica I	kazachstanica I
Kazachstanica II	kazachstanica II
Panama	panama, wewak, cristobali
Pomona	dania, kennewicki, monjakova, mozdok, pomona, proechimys, tropica, tsarotsovo
Pyrogenes	abramis, alexi, biggis, camlo, guaratuba, humptoni, manilae, myocstaris, pyrogenes, robinsoni,

Appendix 1. continued: Current list of leptospiral serogroups and pathogenic serovars.

	varela, zanoni
Ranarum	ranarum
Semaranga*	patoc, semaranga
Shermani	shermani
Tarassovi	atchafalaya, atlantae, bakeri, bravo, chagres, darien, gatuni, guidae, kaup, kisuba, langati, moldaviar, sama, tarassovi, tunis, vietnami, vughia

*Serogroups Andamana and Semaranga belong to *L. biflexa*. The rest of the serogroups belong to *L. interrogans*.

Source: Adapted from Torten (1979).

Appendix 2. Principal leptospiral serovars isolated from cattle.

Serogroup	Serovars
Australis	australis, peruviana
Autumnalis	autumnalis
Ballum	ballum
Bataviae	argentiniensis, bataviae, paidjan
Canicola	canicola, galtoni
Grippotyphosa	grippotyphosa
Hebdomadis	balcanica, goiano, hardjo, hebdomadis, kremastos, saxkoebing, sejroe, szwajizak
Icterohaemorrhagiae	copenhageni, icterohaemorragiae
Kazachstanica I	kazachstanica I
Kazachstanica II	kazachstanica II
Pomona	kennewicki, mozdok, pomona
Pyrogenes	pyrogenes
Semaranga	patoc
Tarassovi	tarassovi

Source: Adapted from Amatredjo and Campbell (1975).

Appendix 3. Details of ecological zones.

ECOLOGICAL ZONES.

I	–	Afro-alpine climate. Climate governed by latitude not moisture. Moorland and grassland or barren land. Not included in the survey.
II	–	Equatorial climate. Humid to dry sub-humid. Moisture index not less than - 10. Forests and bushland.
III	–	Dry sub-humid to semi-arid. Moisture Index -10 to -30. Moist woodland, bushland or "savanna".
IV	–	Semi-arid. Moisture index -30 to-42. Dry forms of woodland and "savanna".
V	–	Arid. Moisture index -42 to -51. Woody vegetation.
VI	–	Very arid. Moisture index -51 to -57 Rangeland of dwarf shrubs and bushed grassland.

(Ecological Zone VII which is true desert, with a moisture index of -57 to-60, does not occur in Kenya).

Source: National Atlas of Kenya, Kenya Government, 1970.

Appendix 4. Leptospira reference cultures* used in the study.

Serogroup	Serovar	Strain
Icterohaemorrhagiae	copenhageni	Wijnberg
Icterohaemorrhagiae	mankarso	Mankarso
Hebdomadis	sejroe	M 84
Autumnalis	autumnalis	Akiyami A
Hebdomadis	wolffi	3705
Hebdomadis	georgia	LT117
Grippotyphosa	grippotyphosa	Moskva V
Pomona	pomona	Pomona
Hebdomadis	hardjo	Hardjoprajitno
Canicola	canicola	Hond Utrecht IV

*Corresponding antisera were also available.

Appendix 5. <u>Arrangement of microtitre plate for titration examination</u>.

Reciprocal serum dilutions

Ref. numbers		50	100	200	400	800	1600	50	100	200	400	800	1600	Ref. numbers
		1	2	3	4	5	6	7	8	9	10	11	12	
	A													
	B													
	C													
	D													
	E													
	F													
	G													
	H													

Antigen:

Source: Adapted from Lewis (1978).

Appendix 6. Distribution of microscopic agglutination titres to 10 leptospiral antigens by sampling areas.

a) Ecological zone II. Area: Nyahururu

		Reciprocal titres									
Antigens*	Sera tested	0	50	100	200	400	800	1600	3200	6400	12,800
1	52	52									
2	52	52									
3	52	52									
4	52	52									
5	52	45	1	2	4						
6	52	52									
7	52	46	4	1	1						
8	52	52									
9	52	45	2	4	1						
10	52	52									

b) Ecological zone II. Area: Ol Kalou

All 15 sera tested were negative to all ten leptospiral antigens

c) Ecological zone II. Area: Nyeri

		Reciprocal titres									
Antigens*	Sera tested	0	50	100	200	400	800	1600	3200	6400	12,800
1	72	72									
2	72	72									
3	72	72									
4	72	72									
5	72	46	2	8	6	7	3				

Appendix 6. continued.

c) continued:

Antigens*	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	6400	12,800
6	72	70	1		1						
7	72	49	2	4	2		4	11			
8	72	72									
9	72	34	4	13	10	6	4	1			
10	72	72									

d) Ecological zone II. Area: Kericho

Antigens*	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	6400	12,800
1	389	389									
2	389	386	2	1							
3	389	389									
4	389	389									
5	389	298	32	26	25	6	2				
6	389	386	3								
7	389	235	27	17	26	21	25	38			
8	389	380	5	2		1	1				
9	389	267	20	44	43	13	1	1			
10	389	388			1						

Appendix 6. continued.

e) Ecological zone II. Area: Kakamega

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	28	27	1								
2	28	28									
3	28	28									
4	28	28									
5	28	27	1								
6	28	27	1								
7	28	28									
8	28	24		3	1						
9	28	27			1						
10	28	27	1								

f) Ecological zone II. Area: Kiambu

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	160	160									
2	160	160									
3	160	160									
4	160	156	3		1						
5	160	148	8	3	1						
6	160	155	4	1							
7	160	148	1	1	4	5	1				
8	160	159			1						
9	160	146	3	5	4	1	1				
10	160	160									

Appendix 6. continued.

g) Ecological zone III. Area: Malindi

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	84	83		1							
2	84	84									
3	84	84									
4	84	84									
5	84	41	14	17	6	4	2				
6	84	84									
7	84	59	6	10	7		1	1			
8	84	84									
9	84	50	10	7	9	6	2				
10	84	84									

h) Ecological zone III. Area: Lamu

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	44	44									
2	44	44									
3	44	44									
4	44	44									
5	44	24	10	8	2						
6	44	44									
7	44	41	3								
8	44	44									
9	44	19	2	8	5	7	2	1			
10	44	44									

Appendix 6. continued.

i) Ecological zone III. Area: Mombasa

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	69	69									
2	69	69									
3	69	68			1						
4	69	67	1	1							
5	69	56	6	4	1	1	1				
6	69	65			4						
7	69	68		1							
8	69	68			1						
9	69	57	2	4	2	4					
10	69	69									

j) Ecological zone III. Area: Kitale

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	173	173									
2	173	173									
3	173	173									
4	173	173									
5	173	151	4	8	7	2	1				
6	173	168	4	1							
7	173	72	16	17	20	22	11	7	3	2	3
8	173	160	11	1	1						
9	173	108	18	23	15	8	1				
10	173	171	2								

Appendix 6. continued.

k) Ecological zone III. Area: Eldoret

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	124	124									
2	124	124									
3	124	124									
4	124	124									
5	124	122	2								
6	124	124									
7	124	118					1	5			
8	124	124									
9	124	113	9	2							
10	124	124									

l) Ecological zone III. Area: Trans Mara

Antigens*	Sera tested	Reciprocal titres									
		0	50	100	200	400	800	1600	3200	6400	12,800
1	212	212									
2	212	212									
3	212	212									
4	212	207	1	3	1						
5	212	156	5	14	14	9	8	4	2		
6	212	184	7	11	5	2	2	1			
7	212	207	2		1	1	1				
8	212	211		1							
9	212	148	5	18	16	10	5	5	5		
10	212	212									

Appendix 6. continued.

m) Ecological zone IV. Area: Nakuru

		Reciprocal titres									
Antigens*	Sera tested	0	50	100	200	400	800	1600	3200	6400	12,800
1	102	102									
2	102	102									
3	102	102									
4	102	102									
5	102	83		3	1	4	10	1			
6	102	100	1	1							
7	102	94		2	4	1	1				
8	102	101	1								
9	102	81		6	2	10	2	1			
10	102	102									

n) Ecological zone IV. Area: Rumuruti

		Reciprocal titres									
Antigens*	Sera tested	0	50	100	200	400	800	1600	3200	6400	12,800
1	71	70		1							
2	71	70		1							
3	71	71									
4	71	70	1								
5	71	47	13	2	2	4	2	1			
6	71	61	6	2	1			1			
7	71	64	2	1			1	1	2		
8	71	64	1	2	2	1	1				
9	71	50	1	10	3	1	3	1	1	1	
10	71	71									

Appendix 6. continued.

o) Ecological zone IV. Area: Dol Dol

Antigens*	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	6400	12,800
1	54	54									
2	,54	54									
3	54	54									
4	54	54									
5	54	48	2	1		3					
6	54	49	5								
7	54	45		1		1	2	5			
8	54	54									
9	54	48		1	3		2				
10	54	54									

p) Ecological zone IV. Area: Kajiado

Antigens*	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	6400	12,800
1	458	458									
2	458	458									
3	458	457		1							
4	458	458									
5	458	299	25	45	36	31	10	12			
6	458	431	10	13	4						
7	458	455		2	1						
8	458	456	1			1					
9	458	300	9	50	44	32	16	7			
10	458	458									

Appendix 6. continued.

q) Ecological zone V & VI. Area: Garissa

All 48 sera tested were negative to all ten leptospiral antigens.

r) Ecological zone V & VI. Area: Marsabit

		Reciprocal titres									
Antigens*	Sera tested	0	50	100	200	400	800	1600	3200	6400	12,800
1	709	709									
2	709	709									
3	709	708	1								
4	709	706	3								
5	709	518	52	65	36	21	15	2			
6	709	655	39	13	2						
7	709	648	23	17	11	7	2	1			
8	709	705	1	1	2						
9	709	509	63	36	48	29	14	10			
10	709	709									

*1. *L. copenhageni*

2. *L. mankarso*

3. *L. sejroe*

4. *L. autumnalis*

5. *L. wolffi*

6. *L. georgia*

7. *L. grippotyphosa*

8. *L. pomona*

9. *L. hardjo*

10. *L. canicola*

Appendix 7a. Distribution of microscopic agglutination titres to *L. copenhageni* in 2,864 sera from cattle in Kenya

			Reciprocal titres								
Ecological zone	Area	Sera tested	0	50	100	200	400	800	1600	3200	$\geq$6400
II	Nyahururu	52	52								
	Ol Kalou	15	15								
	Nyeri	72	72								
	Kericho	389	389								
	Kakamega	28	27	1							
	Kiambu	160	160								
	Zone total	716	715	1							
III	Malindi	84	83		1						
	Lamu	44	44								
	Mombasa	69	69								
	Kitale	173	173								
	Eldoret	124	124								
	Trans Mara	212	212								
	Zone total	706	705		1						
IV	Nakuru	102	102								
	Rumuruti	71	70		1						
	Dol Dol	54	54								
	Kajiado	458	458								
	Zone total	685	684		1						
V & VI	Garissa	48	48								
	Marsabit	709	709								
	Zone total	757	757								
	Grand total	2,864	2,861	1	2						

Appendix 7b. Distribution of microscopic agglutination titres to *L. mankarso* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	$\geq$6400
II	Nyahururu	52	52								
	Ol Kalou	15	15								
	Nyeri	72	72								
	Kericho	389	386	2	1						
	Kakamega	28	28								
	Kiambu	160	160								
	Zone total	716	713	2	1						
III	Malindi	84	84								
	Lamu	44	44								
	Mombasa	69	69								
	Kitale	173	173								
	Eldoret	124	124								
	Trans Mara	212	212								
	Zone total	706	706								
IV	Nakuru	102	102								
	Rumuruti	71	70		1						
	Dol Dol	54	54								
	Kajiado	458	458								
	Zone total	685	684		1						
V & VI	Garissa	48	48								
	Marsabit	709	709								
	Zone total	757	757								
	Grand total	2,864	2,860	2	2						

Appendix 7c. Distribution of microscopic agglutination titres to *L. sejroe* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
	Nyahururu	52	52								
	Ol Kalou	15	15								
II	Nyeri	72	72								
	Kericho	389	389								
	Kakamega	28	28								
	Kiambu	160	160								
	Zone total	716	716								
	Malindi	84	84								
	Lamu	44	44								
III	Mombasa	69	68			1					
	Kitale	173	173								
	Eldoret	124	124								
	Trans Mara	212	212								
	Zone total	706	705			1					
	Nakuru	102	102								
	Rumuruti	71	71								
IV	Dol Dol	54	54								
	Kajiado	458	457		1						
	Zone total	685	684		1						
	Garissa	48	48								
V & VI	Marsabit	709	708	1							
	Zone total	757	756	1							
	Grand total	2,864	2,861	1	1	1					

Appendix 7d. Distribution of microscopic agglutination titres to L. *autumnalis* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	≥6400
II	Nyahururu	52	52								
	Ol Kalou	15	15								
	Nyeri	72	72								
	Kericho	389	389								
	Kakamega	28	28								
	Kiambu	160	156	3		1					
	Zone total	716	712	3		1					
III	Malindi	84	84								
	Lamu	44	44								
	Mombasa	69	67	1	1						
	Kitale	173	173								
	Eldoret	124	124								
	Trans Mara	212	207	1	3	1					
	Zone total	706	699	2	4	1					
IV	Nakuru	102	102								
	Rumuruti	71	70	1							
	Dol Dol	54	54								
	Kajiado	458	458								
	Zone total	685	684	1							
V & VI	Garissa	48	48								
	Marsabit	709	706	3							
	Zone total	757	754	3							
	Grand total	2,864	2,849	9	4	2					

Appendix 7e. Distribution of microscopic agglutination titres to *L. wolffi* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres: 0	50	100	200	400	800	1600	3200	$\geq$6400
II	Nyahururu	52	45	1	2	4					
	Ol Kalou	15	15								
	Nyeri	72	46	2	8	6	7	3			
	Kericho	389	298	32	26	25	6	2			
	Kakamega	28	27	1							
	Kiambu	160	148	8	3	1					
	Zone total	716	579	44	39	36	13	5			
III	Malindi	84	41	14	17	6	4	2			
	Lamu	44	24	10	8	2					
	Mombasa	69	56	6	4	1	1	1			
	Kitale	173	151	4	8	7	2	1			
	Eldoret	124	122	2							
	Trans Mara	212	156	5	14	14	9	8	4	2	
	Zone total	706	550	41	51	30	16	12	4	2	
IV	Nakuru	102	83		3	1	4	10	1		
	Rumuruti	71	47	13	2	2	4	2	1		
	Dol Dol	54	48	2	1		3				
	Kajiado	458	299	25	45	36	31	10	12		
	Zone total	685	477	40	51	39	42	22	14		
V & VI	Garissa	48	48								
	Marsabit	709	518	52	65	36	21	15	2		
	Zone total	757	566	52	65	36	21	15	2		
	Grand total	2,864	2,172	177	206	141	92	54	20	2	

Appendix 7f. Distribution of microscopic agglutination titres to *L. georgia* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
	Nyahururu	52	52								
	Ol Kalou	15	15								
II	Nyeri	72	70	1		1					
	Kericho	389	386	3							
	Kakamega	28	27	1							
	Kiambu	160	155	4	1						
	Zone total	716	705	9	1	1					
	Malindi	84	84								
	Lamu	44	44								
III	Mombasa	69	65			4					
	Kitale	173	168	4	1						
	Eldoret	124	124								
	Trans Mara	212	184	7	11	5	2	2	1		
	Zone total	706	669	11	12	9	2	2	1		
	Nakuru	102	100	1	1						
	Rumuruti	71	61	6	2	1			1		
IV	Dol Dol	54	49	5							
	Kajiado	458	431	10	13	4					
	Zone total	685	641	22	16	5			1		
	Garissa	48	48								
V & VI	Marsabit	709	655	39	13	2					
	Zone total	757	703	39	13	2					
	Grand total	2,864	2,718	81	42	17	2	2	2		

Appendix 7g. Distribution of microscopic agglutination titres to *L. grippotyphosa* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	0	50	100	200	400	800	1600	3200	≥6400
	Nyahururu	52	46	4	1	1					
	Ol Kalou	15	15								
II	Nyeri	72	49	2	4	2		4	11		
	Kericho	389	235	27	17	26	21	25	38		
	Kakamega	28	28								
	Kiambu	160	148	1	1	4	5	1			
	Zone total	716	521	34	23	33	26	30	49		
	Malindi	84	59	6	10	7		1	1		
	Lamu	44	41	3							
III	Mombasa	69	68		1						
	Kitale	173	72	16	17	20	22	11	7	3	5
	Eldoret	124	118					1	5		
	Trans Mara	212	207	2		1	1	1			
	Zone total	706	565	27	28	28	23	14	13	3	5
	Nakuru	102	94		2	4	1	1			
	Rumuruti	71	64	2	1			1	1	2	
IV	Dol Dol	54	45		1		1	2	5		
	Kajiado	458	455		2	1					
	Zone total	685	658	2	6	5	2	4	6	2	
	Garissa	48	48								
V & VI	Marsabit	709	648	23	17	11	7	2	1		
	Zone total	757	696	23	17	11	7	2	1		
	Grand total	2,864	2,440	86	74	77	58	50	69	5	5

Appendix 7h. Distribution of microscopic agglutination titres to *L. pomona* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
	Nyahururu	52	52								
	Ol Kalou	15	15								
II	Nyeri	72	72								
	Kericho	389	380	5	2		1	1			
	Kakamega	28	24		3	1					
	Kiambu	160	159			1					
	Zone total	716	702	5	5	2	1	1			
	Malindi	84	84								
	Lamu	44	44								
III	Mombasa	69	68			1					
	Kitale	173	160	11	1	1					
	Eldoret	124	124								
	Trans Mara	212	211		1						
	Zone total	706	691	11	2	2					
	Nakuru	102	101	1							
	Rumuruti	71	64	1	2	2	1	1			
IV	Dol Dol	54	54								
	Kajiado	458	456	1			1				
	Zone total	685	675	3	2	2	2	1			
	Garissa	48	48								
V & VI	Marsabit	709	705	1	1	2					
	Zone total	757	753	1	1	2					
	Grand total	2,864	2,821	20	10	8	3	2			

Appendix 71. Distribution of microscopic agglutination titres to *L. hardjo* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
II	Nyahururu	52	45	2	4	1					
	Ol Kalou	15	15								
	Nyeri	72	34	4	13	10	6	4	1		
	Kericho	389	267	20	44	43	13	1	1		
	Kakamega	28	27			1					
	Kiambu	160	146	3	5	4	1	1			
	Zone total	716	534	29	66	59	20	6	2		
III	Malindi	84	50	10	7	9	6	2			
	Lamu	44	19	2	8	5	7	2	1		
	Mombasa	69	57	2	4	2	4				
	Kitale	173	108	18	23	15	8	1			
	Eldoret	124	113	9	2						
	Trans Mara	212	148	5	18	16	10	5	5	5	
	Zone total	706	495	46	62	47	35	10	6	5	
IV	Nakuru	102	81		6	2	10	2	1		
	Rumuruti	71	50	1	10	3	1	3	1	1	1
	Dol Dol	54	48		1	3		2			
	Kajiado	458	300	9	50	44	32	16	7		
	Zone total	685	479	10	67	52	43	23	9	1	1
V & VI	Garissa	48	48								
	Marsabit	709	509	63	36	48	29	14	10		
	Zone total	757	557	63	36	48	29	14	10		
	Grand total	2,864	2,065	148	231	206	127	53	27	6	1

Appendix 7j. Distribution of microscopic agglutination titres to *L. canicola* in 2,864 sera from cattle in Kenya.

Ecological zone	Area	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
II	Nyahururu	52	52								
	Ol Kalou	15	15								
	Nyeri	72	72								
	Kericho	389	388			1					
	Kakamega	28	27	1							
	Kiambu	160	160								
	Zone total	716	714	1		1					
III	Malindi	84	84								
	Lamu	44	44								
	Mombasa	69	69								
	Kitale	173	171	2							
	Eldoret	124	124								
	Trans Mara	212	212								
	Zone total	706	704	2							
VI	Nakuru	102	102								
	Rumuruti	71	71								
	Dol Dol	54	54								
	Kajiado	458	458								
	Zone total	685	685								
V & VI	Garissa	48	48								
	Marsabit	709	709								
	Zone total	757	757								
	Grand total	2,864	2,860	3		1					

Appendix 8: **Distribution of microscopic agglutination titres to the 10 leptospiral antigens in 2,864 bovine sera by ecological zones in Kenya.**

Antigen	Ecological zone	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	≥ 6400
a) L. copenhageni	II	716	715	1							
	III	706	705		1						
	IV	685	684		1						
	V & VI	757	757								
		2,864	2,861	1	2						
b) L. mankarso	II	716	713	2	1						
	III	706	706								
	IV	685	684		1						
	V & VI	757	757								
		2,864	2,860	2	2						
c) L. sejroe	II	716	716								
	III	706	705			1					
	IV	685	684		1						
	V & VI	757	756	1							
		2,864	2,861	1	1	1					
d) L. autumnalis	II	716	712	3		1					
	III	706	699	2	4	1					
	IV	685	684	1							
	V & VI	757	754	3							
		2,864	2,849	9	4	2					

Appendix 8. continued: Distribution of microscopic agglutination titres to the 10 leptospiral antigens in 2,864 bovine sera by ecological zones in Kenya.

Antigen	Ecological zone	Sera tested	Reciprocal titres 0	50	100	200	400	800	1600	3200	≥6400
e) *L. wolffi*	II	716	579	44	39	36	13	5			
	III	706	550	41	51	30	16	12	4	2	
	IV	685	477	40	51	39	42	22	14		
	V & VI	757	566	52	65	36	21	15	2		
		2,864	2,172	177	206	141	92	54	20	2	
f) *L. georgia*	II	716	705	9	1	1					
	III	706	669	11	12	9	2	2	1		
	IV	685	641	22	16	5			1		
	V & VI	757	703	39	13	2					
		2,864	2,718	81	42	17	2	2	2		
g) *L. grippotyphosa*	II	716	521	34	23	33	26	30	49		
	III	706	565	27	28	28	23	14	13	3	5
	IV	685	658	2	6	5	2	4	6	2	
	V & VI	757	696	23	17	11	7	2	1		
		2,864	2,440	86	74	77	58	50	69	5	5
h) *L. pomona*	II	716	702	5	5	2	1	1			
	III	706	691	11	2	2					
	IV	685	675	3	2	2	2	1			
	V & VI	757	753	1	1	2					
		2,864	2,821	20	10	8	3	2			

Appendix 8. continued: Distribution of microscopic agglutination titres to the 10 leptospiral antigens in 2,864 bovine sera by ecological zones in Kenya.

Antigen	Ecological zone	Sera tested	Reciprocal titres								
			0	50	100	200	400	800	1600	3200	≥6400
i) *L. hardjo*	II	716	534	29	66	59	20	6	2		
	III	706	495	46	62	47	35	10	6	5	
	IV	685	479	10	67	52	43	23	9	1	1
	V & VI	757	557	63	36	48	29	14	10		
		2,864	2,065	148	231	206	127	53	27	6	1
j) *L. canicola*	II	716	714	1		1					
	III	706	704	2							
	IV	685	685								
	V & VI	757	757								
		2,864	2,860	3		1					

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