A CLINICAL AND SEROLOGICAL INVESTIGATION INTO LEPTOSPIRAL INFECTION IN DOGS AND CATS

by

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DECLARATION

I declare that the work presented in this thesis has been carried out by me. The clinical aspects were carried out under supervision by Dr. A.S. Nash, Department of Veterinary Medicine, University of Glasgow. The serological aspects were carried out by me at the Veterinary Research Laboratories, Stormont, Belfast, by kind permission of Professor J.B. McFerran.

C.A. AGUNLOYE

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SUMMARY

CATS

This work was undertaken to determine the prevalence of leptospiral infection in cats in the Glasgow area and to assess the relationship of such serological evidence with actual disease. A total of 87 cats were sampled. Of these, 15 were pure bred and 72 were domestic cats, 46 were male and 41 female. The age range was 8 weeks to 19 years.

Eight (9.2%) of the 87 cats sampled were serologically positive to three leptospiral serovars. Five (62.5%) of these cats were seropositive to *Leptospira hardjo*. Two (25%) were seropositive to *L.autumnalis*. One cat was positive to *L.icterohaemorrhagiae*. This is the first serological survey of leptospiral infection in cats in the Glasgow area and the first report of *L.autumnalis* infection in cats in the U.K. A paired serum sample demonstrated a recent infection in one of the seropositive cats. The major clinical sign shown by this cat was ascites. Active leptospiral infection could not be confirmed in the other cases. Four of the 5 cats seropositive to *L.hardjo* were from rural areas.

This confirmed that a small proportion of the cat population does become infected with leptospirae and that this may occasionally result in clinical disease. In view of the lack of a definitive disease description of leptospiral infection in cats, there is need for further studies into this disease. In addition, there is a need to investigate the factors which may predispose to leptospiral disease in cats.

DOGS

One hundred and fifty dogs from the Glasgow area were examined for antibodies to 11 leptospiral serovars and also for clinical

signs of infection. This was to determine the prevalence and types of leptospiral infection in both vaccinated and unvaccinated dogs. Seventy-two of the dogs were male and 78 were female. One hundred and thirty eight were pure bred and 12 were crossbred. Ages ranged from 8 weeks to 14 years.

Twenty nine (19.8%) of these dogs had positive leptospiral titres to 5 leptospiral serovars. Eighteen (48.6%) of the seropositive dogs reacted to *L.icterohaemorrhagiae*, 9 (24.3%) to *L.bratislava*, 8 (21.6%) to *L.canicola*. One was positive to *L.hardjo*. Sixteen of the seropositive dogs were male and 13 were female with an age range from 24 weeks to 12 years.

Seventy two (48.0%) of the sampled population had been vaccinated and in most cases revaccinated within the previous 12 months (Group A). Of these, only 15 (20.8%) had antibody titres to leptospiral antigens. Eight (47.1%) showed a positive reaction to *L.icterohaemorrhagiae*, 5 (29.4%) were seropositive to *L.canicola*, 2 had antibodies to *L.bratislava* and one was positive to *L.hardjo*. Fifty six of the 150 dogs (37.4%) had been initially vaccinated against leptospirosis but not revaccinated within the previous 12 months (Group B). Of these, 7 (12.5%) had antibodies to leptospiral antigens. They were variously positive to *L.icterohaemorrhagiae*, *L.canicola* and *L.bratislava*. However, there is no significant difference ($P>0.5$) in the number of seropositive dogs in groups A and B.

Six adult dogs in the sampled population had been recently vaccinated (Group C). Four had positive leptospiral antibodies to *L.icterohaemorrhagiae* and *L.bratislava* and were from the same kennel. Sixteen (10.7%) of the 150 dogs had never been vaccinated against leptospirosis or had an unknown vaccination history (Group D). Of

these, 3 (18.8%) had positive leptospiral titres. Two were positive to *L.icterohaemorrhagiae* and one to *L.bratislava*.

Of the seropositive dogs, 2 had evidence of active infection with leptospiral organisms, one from Group B and the other from Group D. The former, an imported dog, (vaccinated, but not known to have been revaccinated within the previous 12 months,) had a high antibody titre to *L.icterohaemorrhagiae* and classical signs of leptospirosis.

This study confirmed that not all fully vaccinated dogs have antibody to leptospiral antigens, as detected by the microscopic agglutination test (MAT), to bacterin serovars. Also, vaccinated animals are not free from infection with bacterin and non-bacterin serovars and illness is possible in vaccinated dogs, although the risk of infection is lower. Unvaccinated and inadequately vaccinated dogs are at greater risk of developing active infection.

In this work a large proportion of seropositive dogs had antibodies to the *Australis* group, a non-vaccine serovar. There is need for further work to associate such infections with disease to provide a basis for improving future vaccination programmes.

CHAPTER I

FELINE LEPTOSPIROSIS

1.1. INTRODUCTION

There is serological evidence of the occurrence of feline leptospiral infection in various parts of the world and many pathogenic leptospiral serovars have been associated with it (Table 1). In a few instances the infecting leptospiral organisms have been successfully isolated (Table 2). However, there have been very few reports of clinical disease in cats due to leptospiral infections despite the fact that reported infection rates ranged from 1.4 per cent (Jones, 1964) to 30 per cent (Esseveld *et al.*, 1940). In the few reported clinical cases (Rees, 1964; Carlos *et al.*, 1971; Bryson and Ellis, 1976), there have been no consistent clinicopathological signs associated with the disease. Moreover, experimental studies in cats have produced little or no clinical evidence of infection (Fessler and Morter, 1964; Jones, 1964; Shophet and Marshall, 1980; Larsson *et al.*, 1985).

In the U.K., Hemsley (1956) first reported interstitial nephritis in 3 cats seropositive to *Leptospira canicola*. The first serological survey was conducted in the Bristol area by Lucke and Crowther (1965). There have been no reports of any serological survey of feline leptospiral infection in the Glasgow area despite the presence of leptospiral infections in other susceptible hosts (Michna, 1970).

The present study was therefore designed to investigate the following:

(1) The prevalence and type of leptospiral infections in cats in the Glasgow area

(2) The relationship (if any) between leptospiral infection and disease in the cat

(3) The epizootiological factors that may predispose to infection.

TABLE 1: Reported serological surveys of feline leptospirosis.

	Reference	Country	No.of cats examined	% Infection rate	Implicated serovars	Other information
1.	Esseveld *et al.* (1940)	Indonesia	500	30	*L.bataviae; L.javanica L.icterohaemorrhagiae*	5.6% of the cats were urinary shedders
2.	Mochmann (1955)	East Germany	15	13.3	not reported	-
3.	Fennestad (1956)	Denmark	86	9.3	*L.bataviae; L.ictero-haemorrhagiae; L.poi; L.saxkoebing*	-
4.	Murphy *et al.* (1958)	U.S.A.	350	4.9	*L.autumnalis; L.pomona L.djasiman; L.sentot*	Survey in Pennsylvania
5.	Jones (1964)	U.S.A.	139	1.4	*L.canicola; L.ictero-haemorrhagiae*	-
6.	Lucke & Crowther (1965)	U.K.	118	6.8	*L.canicola; L.ictero-haemorrhagiae; L.minimini L.javanica; L.pomona; L.bratislava*	Bristol area
7.	Carlos *et al.* 1971	Philippines	8	12.5	*L.grippotyphosa*	All 8 cats febrile and jaundiced.
8.	Watson & Wannan (1973)	Australia	100	6	*L.hardjo; L.wolffi; L.grippotyphosa*	Survey in Sydney using rapid slide agglutation test
9.	Modric (1978)	Yugoslavia	112	21.4	*L.grippotyphosa; L.sejroe; L.australis; L.pomona; L.canicola L.icterohaemorrhagiae; L.tarassavi L.saxkoebing; L.ballum*	Mainly in Zagreb
10.	Everard *et al.* (1979)	Trinidad	84	12.5	*L.canicola; L.ictero-haemorrhagiae; L.hebdomadis*	-

TABLE 2. Leptospires isolated from naturally-infected cats.

	Reference	Country	Serotype	Source Material
1.	Mertens (1938)	Indonesia	*L.bataviae*	urine
2.	Esseveld *et al.* (1940)	Indonesia	*L.javanica*	urine
3.	Ferris & Andrews (1965)	U.S.A.	*L.pomona*	urine
4.	Carlos *et al.* (1971)	Philippines	*L.grippotyphosa*	urine
5.	Bryson & Ellis (1976)	U.K.	*L.bratislava*	liver thoracic fluid aqueous tumour
6.	Modric (1978)	Yugoslavia	*Australis* serogroup	kidney
7.	Trifunovic & Nesic (1986)	Yugoslavia	*L.pomona*	kidney

1.2. REVIEW OF LITERATURE

INTRODUCTION

Leptospiral infections are uncommon in cats and very little is known about their clinical significance. Occasional infections have however been reported to be fatal (Rees, 1964; Mason *et al.*, 1972; Bryson and Ellis, 1976). From these 4 cases, it would appear to be a severe condition affecting many organs.

AETIOLOGY AND THE MORPHOLOGY

Leptospiral organisms are Gram-negative bacteria commonly referred to as spirochaetes. Structurally, they are slender, motile, helically coiled organisms, measuring 6-20µm in length and about 0.1µm in diameter. However, some isolates of the genotype *L.interrogans* have had higher measurements and Hovind-Hougen (1986) suggested that more work is required to provide accurate details on the morphology and classification of leptospires. When viewed either by dark ground microscopy or conventional light microscopy at a magnification of x250 or above, the organism has a wavy outline and is hooked at one or both ends (Faine and Stallman, 1982).

The agent associated with feline leptospirosis is *L.interrogans* and various serovars have been identified by serological means. However only a few have been reported isolated by cultural means. These are presented in Table 2.

HISTORICAL BACKGROUND AND DISTRIBUTION OF LEPTOSPIRAL INFECTIONS

Leptospiral infection in cats was first described by Mertens (1938) following the isolation of leptospiral organisms from a cat in Indonesia. Since then serological surveys have been conducted in several countries (Table 1). In some surveys (Lucke and Crowther, 1965; Carlos *et al.*, 1971), it has been difficult to associate serological evidence of infection with actual disease. Probably as a result of these apparently low infection rates, very few other sero-epidemiological surveys have been conducted.

SOURCES AND ROUTES OF INFECTION

Whereas the sources of and routes of leptospiral infection in other domestic species are well documented, water-borne infection being the most common route (WHO 1982), this information is lacking for the cat. In an experiment to demonstrate a predator chain transmission of *L.ballum*, cats became leptospiruric after consuming infected mice but no overt clinical signs were observed (Shophet and Marshall, 1980). It is not clear whether the leptospires from these cats were infective for other cats.

The probability of cats spreading infection via urine is low because of their fastidious nature. Spraying by male cats, however, remains a possibility.

Mason *et al.* (1972) described a suspected case of *L.pomona* in a cat kept on a dairy farm and suspected that cattle were the source of infection. The cat was "unwell" and jaundiced. Michna (1970) also described jaundice and fever in a rural cat which had a high agglutinin titre to *L.sejroe*. More recently Trifunovic and Nesic (1986) described an

outbreak of *L.pomona* infection in dairy cattle in which 14 out of 47 farm cats were leptospiruric. Infection was thought to have been acquired from cattle.

CLINICAL SIGNS IN LEPTOSPIRAL INFECTIONS

Natural infections:

There are so few reports of clinical leptospirosis in cats that it is impossible to recognise a specific disease picture. Moreover, many of these cases were not recognised as such in life (Rees, 1964; Mason *et al.*, 1972; Bryson and Ellis, 1976).

Dullness, anorexia and pyrexia have been reported in suspected cases (Hemsley, 1956; Rees, 1964). Jaundice was noted in 2 suspected cases reported by Mason *et al.* (1972) and similarly in the single cases reported by Rees (1964) and Michna (1970). Carlos *et al.* (1971) isolated *L.grippotyphosa* from the urine of one out of 8 cats with jaundice and fever.

Hemsley (1956) observed vomiting in 2 out of 3 cats that were seropositive to *L.canicola*. One of these cats was also diarrhoeic. Mason *et al.* (1972) also reported vomiting and diarrhoea in one case. Ascites and hydrothorax were observed in one cat (Bryson and Ellis, 1976).

Central nervous system signs have also been reported in a few naturally-infected cats. Hemsley (1956) observed fits in one case and lower jaw chorea in another which had a high titre to *L.canicola*. One dead cat from which *L.bratislava* was isolated had CNS lesions but it is not clear if the cat showed signs of CNS disturbance in life (Bryson and Ellis, 1976).

Experimental infections:

There have been very few reports of attempts to induce leptospiral infection in the cat. Fessler and Morter (1964) used live cultures of *L.pomona* and *L.ballum*. Jones (1964) used both killed and live *L.icterohaemorrhagiae* and *L.canicola* cultures but failed to demonstrate any antibody response in these cats. Modric (1978) used live *L.australis*, *L.pomona* and *L.icterohaemorrhagiae* and although the cats showed an antibody response, there were only mild clinical signs and pathological changes were confined to the liver, lungs and kidney. Shophet and Marshall (1980) fed mice infected with *L.ballum* to cats and demonstrated a seroconversion. *L.icterohaemorrhagiae* and *L.canicola* were inoculated subcutaneously into cats by Larsson *et al.* (1985). Apart from a few cats which became pyrexic (Shophet and Marshall, 1980; Larsson *et al.*, 1985), and 3 cats which had histological evidence of interstitial nephritis (Fessler and Morter, 1964; Shophet and Marshall, 1980,) experimentally-infected cats remained healthy.

PATHOGENESIS OF LEPTOSPIRAL INFECTION IN THE CAT

Reports considering the pathogenesis of feline leptospirosis are scanty. It is difficult to assess the incubation period since most attempts to produce the disease experimentally have been unsuccessful (Fessler and Morter, 1964; Shophet and Marshall, 1980; Larsson *et al.*, 1985). From studies in other species, it is known that leptospires penetrate intact mucous membranes, abrasions of skin and sometimes through the alimentary tract of the susceptible host (Michna, 1970). After successfully evading the host's innate defence mechanisms, leptospires multiply in the blood stream and the liver (Jungheer, 1944). This usually coincides with the period of pyrexia. Migration to the parenchymatous

organs then occurs. In most susceptible hosts, leptospires tend to localise in the kidney but the reason for this preference is largely unknown. However, certain leptospires have been shown to utilize urea for their metabolism (Kadis and Pugh, 1974). Leptospires were isolated from liver, thoracic fluid and aqueous humour of a cat that died of *L.bratislava* infection. The presence of leptospires was also demonstrated in the lungs, kidney and brain of the same cat using fluorescent antibody technique (Bryson and Ellis, 1976).

The production of toxins by some pathogenic leptospires has been reported (Knight *et al.,* 1973; Arean *et al.,* 1964; Thompson and Manktelow, 1986) but the actual role of these toxins in the pathogenesis of leptospirosis is obscure.

The leptospiraemic phase in infected cats is closely followed by the production of agglutinating antibodies against leptospiral antigens within the first two weeks of infection. Fessler and Morter (1964) demonstrated agglutinin titres to *L.pomona* from day 8 in most of their infected cats. In one cat, the titre persisted for nearly 9 weeks when the experiment was terminated. Larsson *et al.* (1985) found agglutinin titres from day 7 in 5 *L.icterohaemorrhagiae* infected cats and in one out of 5 cats infected with *L.canicola*. Cats exposed to both serovars maintained agglutinin titres of 1:100 for 56 days post-exposure before they were euthanased.

There is very little information on types of antibody produced in feline leptospirosis. Shophet and Marshall (1980) found anti-ballum IgM in the serum of cats fed with either whole infected mice, whereas IgG was present only in cats fed infected offal. The reason for this disparity is unknown.

The pathogenesis of jaundice observed in a few natural cases of feline leptospirosis (Rees, 1964; Mason *et al.*, 1972) has not been investigated, although hepatic lesions and jaundice are inconsistent findings (Bryson and Ellis, 1976). Hepatomegaly was present in cats infected with *L.pomona* (Fessler and Morter, 1964). Histologically, there was pronounced perilobular degeneration. Similar findings were reported by Bryson and Ellis (1976) in a cat infected with *L.bratislava*. Neither of these cats was jaundiced. An electron microscopic study of red blood cell destruction in leptospiraemic hamsters infected with *L.pomona* revealed that this destruction was extravascular and that erythrophagocytosis and RBC sequestration occur in the liver and spleen (Thompson and Manktelow, 1986).

Neurological manifestations that were observed in 2 suspected cases by Hemsley (1956) may have been due to the localization of the organism in brain tissue. Bryson and Ellis (1976) described subdural and perivascular haemorrhage in the brain of a cat and demonstrated a strong fluorescent antibody reaction with *L.bratislava* in the brain tissue. Lucke and Crowther (1965) reported a history of "fits" in a cat with a titre of 1:100 to *L.mini*.

Although the kidney is the major target organ of leptospiral infection in most susceptible hosts, renal lesions have not been a consistent finding in feline leptospirosis. Lucke and Hunt (1965) concluded that leptospiral infection was not important in the pathogenesis of chronic interstitial nephritis in cats as they could only demonstrate agglutinin titre in one out of 85 cats with evidence of renal damage. This cat was suffering from severe chronic nephritis and had a titre of 1:1000 to *L.bratislava*. Conversely, Bryson and Ellis (1976) found

no pathological changes in the kidneys of a cat that died of *L.bratislava* infection.

There are conflicting reports on the renal lesions produced in cats infected with the leptospiral organism. Hemsley (1956) reported mild proteinuria in 3 cats with positive titres to *L.canicola* and observed glomerulonephritis in the kidney of one of the cats. Interstitial nephritis was described in *L.pomona* infected cats by Fessler and Morter (1964). With the same serotype, *L.pomona*, Mason *et al.* (1972) noted focal interstitial nephritis in one case, tubular nephrosis in another.

Urinary shedding of leptospires has been observed in naturally-infected cats (Carlos *et al.*, 1971; Trifunovic and Nesic, 1986) and following experimental infections (Fessler and Morter, 1964; Shophet and Marshall, 1980; Larsson *et al.,* 1985). The duration of leptospiruria, however, varies. Shophet and Marshall (1980) observed urinary shedding of leptospirae in *L.ballum* infected cats from 12 days post-infection continuing until the 47th day, when the experiment was terminated. Although Fessler and Morter (1964) did not record the onset, one cat infected with *L.pomona* was still leptospiruric 8 weeks post-infection. The public health implications of feline leptospiruria is yet to be examined. Out of 791 cases of human leptospirosis reported in the USA over a 14 year period, 2 cases were suspected to have been acquired from cats (Kaufmann, 1976).

EPIDEMIOLOGICAL CONSIDERATIONS

It has been estimated that 41 per cent of cats in U.K. supplement their food by feeding on mice and birds (Fennell, 1975). The close association between cats and rodents has stimulated a few studies into feline leptospirosis since rodents in many parts of the world are known

maintenance hosts of several leptospires. However, a surprisingly low infection rate (5.5%) with *L.pomona* in feral cats in a rural area of southern Illinois was reported by Ferris and Andrews (1965). A prey-predator transmission of infection using infected mice was successful in so far as the cats became leptospiruric and 2 showed histological evidence of interstitial nephritis but no clinical signs of infection (Shophet and Marshall, 1980).

It is still not known whether cats act as accidental or maintenance hosts when they are infected with pathogenic leptospires. Furthermore, there is no information on sex, age or breed susceptibility. Hemsley (1956) found that 2 out of the 3 cats seropositive to *L.canicola* were over 12 years of age. Nothing is known about any seasonal incidence of leptospiral infections in cats.

THE SITUATION IN BRITISH ISLES

Early investigations by Hemsley (1956) associated chronic nephritis in 3 cats with high serological titres to *L.canicola*. He observed that 3 out of 6 cats with clinical signs of chronic nephritis had positive agglutinin titres to *L.canicola* but none to *L.icterohaemorrhagiae*. This worker also reported the work of Broom (1955, unpublished) who found that 4 out of 180 cats tested against 6 leptospiral antigens (*L.icterohaemorrhagiae*, *L.pomona*, *L.canicola*, *L.sejroe*, *L.grippotyphosa* and *L.bataviae*) had positive agglutinin titres to *L.icterohaemorrhagiae*. Two of these 4 cats were also positive to *L.canicola*.

In the first reported serological survey in the U.K., Lucke and Crowther (1965), using a battery of formalin-fixed antigens (13 serotypes), found a 6.8 per cent infection rate in 118 cats from the Bristol area. These workers found evidence of renal disease in 3 out of

8 cats with agglutination titres to leptospiral antigen, one of which was positive to *L.bratislava*. Bryson and Ellis (1976) in Northern Ireland reported a fatal case of *L.bratislava* infection in a cat. The maintenance host of *L.bratislava* in the U.K. is unknown. However, serological and cultural evidence indicates that infection is widespread in pigs, horses, badgers (*Meles meles*), mink (*Lutreola lutreola*), foxes (*Vulpes vulpes*), brown rats (*Rattus novegicus*), hedgehogs (*Erinaceus europaenus*) and grey squirrels (*Sciurus carolensis*), (Hathaway *et al.*, 1983 a&b).

Michna (1970), in Glasgow, found agglutinin titres to the *sejroe* serogroup in the serum of a pyrexic and severely jaundiced Siamese cat.

Fennell (1975) in a demographic survey of the domestic cat population in the U.K. observed that 41 per cent of cats feed on birds and mice. In a study of predation by 70 domestic cats in a typical English village, Churcher and Lawton (1987) found that 75 per cent of the annual catch were small mammals (wood mouse, field vole, common shrew, bank vole etc.). The significance of this observation on the epizootiology of feline leptospirosis is not clear. Leptospiral infections in British rodents are well documented (Broom and Coghlan, 1958; Twigg *et al.*, 1968; Little and Salt, 1975; Hathaway *et al.*, 1983a). In addition, *L.icterohaemorrhagiae* has been isolated from rats in cases of human and canine leptospirosis (Clegg and Heath, 1975). However, Hathaway *et al.* (1982a) could find no evidence of leptospiral infection in 272 house mice (*Mus musculus*) in the south east of England.

There are no reports of recent serological surveys for leptospiral antibody in cats despite recent evidence of this disease in other susceptible hosts in the U.K. (Pritchard, 1986).

TREATMENT AND CONTROL OF FELINE LEPTOSPIROSIS

Attempts to treat the small number of cats reported to have leptospiral infections have all been unsuccessful. In the 2 suspected cases reported by Mason *et al.* (1972), the first case was treated with a combination of penicillin, streptomycin and a liver extract and the second case with 200mg of ampicillin trihydrate plus fluid therapy. Both animals deteriorated and died.

Cats are not routinely vaccinated against leptospirosis.

DIAGNOSIS OF FELINE AND CANINE LEPTOSPIROSIS

The diagnosis of leptospiral infection in cats and dogs is basically the same except for the fact that clinical signs of leptospirosis in cats are not well defined, whereas the clinical signs of leptospirosis in dogs are well recognised and therefore play a major role in arriving at a tentative diagnosis.

Other methods of diagnosis include the microscopical demonstration of the organism, culture techniques and serology. Since, at present, there is no clear clinical disease picture the diagnosis of feline leptospiral infection is dependent on laboratory investigation using microscopic, cultural and serological tests. Conclusive diagnosis is usually based on a combination of the above (Ellis, 1986b). However, in the cat most reported diagnoses have been based on serological evidence (Hemsley, 1956; Rees, 1964; Michna, 1970; Mason *et al.*, 1972).

Microscopy

Darkground microscopy is routinely used in an attempt to demonstrate leptospirae in body fluids such as urine, plasma and

thoracic fluid and sometimes contaminated water. Ellis (1986b) contended that the contamination of these fluids with other debris makes it unreliable for diagnostic purposes. Wolff (1954) recommended that blood and cerebrospinal fluid should be examined within the first 8 days of overt clinical disease and thereafter urine, examined by darkground microscopy, for the presence of leptospirae at a x200-300 magnification.

Leptospiral organisms stain very poorly with the usual bacterial stains because of their very thin nature. Silver stains are usually used for demonstrating leptospirae in paraffin sections. Young's (1969) modification of Warthin and Starry's method is the most widely used. A recent modification has been advanced (Elliot, 1988) incorporating 0.5% iodine into Young's (1969) method. Intact leptospirae are required for certainty in identification.

The fluorescent antibody technique (FAT) has been described as the most useful tool for demonstrating leptospiral organism in tissue sections (Ellis, 1986b). Bryson and Ellis (1976) used this technique in diagnosing a case of leptospirosis in a dead cat. Unlike darkground microscopy, the FAT test does not require the organism to be viable.

The immunoperoxidase staining technique has been used in the diagnosis of leptospirosis in pigs (Ellis *et al.*, 1983). These workers concluded that there was good correlation between culture results and the results of the immunoperoxidase staining method.

Cultural techniques

Culture is the best method of confirming leptospiral infection when a tentative diagnosis is based on the clinical signs. A review of the useful media available has been provided by Turner (1970b). Broadly,

the leptospiral culture media can be subdivided into 3 types: liquid, semi-solid and solid media. Only the first 2 types are routinely used.

Leptospiral organisms are very exacting nutritionally, utilizing long chain fatty acids as their main source of carbon, and ammonium salts as their source of nitrogen. They also require additional growth factors such as vitamins B12 and B1 (Palmer, 1988).

The use of rabbit serum dates back to the work of Fletcher (1928). Rabbit serum is now known to contain high concentrations of bound vitamin B12, a growth promoter for leptospiral organisms. However, some rabbit sera contains antileptospiral antibody which may be lethal to leptospirae. Pooled inactivated rabbit serum is now routinely used (Wolff, 1954). Johnson and Harris (1967) used commercially available vitamin B12 and Tween 80 (polyoxyetheylene sorbitan monoleate) as a source of their fatty acid. However, Ellis (1986b) recommended a mixture of Tween 40 (polyoxyetheylene sorbiton monopalmitate) and Tween 80 to provide fatty acid for growth of leptospirae.

Johnson and Rogers (1964) observed that purine bases were incorporated with the nucleic acid of leptospirae whereas pyrimidine bases were not. They showed that 5-fluorouracil (uracil is a pyrimidine) is lethal to various micro-organisms but not to leptospiral organisms at concentrations of 200-400 ug/ml. This substance is now routinely incorporated into selective media for leptospires in addition to low concentrations of bacitracin, neomycin, amphotericin B and occasionally nalidixic acid for their anti-bacterial actions.

The optimum temperature for growth of leptospires is 28-30°C. In laboratory-adapted cultures, dense cultures are usually attained within 7 days in liquid media and subculturing is required.

Isolation of leptospirae from clinical material

Successful isolation of leptospirae is dependent on collection of the correct material at the appropriate time and inoculating the optimum quantity into a suitable medium.

Blood and cerebrospinal fluid is best obtained at the onset of the disease (1-8 days) and 2 drops inoculated into 7-10ml semi solid media (Turner, 1970b). Urine culture is usually successful at a later stage in the course of illness. Isolation from various parenchymatous organs such as the kidney liver and lungs, can be attempted from post-mortem material. Ellis (1986b) also recommended culture of the adrenal gland.

Isolation by inoculation into laboratory animals

Certain authors (Wolff, 1954; Turner, 1970b) have propounded that this method should be used for strains that do not adapt very well to routine laboratory media until a suitable media is found. Twenty one day old hamsters are usually used.

Serological diagnosis of leptospirosis

A recent review of this method has been published by Palmer (1988). Serological diagnosis of leptospirosis can be divided into two groups - the genus specific and the group specific tests.

The genus specific tests use a combination of different leptospiral serovars or the biflexa group of antigens. Such combinations of leptospirae should include all types known to exist in that environment. However, these methods cannot differentiate infecting serogroups although they are able to detect infections at a fairly early stage. They include the macroscopic slide agglutination test, complement fixation

test and the genus specific ELISA such as the Dot ELISA described by Watt *et al.* (1988).

The group specific test involves screening sera against a battery of antigens known to occur in a particular environment in addition to some that do not occur, usually selecting a strain to represent a serogroup. The most widely used is the microscopic agglutination test (MAT) using live or killed antigen. Its sensitivity has been questioned by various authors (Heath and Box, 1965; Negi *et al.*, 1971; Hartman *et al.*, 1984b). It is however, at present, the test recommended by WHO. A group specific ELISA test has been described by Hartman *et al.* (1984a) for the diagnosis of canine leptospirosis. These methods can detect infecting leptospires to the serogroup level although low levels of cross-agglutination may occur. In the dog, agglutination titres are not usually detectable (using the MAT) until day 11 post infection (Taylor *et al.*, 1970). Administration of large doses of antibiotics in the early stages of infection may cause the MAT titre to remain low (Wolff, 1954; Turner, 1970a; Hartman *et al.*, 1984b).

New developments in the diagnosis of leptospirosis include the use of monoclonal antibody and bacterial restriction DNA analysis (Terpstra *et al.*, 1986).

1.3. MATERIALS AND METHODS

Animals

The cats included in this study were all referred to the Small Animal Clinic of the University of Glasgow Veterinary School between September 1987 and October 1988. Records of breed, age, sex, residential address, previous and immediate medical history were obtained and recorded. Each animal was given a thorough clinical examination and the clinical findings recorded.

Blood collection and processing

Following clinical examination, blood samples were obtained by jugular venipuncture. The cat was wrapped in a strong laboratory coat, leaving the head and neck exposed, and held either in an upright position or, for more difficult cats, supine. The jugular vein was identified by palpation with or without previous fur plucking. The method of sampling was as described by Kirk and Bistner (1985), using a 21 gauge, one inch needle, fitted to a 10ml disposable syringe. The aim was to collect 14ml of blood by totally filling the syringe but this was not possible or advisable in all cases. The final total volume of blood collected depended upon the co-operation of the cat and the discretion of the clinician.

Blood was transferred immediately to EDTA tubes for haematology, lithium heparin tubes for biochemistry and virology, and sterile 10ml plain tubes for serology. The serology aliquot was allowed to clot for about 2 hours. After clotting, serum was separated by centrifugation at 1,500g for 15 minutes. Serum so prepared was then stored at -20°C.

Haematological examination was carried out in the Department of Veterinary Pathology, Glasgow University Veterinary School and using a Coulter^{R}ZX6 the following parameters were measured: haematocrit value, haemoglobin content, total red blood cell count, total white blood cell count, and differential white blood cell count. Biochemical examination was carried out in the Department of Veterinary Clinical Biochemistry, Glasgow University Veterinary School, and using a Cobas Mira^{R} autoanalyser (Roche) the following plasma determinations were made: urea, creatinine, sodium, chloride, potassium, inorganic phosphate, bilirubin, alkaline phosphatase (AP), alanine amino transferase (ALT) and aspartate amino transferase (AST). In addition, total blood protein, albumin and globulin fractions were quantified using Technicon continuous flow auto analyser.

Virological testing for feline leukaemia virus (FeLV) antigen, feline immunodeficiency virus (FIV) antibody and feline infectious peritonitis virus (FIP) antibody was carried out by the Feline Virus Unit, University of Glasgow Veterinary School whenever a sufficient volume of blood was available.

A second serum sample was obtained whenever possible if the cat was presented for a follow-up examination.

Urine sampling and testing

Urine samples were obtained from cats by manual expression of the bladder or catheterization using a 4FG nylon catheter (Portex^{R}). Urine samples were transferred into sterile detergent free bottles. Routine urine analysis was carried out in the laboratory of the Department of Veterinary Medicine, Glasgow University Veterinary School. The following parameters were measured or examination made:

specific gravity, using a refractometer, and bilirubin and protein content by standard techniques. Occasionally, centrifuged urine deposits were stained and examined. Urine samples from animals suspected of having a possible leptospiral infection and which had not received previous antibiotic therapy, were immediately examined by dark-ground microscopy for the presence of leptospires using a Leitz^{R} microscope equipped with a dark ground condenser at X400. Culture of suspected positive urine samples was attempted in the Bacteriology laboratory, Department of Veterinary Pathology, University of Glasgow Veterinary School.

Other clinical diagnostic aids

The following were used when deemed necessary: radiography, electrocardiography and ultrasonography. Animals requiring further assessment or specialist investigation and treatment were admitted to the feline ward of the Department of Veterinary Medicine, Glasgow University Veterinary Hospital.

Fluorescent antibody technique (FAT)

Sections of liver, kidney and lungs collected from two jaundiced cats at necropsy, fixed in 10% methanol, were submitted to the Veterinary Research Laboratories, Stormont, Belfast, for determination of the presence of leptospires using fluorescein-labelled antibodies to *L.hardjo* and *L.bratislava*.

Microscopic agglutination test (MAT)

The MAT was carried out by the author at the Veterinary Research Laboratories in Stormont, Belfast. Adequate precautions were taken at each stage of testing to reduce any risk of laboratory workers

becoming infected. Protective clothing, including gloves and face mask were worn at all times. Disinfection was carried out promptly whenever spillage occurred and at the end of each day's work. All instruments used were soaked in concentrated disinfectant overnight.

Antigens used: Six to 7 day old dense cultures of 11 serotypes grown in liquid media (Ellis, 1986b) were used. Strains of leptospiral antigen employed in this work are listed in Table 3. Antigens were regularly checked for density and viability before use. The identity of each strain was confirmed by testing against the homologous sera raised in rabbits.

Procedure: The MAT was done in two stages -

a. *Screening test*

This was done to eliminate negative samples from the end point titration. U-shaped Cooke's^{R} Microtitre plates were used for the test and normal saline used as the diluent. The microtitre plates were marked to take 2 serum dilutions, 1/30 and 1/300. Each plate could accommodate 4 serum samples. Dilutions were made as described by Wolff (1954) with a few modifications. Instead of using droppers for the dilutions, calibrated micro-pipettes were used to deliver and transfer accurate volumes. Serum samples were diluted before the addition of antigens. Plates containing these antigen-serum mixtures were then stacked in layers of not more than 6 and incubated at 30°C for 2 hours. After incubation, multiple droppers, the one handle titre tex^{R} diluter, were used to transfer drops of the antigen-serum mixtures to 0.1mm thick microscope slides. The results were read using a darkground

TABLE 3. **Leptospiral antigens and strains used.**

	Antigen	Strain
1.	*L.canicola*	S/80/1090
2.	*L.icterohaemorrhagiae*	AB 102
3.	*L.pomona*	S/80/1503
4.	*L.ballum*	type culture
5.	*L.hardjo bovis*	S/80/1441
6.	*L.bratislava*	S/82/834
7.	*L.autumnalis*	Akiyami A
9.	*L.grippotyphosa*	CH 31
10.	*L.javanica*	type culture
11.	*L.cynopteri*	3522C

microscope equipped with a X15 eye piece and a X10 objective lens. Positive reactions were recorded when at least 50 per cent of leptospires in either the 1/30 or 1/300 dilutions had agglutinated. A titre of 1/30 and above was considered positive (Turner, 1970a). In all cases, the 1/300 dilution was examined even when there was no agglutination at the 1/30 dilution in the event of a prozone phenomenon. All positive and doubtful samples were subjected to the end point titration.

b. *End point titration*

The dilutions were made as described by Wolff (1954) with a few modifications. Calibrated volumes of saline, serum and the appropriate antigen were delivered into microtitre plates in that order. Serum at dilutions of 1/10, 1/30, 1/100, 1/300, 1/1000, 1/3000, 1/10000, 1/30000 were used for the test. After the addition of the appropriate antigen, plates were stacked in layers of 6 or less, covered and incubated at 30°C for 2 hours.

The results were read using darkground microscopy. The end point was taken as the dilution at which at least 50 per cent of the leptospires were agglutinated.

The results were analysed using percentages and the chi-square test.

1.4. RESULTS

Serum samples from 87 cats were examined for antibodies to 11 leptospiral serovars (Table 3). Fifteen were purebred and 72 were domestic cats, with an age range of 8 weeks to 19 years. Sixty-three were domestic short hair, 9 domestic long hair, 7 Siamese, 3 Persian, 2 each were Burmese and Ragdoll, and one Birman. A summary of the age and sex of the cats is presented in Table 4.

SEROPOSITIVE CATS

Eight cats were positive to various leptospiral serovars at a titre of 1/30 and above. Details of these cats are summarised in Table 5. Five of the cats were positive to *L.hardjo*, 2 to *L.autumnalis* and one to *L.icterohaemorrhagiae*. Ages of seropositive cats ranged from 10 months to 10 years. Cat no.1 was negative initially but had a titre of 1/100 to *L.hardjo* when resampled 8 days later. This animal was again negative after an interval of over 3 months. Cat no.2 was still positive to *L.hardjo* at the same titre, 1/30, when resampled after 2 months. One other cat, positive to *L.bratislava* at a titre of 1/10, was not included in the series. Seven months later, serum from a rat, killed at the cattery where this cat lived, was also found to be positive to *L.bratislava* at a titre of 1/10.

CLINICAL SIGNS AND LABORATORY FINDINGS IN SEROPOSITIVE CATS

All 8 cats had varying reductions in appetite and 3 cats were totally anorexic (cat nos. 3,5 and 6). All the cats except cat no.7 showed some degree of lethargy and weight loss. Three of the cats

TABLE 4. Age and sex of cats sampled.

Age in years	Male (neuter)	Female (spayed)	Total
0.1-0.5	3	2	5
0.6-1.0	5(2)	3(2)	8
1.1-2.0	7(4)	2(2)	9
2.1-3.0	3(2)	2(1)	5
3.1-4.0	7(6)	5(4)	12
4.1-5.0	1(1)	1(1)	2
5.1-6.0	2(2)	3(1)	5
6.1-7.0	1(1)	0	1
7.1-8.0	3(3)	3(2)	6
8.1-9.0	4(4)	2(2)	6
9.1-10	3(2)	3(3)	6
>10 years	7(7)	15(14)	22
TOTAL	46(34)	41(32)	87

TABLE 5. **Summary of details of seropositive cats.**

Cat number	Breed	Age	Sex	Reciprocal of titre	Reacting Serovar	Other information
1	DLH	8 yr	MN	100	*L.hardjo*	Previous sample negative Resampled after 3.5 months: negative
2	DSH	4 yr	FS	30	*L.hardjo*	Resampled after 2 months: still positive
3	DSH	8 yr	FS	30	*L.hardjo*	-
4	Siamese	10 yr	FS	30	*L.hardjo*	-
5	DSH	6 yr	F	30	*L.hardjo*	-
6	DSH	10 mth	M	300	*L.autumnalis*	-
7	Burmese	9 yr	MN	300	*L.autumnalis*	-
8	DSH	8 yr	F	100	*L.icterohaemorrhagiae*	

DLH : domestic long hair
DSH : domestic short hair
M, MN : male, male neutered
F, FS : female, female spayed

were polyuric and polydipsic when first presented (cat nos. 4, 7 and 8). Later in the course of his illness, cat no.1 also had an increased thirst, and developed a preference for eating soda scones. Vomiting was observed in 3 cases (cat nos. 1, 4 and 6). Dyspnoea was reported in 2 cats (cat nos. 4 and 8) which were also ataxic and had alopecia of the ventral abdomen. On clinical examination, 2 of the cats had ascites detectable on ballotment of the abdomen (cat nos. 1 and 2). Cat no.1 also had a palpable liver. Rectal bleeding, periorbital irritation and gingivitis was reported in cat no.7.

Haematological examination revealed a mild anaemia (haematocrit 0.25-0.29 l/l) in 2 seropositive cats (cat nos. 1 and 8) and varying degrees of leukocytosis (total WBC counts in excess of $15x10^9$/l) in 5 cases (cat nos. 1, 3, 4, 6 and 8). There was a marked leukopaenia in cat no.7 (WBC $1.4x10^9$/l). Platelet clumping was reported in 2 of the cats (cat nos. 7 and 8) and a mild eosinophilia was present in cat no.6. Haematological examination of the ascitic fluid in cat no.1 revealed the presence of red blood cells and white blood cells. The latter were initially predominantly lymphocytes but in a sample taken 8 days later they were mainly neutrophils.

Blood biochemistry was performed on samples obtained from 6 of the 8 seropositive cats. Two of the cats were mildly azotaemic (plasma urea 12-20mmol/l, cat nos. 2 and 8), and cat no.1 became mildly azotaemic later in the course of illness. Plasma creatinine levels were slightly raised (150-200umol/l) in cat nos. 1 and 7, the latter having known to have been previously azotaemic. Serum enzymes were raised in all the 6 sampled cats (cat nos. 1, 2, 4, 6, 7 and 8). Three cats (cat nos. 2, 4 and 8), were proteinuric, especially cat no.2. Blood electrolyte

levels were normal in all 6 cats sampled. The laboratory findings for cat no.1 are summarised in Table 6.

DIAGNOSIS AND FOLLOW-UP OF SEROPOSITIVE CATS

At the time of clinical examination, leptospirosis was not suspected in any of the seropositive cats. Based on clinical examination and laboratory findings, a diagnosis was made in 6 of the 8 seropositive cats and details are presented in Table 7. Two cases (cat nos. 4 and 8) had pancreatic and hepatic tumours with metastasis. Cat no.5 had a mammary tumour and cat no.6 had lymphosarcoma. Intractable gingivitis was diagnosed in cat no.7, which was referred with a tentative diagnosis of renal failure. Although the animal had been previously slightly azotaemic, there was no clinical evidence of renal failure, and post-mortem examination confirmed that the kidneys were normal. The cat showed haematological evidence of immunosuppression (total WBC $1.4x10^9/l$) and later was confirmed as FIV positive.

All the 8 cats were admitted to the Veterinary Hospital for follow-up and treatment. Six of the cats received antibiotic therapy. Cat no.5 was euthanazed soon after admission. Only 2 of the cats were discharged. Cat no.1 was still ascitic but otherwise well, 3 months after initial presentation. He was FIP, FeLV and FIV negative both initially and after 3 months. Culture of the ascitic fluid for acid fast organisms was negative. Serum samples from 2 cats and 2 dogs in the same household were also negative for leptospiral antibodies. Unfortunately, cat no.1 was subsequently lost to further follow-up.

TABLE 6. Initial and follow-up laboratory findings in Cat Number 1.

	18/5/88	25/8/88	12/9/88	Normal value [+]
(a) Blood				
P.C.V. (l/l)	**0.29**	**0.28**	**0.29**	>0.30
total W.B.C. ($x10^9$/l)	**19.1**	**21.8**	**16.8**	<17.0
% neutrophils	**3.5**	**62**	**68**	70
% lymphocytes	**57**	**9**	**27**	30
urea (mmol/l)	**9.1**	**12.8**	**13.9**	<10.0
creatinine (umol/l)	**ND***	**187**	**119**	<150
ALT (IU/L)	**42**	**133**	**73**	<40
albumin (g/l)	**28**	**36**	**34**	35-40
globulin (g/l)	**37**	**42**	**41**	30-35
(b) Peritoneal fluid				
albumin (g/l)	**20**	**30**	**33**	-
globulin (g/l)	**20**	**25**	**18**	-
(c) Virology				
FeLV antigen	**-ve**	**-ve**	**ND**	-ve
FIV antibody	**-ve**	**-ve**	**ND**	-ve
FIP antibody	**-ve**	**-ve**	**ND**	-ve
(d) Leptospira serology				
18/5/88	**0**	**ND**	**0**	-ve
25/5/88	**1/100** *L.hardjo*			

ND : not done

[+] normal figures as quoted by laboratories at Glasgow University Veterinary School

TABLE 7. **Diagnosis or major clinical finding, management, outcome and post-mortem findings in seropositive cats.**

Cat Number	Diagnosis or Major Clinical Finding	Management	Outcome	Post-mortem findings
1	Ascites	Drainage of abdominal fluid antibiotic therapy	Discharged; still ascitic	-
2	Protein losing nephropathy	Antibiotic and diuretic therapy	Discharged	-
3	Chronic weight loss	Antibiotic, corticosteroid and fluid therapy	Euthanazed	No post-mortem
4	Hepatic tumour	Fluid therapy	Euthanazed	Undifferentiated hepatic neoplasia; interstitial renal fibrosis
5	Mammary tumour	None. Euthanazed soon after admission	Euthanazed	No post-mortem
6	Lymphosarcoma	Antibiotic and corticosteroid therapy	Euthanazed	No post-mortem
7	Gingivitis; FIV +ve	Antibiotic therapy	Euthanazed	Gingivitis
8	Pancreatic adenocarcinoma	Antibiotic therapy	Euthanazed	Pancreatic adenocarcinoma

RESULT OF THE FLUORESCENT ANTIBODY TECHNIQUE (FAT)

Specimens of liver taken at post-mortem examination from 2 jaundiced cats, (hospital numbers 106393 and 107022), were examined by FAT and found to be negative. Neither cat had evidence of leptospiral antibody when serum was tested using the MAT.

1.5. DISCUSSION

In this study, the first serological survey of cats from the Glasgow area, 8 (9.2%) of 87 cats tested were seropositive to a variety of leptospiral serovars. This infection rate is slightly higher than that of 6.8 per cent reported by Lucke and Crowther (1965) in cats from the Bristol area. In addition, the results have yielded the first serological evidence of *L.autumnalis* infection in cats in the U.K. In this survey, there did not appear to be any age or breed predisposition to infection.

Five (62.5%) of the seropositive cats in this study reacted to *L.hardjo* antigen. Cattle are reported to be the maintenance host of serovar *hardjo* in the U.K. (Pritchard, 1986). In addition, *L.hardjo* is reported to be the most prevalent serovar in man (Waitkins, 1986). Four of these 5 cats lived in rural areas and infection could have been acquired from cattle. However, the MAT used in this work is a group specific test and infection in seropositive cats could be due to serovar *saxkoebing*, another member of the Hebdomadis group, which has been isolated from field voles, bank voles, and wood mice in the U.K. (Little *et al.*, 1987). These small mammals constitute 38 per cent of the prey of domestic cats in a typical British village (Churcher and Lawton, 1987). The possibility of a prey-predator transmission of infection has been previously demonstrated (Shophet and Marshall, 1980). Infection in these cats could have been acquired from cattle or rodents, although the actual source of infection of seropositive cats in this work remains obscure.

Two (25%) of the seropositive cats in this work reacted to the *L.autumnalis* antigen. The maintenance host of this serovar in the U.K. is largely unknown, although Hathaway *et al.* (1982b) recorded serovar

autumnalis as the most prevalent serovar in a serological survey of sheep. However, they concluded that these were probably due to cross reactions from the *autumnalis* serogroup. In the present work, although *L.bratislava* (an australis serogroup) was included in the battery of antigens used for screening the cats, none of them reacted to the australis serotype. Both the seropositive cats had relatively high titres to *L.autumnalis* (1:300). This serotype has been isolated from pigs in the U.K. (Pritchard, 1986). It has, however, not been implicated in feline infections.

One of the seropositive cats reacted positively to *L.ictero-haemorrhagiae* antigen. Rats are known maintenance hosts of *L.ictero-haemorrhagiae* in the U.K. (Twigg, 1973). Infection could have been due to *L.copenhageni*, another member of the icterohaemorrhagiae serogroup, which had been isolated from pigs in the U.K. (Pritchard, 1986). One cat was positive to *L.bratislava* at a titre of 1/10. This was most probably a non-specific reaction as previous serum samples from this cat were negative for leptospiral antibodies. However, in a review of the diagnosis of leptospirosis, Turner (1970a) suggested that titres as low as 1/10 could be the result of past infection and in view of a rat on the same premises having a titre of 1/10 to *L.bratislava* several months later, the titre in the cat might have been more significant.

In the 8 seropositive cats, recent leptospiral infection could only be presumed in cat no.1, which had a titre of 1/100 to *L.hardjo* and 8 days earlier had been negative for leptospiral antibodies. However, leptospirosis was not suspected at the time of clinical examination. The major clinical sign in this cat was ascites. There was a mild anaemia and a leukocytosis. An enlarged liver was palpated and a slightly raised level of plasma ALT was reported, suggestive of impaired hepatic

function. The cat was, however, not jaundiced. A few suspected cases of natural infection in cats have been reported to be icteric (Rees, 1964; Carlos *et al.*, 1971; Mason *et al.*, 1972). The pathogenesis of jaundice in feline leptospirosis is unknown.

The clinical signs shown by cat no.1 could not be explained by conventional causal agents such as feline leukaemia virus, feline infectious peritonitis and infection by acid fast organisms. One other possible disease, feline lymphocytic cholangitis, can only be confirmed by liver biopsy (Lucke and Davies, 1984), and this was refused by the owner. The antibody level to *L.hardjo* was rather low (1/100), but this could have been due to the early management of the case with antibiotics by the referring veterinarian. Babudieri (1961) reported that management of leptospiral infections with antibiotics may impede seroconversion. Also, Shophet (1979) reported low titres (<1:100) in actively infected cats. The increased appetite and preference for certain food cannot be explained. The owner, however, reported that the cat later lost interest in hunting, which was keenly pursued before the illness. Ascites of undisclosed amount was reported in a cat which died of apparent *L.bratislava* infection (Bryson and Ellis, 1976). Although the initial blood biochemistry did not reveal impaired renal function, the owner later reported an increased fluid intake and the cat became mildly azotaemic and had a slightly raised blood creatinine level, although the urine remained relatively concentrated. Fessler and Morter (1964) had reported interstitial nephritis in a cat experimentally-infected with *L.pomona*, although the blood urea in this experimental cat was normal. Cat no.1 was an outdoor hunting cat living in a semi-rural environment. The source of his infection could have been cattle or rodents. It is unfortunate that no definitive diagnosis was reached and that cat no.1

was lost to further follow-up as this was the one case in the series which bore greatest similarity to other reported clinical cases of feline leptospirosis.

One cat (cat no.2) maintained the same level of antibody to *L.hardjo* over a 2 month period, which is indicative of a past infection. Although a diagnosis of a protein-losing nephropathy was reached in this case, it is not impossible that this could have resulted from the previous leptospiral infection, although this has never been suggested as a cause of the nephrotic syndrome nor has proteinuria been reported as a clinical finding in feline leptospirosis. Larsson *et al.* (1985) were able to demonstrate agglutinin titres in their cats experimentally infected with *L.canicola* for up to 12 weeks post infection. The duration of immunity to natural leptospiral infection in cats is not known.

The lack of a paired serum sample or the isolation of leptospires from the other 6 cases makes the diagnosis of leptospirosis inconclusive.

There was evidence of renal damage in some of the seropositive cats. Three of these cats had been polyuric and polydipsic (cat nos. 4, 7 and 8). Plasma urea and creatinine levels were raised in 2 of these cases (cat nos. 7 and 8). Cat no.1 later became mildly azotaemic. Post-mortem findings indicated an interstitial renal fibrosis in cat no.4, which had been seropositive to *L.hardjo*. One of the cats infected with *L.pomona* reported by Fessler and Morter (1964) had been polydipsic and polyuric and necropsy revealed an interstitial nephritis. No kidney lesions were, however, found in the present study in cat nos. 7 and 8, which had positive titres to *L.autumnalis* and *L.icterohaemorrhagiae*, respectively. These changes could not be ascribed *per se* to leptospiral infection, since very little is known about the disease in cats (Povey,

1985). Hemsley (1956) had reported evidence of chronic nephritis in 3 *L.canicola* seropositive cats but there was no clear evidence of a cause and effect relationship.

None of the seropositive cats in this study was jaundiced, although 6 of the cats sampled in the course of the survey were jaundiced. Examination of organs from 2 of these cats by fluorescent antibody technique was negative. While jaundice has been reported in a few suspected cases of feline leptospirosis, (Rees, 1964; Carlos *et al.*, 1971) there is no evidence that this has been reproduced experimentally. However, blood biochemistry indicated impaired hepatic function in 6 of the seropositive cats (cat nos. 1, 2, 4, 6, 7 and 8).

Fifty per cent of the seropositive cats in this work had neoplasia (cat nos. 4, 5, 6 and 8), and later, cat no.7 was found to have FIV infection. The significance of this observation is unknown but could be related to tumour-induced immunosuppression at the time of leptospiral infection. There are no reports of immunosuppressed cats being used in experimental studies.

From this work there is evidence that leptospiral infection does occur as a disease entity in the feline population. However, there is still a need to define the type of disease that leptospiral organisms produce in cats and the epizootiological factors affecting the spread of the infection. Cats have been implicated in 2 cases of human leptospirosis in the U.S.A. (Kaufmann, 1976). There was no indication of human infection from the seropositive cats in the present study although not all the owners were questioned about their own health at the time of presentation of the cats. There is still a need to investigate the public health implications, if any, of feline leptospirosis.

CHAPTER II

CANINE LEPTOSPIROSIS

2.1. INTRODUCTION

Canine leptospirosis is a zoonosis of worldwide distribution. The first evidence of infection in the U.K. was reported by Okell *et al.* (1925) in England. Since then, clinical and serological studies have been conducted in dogs in Scotland and England to determine infection rate and evidence of disease. Stuart (1946) in Scotland, reported a 46 per cent infection rate and Broom and McIntyre (1948) a 26 per cent infection rate in England. In 1962 Weaver described an outbreak of leptospirosis in a greyhound kennel. Later, Michna and Ellis (1973) found a 33 per cent infection rate in a group of unvaccinated group of Glasgow dogs. These infections were all associated with serotypes *L.canicola* and or *L.icterohaemorrhagiae*.

The first evidence that infection might be caused by a serovar other than the components of commercial bacterins (*L.canicola* and *L.icterohaemorrhagiae*) in the U.K. was given by Thomas (1980) who found a 4-fold rise in titre to *L.bratislava* in a dog with jaundice. Reports from other parts of the world also indicate that non-vaccine serovars may cause clinical disease in the dog (Keenan *et al.*, 1978; Cole *et al.*, 1982; Everard *et al.*, 1987). Recent bacteriological and serological evidence of infection of dogs by a wide variety of serovars in the U.K. was provided by Pritchard (1986). However, it is not yet known if these serovars are associated with disease in dogs.

This study was therefore designed to determine the following:

(1) The type and prevalence of leptospiral antibodies currently present in Glasgow dogs,

(2) whether such infections are associated with clinical disease, and

(3) the clinical and epizootiological factors associated with the disease.

2.2. REVIEW OF LITERATURE

INTRODUCTION

Canine leptospirosis caused by *L.interrogans* is an important zoonotic disease affecting dogs worldwide, eliciting a wide range of clinical signs, usually associated with hepatorenal failure. The commonly incriminated serovars are *L.canicola* and *L.icterohaemorrhagiae*, although *L.grippotyphosa*, *L.ballum*, *L.pomona* and *L.bratislava* can also cause disease in the dog (Baldwin and Atkins, 1987). The disease can present as an acute haemorrhagic syndrome, an acute hepatic failure with jaundice, or as acute renal failure with uraemia.

THE AETIOLOGICAL AGENT

The commonest causal agents of canine leptospirosis are the serovars *L.canicola* and *L.icterohaemorrhagiae*. However, in recent years, more serotypes are being isolated from dogs and associated with disease (Thomas, 1980; Baldwin and Atkins, 1987).

L.canicola and *L.icterohaemorrhagiae* appear to be distributed worldwide and to be uniformly infective in dogs. Other serotypes which infect dogs seem to be area dependent, usually reflecting the serotypes found in other susceptible hosts in that environment.

The known infecting serovars as reported in recent serological surveys in some parts of Europe are shown in Table 8.

HISTORICAL BACKGROUND AND SYNONYMS OF CANINE LEPTOSPIROSIS

The first report of canine leptospirosis due to *L.icterohaemorrhagiae* came from the German workers, Krumbein and Frieling (1916), who associated it with 2 cases of Weil's disease in man. In the

TABLE 8. Recent serological evidence of canine leptospiral infection in some parts of Western Europe.

	Reference	Country	No.of dogs sampled	% positive	Incriminated serovars
1.	Dabalis (1986)	Greece	132	22.7	*L.canicola* *L.icterohaemorrhagiae*
2.	Gaumont & Trap (1986)	France	21	38.1	*L.canicola* *L.icterohaemorrhagiae*
3.	Andreani *et al.* (1986)	Italy	498	31.5	*L.icterohaemorrhagiae* *L.canicola* *L.bratislava* *L.hardjo*
4.	Desmecht (1986)	Belgium	631	19.3	*L.icterohaemorrhagiae* *L.canicola* *L.ballum* *L.javanica* *L.australis* *L.pyrogenes* *L.hebdomadis*

TABLE 8. contd.

	Reference	Country	No.of dogs sampled	% positive		Incriminated serovars
5.	Schonberg *et al.* (1986)	Federal Republic of Germany	299	8.4		*L.copenhageni* *L.saxkoebing* *L.grippotyphosa* *L.autumnalis* *L.canicola* *L.tarrasovi* *L.hardjo*
6.	Pritchard (1986)	United Kingdom	1.368	32.8	*	Icterohaemorrhagiae Canicola Hebdomadis Australis Javanica Ballum Tarrasovi Pyrogenes

* Serogroups

U.K., Okell *et al.* (1925) found the same serotype in association with a canine condition called "yellows", probably descriptive of the jaundice present in this condition. Other synonyms include enzootic jaundice (Okell *et al.*, 1925), Stuttgart disease and canine typhus (Alston and Broom, 1958). In 1934, Schuffner isolated another serotype, *L.canicola*, the causal agent of human canicola fever.

MODE OF TRANSMISSION OF CANINE LEPTOSPIROSIS

Canine leptospirosis can be spread directly and indirectly and the modes of transmission were reviewed by Baldwin and Atkins (1987). Venereal transmission has been described in cattle (Sleight and Williams, 1961) and vertical transmission has been implicated in man (Coghlan and Bain, 1969) and in cattle (Ellis *et al.*, 1983). It is not clear if these two modes of transmission occur in dogs, although Cole *et al.* (1982) described infection in young puppies caused by *L.ballum* and *L.grippotyphosa*.

Serological surveys worldwide have consistently indicated a higher male to female ratio of sero-positive dogs (McIntyre and Montgomery, 1952; Weaver, 1962; Torten *et al.*, 1971 and Ryu, 1976). The possibility that this may result from the male dog's habit of sniffing other dog's genitalia (McIntyre and Montgomery, 1952) remains conjectural but Baker and Little (1948) did suggest inhalation as a possible means of dissemination of leptospiral organisms among cattle.

Other sources of infection include contamination of the environment by rats (Thiermann, 1977; 1980) and by cattle (Mackintosh *et al.*, 1980). In addition, leptospires have been isolated from flies and ticks so these might play a role in transmission of infection (Michna, 1970).

PATHOGENESIS OF LEPTOSPIRAL INFECTION IN THE DOG

Acute and chronic leptospirosis have been described in the dog. The morphological and histological changes are, however, dependent on the infecting serovars (Michna, 1970; Baldwin and Atkins, 1987).

After successful invasion of the host by penetration through intact mucous membrane, abraded skin or following ingestion of contaminated material (Michna, 1970), leptospirae invade the blood stream leading to a leptospiraemia, and multiply primarily in the liver (Hanson, 1976). This usually coincides with a period of pyrexia and congestion of the visible mucous membranes.

The incubation period of the disease varies from 2-11 days (Low *et al.*, 1956a; Anderson, 1967) depending on the number of invading pathogens, their virulence, and the susceptibility of the host (Michna, 1970).

Hepatic lesions and jaundice have been observed in dogs infected with *L.icterohaemorrhagiae* (Low *et al.*, 1956a; Gleiser, 1957). Both these workers attributed the jaundice to hepatocellular injury and possibly intrahepatic biliary stasis. Bishop *et al.* (1979) described a form of chronic active hepatitis in 5 dogs infected with *L.grippotyphosa*.

Invasion of the kidney by leptospirae leads to the development of an acute or chronic interstitial nephritis depending partly on the severity of infection. Acute interstitial nephritis is usually characterised by infiltration of the interstitium by lymphocytes and plasma cells, resulting in acute renal failure due to pressure of the infiltrate preventing adequate glomerular filtration and tubular patency (Jones and Hunt, 1983). Chronic interstitial nephritis (CIN), an insidious and progressive condition, is usually characterised by interstitial fibrosis, possibly resulting from

damage caused by earlier infection with *L.canicola* (McIntyre and Montgomery, 1952; Anderson, 1967; Morrison and Wright, 1976).

McIntyre and Montgomery (1952) described a gradual progression from acute to chronic interstitial nephritis, whereas Low *et al.* (1967) could find no evidence of this in a long term study of dogs experimentally infected with *L.canicola*. However, none of these animals developed classical clinical acute or chronic renal failure. Taylor *et al.* (1970), in another attempt to induce experimental infection, observed a few leptospirae in the tubular lumina but not in the interstitial spaces, although amorphous granules were observed and these were considered to be leptospiral organisms whose morphology had changed. Torten *et al.* (1967) reported the detection of auto-antibodies to kidney tissue in the serum of *L.canicola* infected dogs. Morrison and Wright (1976) detected antigen and antileptospiral antibody in interstitial infiltrates but did not find evidence of auto-antibody to kidney tissue in dogs suffering from acute leptospiral nephritis. In more recent work on chronic interstitial nephritis, Spencer and Wright (1981) concluded that neither immune complex deposition nor the formation of auto-antibodies were involved in the pathogenesis of this disease. The pathogenesis of chronic interstitial nephritis in the dog therefore remains controversial. Arean (1962) highlighted the discrepancy between the mild histological changes in the kidney and liver and the severity of functional impairment in some infected dogs and suggested the involvement of a toxin or toxin-like substance.

Intestinal intussusception has been associated with canine leptospirosis (Gleiser, 1957; Hartman *et al.*, 1986) but the pathogenesis of this is not clear.

NECROPSY FINDINGS

The type and distribution of lesions in canine leptospirosis depends on the infecting serovars and the stage of the disease.

The most common gross findings following infection with *L.icterohaemorrhagiae* are generalised icterus and haemorrhage. Enlargement and focal white spotting of the kidney may occur (Low *et al.*, 1956b; Gleiser, 1957; Hartman *et al.*, 1986). Secondary broncho-pneumonia and intussusception of the intestine have also been noted (Gleiser, 1957; Hartman *et al.*, 1986; Baldwin and Atkins, 1987).

Histologically, in the liver there is dissociation of the hepatocytes and areas of necrosis surrounded by lymphocytic infiltration. Intrahepatic bile stasis and severe hepatocellular injury may be evident in icteric dogs (Low *et al.*, 1956a; Gleiser, 1957).

In *L.canicola* infection, the primary lesions are renal, with uraemic changes in other organs and tissues when severe uraemia is present. In acute interstitial nephritis, the kidneys are swollen with a pale, mottled appearance, especially at the cortico-medullary junction (McIntyre and Montgomery, 1952; Taylor *et al.*, 1970). Histologically, there is an interstitial nephritis with large areas of intense interstitial infiltration of lymphocytes and plasma cells. In subacute and chronic cases, varying degrees of interstitial fibrosis are found (Anderson, 1967). Occasional glomerular lesions may be seen including thickening of the Bowman's capsule and atrophy of affected glomeruli (Hanson, 1976). Metastatic calcification of the lungs and pleura due to uraemia have been reported (Hartman *et al.*, 1986).

IMMUNITY IN CANINE LEPTOSPIROSIS

Much information is available on the humoral immune response to leptospirosis (Heath and Box, 1965; Huhn *et al.*, 1975; Bey and Johnson, 1978; 1982; Hartman *et al.*, 1984a). However, the role of cell-mediated immunity in leptospirosis is still largely unknown (Bey and Johnson, 1982).

The microscopic agglutination test (MAT) is recommended by WHO for measuring antibody response in leptospirosis. However, it is well known that vaccinated animals or those which have recovered from leptospirosis generally have low or no agglutinating antibody as detected by the MAT (Heath and Box, 1965; Negi *et al.*, 1971; Hartman *et al.*, 1984b). Based on a comparative study of immunity in experimentally vaccinated dogs and naturally infected dogs, Heath and Box (1965) concluded that two types of antibody, one protective and the other agglutinating, are both important in the development of immunity to canine leptospirosis. Similarly, in cattle, Negi *et al.* (1971) observed that although calves vaccinated against *L.pomona* did not develop detectable MAT titres, they had protective antibody as detected by the hamster protection test. They also observed that protection was provided by both IgG and IgM. In a review of immune response to leptospiral vaccination in cattle, Hanson (1973) stressed the need for reliable tests for measurement of the immune response in vaccinated animals.

Following controversy on the type of antibody detected by the MAT, Morris and Hussaini (1974) demonstrated agglutinating activity in both the IgM and IgG classes. Similar observations were reported by Negi *et al.* (1971). Little information is available on the classes of antibody detected by the MAT in the dog. Nevertheless, Hartman *et al.* (1984a) observed that the MAT was negative in dogs primarily vaccinated

with *L.canicola* despite a rise in IgM and IgG detected by an ELISA test. Positive MAT titres were, however, detected in dogs primarily vaccinated against *L.icterohaemorrhagiae*.

Cross immunity has been described in leptospiral infections. Alexander (1976), in a review of immunity in leptospirosis, concluded that cross immunity does occur within serogroups and to a lesser extent between serogroups depending on the virulence of the infecting serovar. Plesko (1974) demonstrated cross immunity between two lipase positive leptospiral strains, *Icterohaemorrhagiae* and *Javanica* serogroups and two lipase negative strains, serogroups *Ballum* and *Canicola*.

Investigations have been made into the antigenic components of the leptospiral organism in order to improve the immunogenicity of vaccines available for protection against leptospirosis. In a comparison of three such antigenic components, namely, the outer envelope (OE), the protoplasmic cylinder (PC) and the leptospiral whole cell (WC), Bey and Johnson (1982) found no difference in the humoral immune response of vaccinated dogs although the PC sensitized the greatest number of lymphocytes. Earlier, Glosser *et al.* (1974) had shown that the OE was more efficient in protecting hamsters against leptospirosis.

CANINE LEPTOSPIROSIS IN BRITAIN

Okell *et al.* (1925) were the first to report an outbreak of leptospirosis in dogs infected with *L.icterohaemorrhagiae*. Stuart (1946) found that 46 per cent of Glasgow dogs were serologically positive to *L.canicola* and *L.icterohaemorrhagiae*. Broom and McIntyre (1948) reported a 26 per cent infection rate in England. In a follow-up study in Scotland, McIntyre and Stuart (1949) showed a good correlation between renal disease and canine leptospirosis. In a survey of 416 dogs, these

workers reported a higher rate of infection due to *L.canicola* (35%) than to *L.icterohaemorrhagiae* (5%) and proposed the prognostic use of blood urea levels in *L.canicola* infection. This study also included the comment that vaccination against *L.icterohaemorrhagiae* was by then routine practice in some kennels. Broom and Joshua (1949) and Cunningham *et al.* (1957) also confirmed the findings of McIntyre and Stuart (1949) with regard to the higher incidence of *L.canicola* infection.

Broom and Joshua (1949) in England reported a higher incidence of canine leptospiral infections in the colder months and this was confirmed in Scotland by Weaver (1962) and by Nash (1976, unpublished observation). However in a review of canine leptospirosis, Alston and Broom (1958) commented that they did not observe a seasonal incidence. A higher male to female ratio in canine leptospirosis has been demonstrated by several workers in the U.K. (Broom and Joshua, 1949; Cunningham *et al.*, 1957; Weaver, 1962).

In all of the early surveys of canine leptospirosis in the U.K. investigation was confined to the two antigens, *L.canicola* and *L.icterohaemorrhagiae* (Stuart, 1946; Broom and McIntyre, 1948; McIntyre and Stuart, 1949; Broom and Joshua, 1949; Cunningham *et al.*, 1957). Later, Michna and Ellis (1973) used 14 antigens in screening sera from a mixture of vaccinated and unvaccinated dogs in the Glasgow area. Positive results were confined to *L.canicola* and *L.icterohaemorrhagiae* antigens, with a much higher proportion (88%) positive to *L.canicola*.

The first indication that other serovars might be involved arose in a report by Thomas (1980) of an English dog with malaise and jaundice which showed a four fold rise in antibody level to *L.bratislava*. Recently, many more serotypes have been shown to be infective for dogs in the U.K. Pritchard (1986) reported the cultural isolation of *L.bratislava*,

L.pomona, *L.hardjo*, *L.tarassovi*, *L.icterohaemorrhagiae* and *L.canicola* from dogs and positive serological evidence of the serogroups *Icterohaemorrhagiae*, *Canicola*, *Hebdomadis* *Australis*, *Autumnalis*, *Javanica*, *Ballum*, *Tarrasovi* and *Pyrogenes*. Contrary to the findings of earlier workers (Broom and McIntyre, 1948; Michna and Ellis, 1973), a higher proportion of dogs were positive to *L.icterohaemorrhagiae*.

With the exception of the findings of Thomas (1980) of *L.bratislava* in a jaundiced dog and the observation of Ellis (1986b) that *L.bratislava* may be associated with infertility and abortion in breeding kennels, clinical disease caused by the other serotypes has not been described.

Waitkins (1986) reported 90 confirmed cases of human leptospirosis in a 12 month period in the U.K. Six cases were due to *L.canicola* and 5 of these people had been in contact with dogs.

VACCINATION

Vaccination against canine leptospirosis has been routine in Britain for over 30 years, using bivalent vaccines containing killed *L.icterohaemorrhagiae* and *L.canicola* organisms and the disease is seldom, if ever, seen in appropriately vaccinated dogs (A.S.Nash, 1988, unpublished observation).

CLINICAL SIGNS OF CANINE LEPTOSPIROSIS

Leptospiral infections in dogs may present in 3 forms, an acute haemorrhagic syndrome, a less acute icteric syndrome and the uraemic syndrome. Each form is dependent on the pathogenicity and virulence of the infecting serovar, and the host's immune status. In dogs, many leptospiral infections are inapparent or produce mild illness, but acute

infections with *L.canicola* and *L.icterohaemorrhagiae* are well documented (Weaver, 1962; Baldwin and Atkins, 1987).

As the primary target organs involved in canine leptospiral infections are the liver and kidneys, the resultant diseases are usually associated with dysfunction of one or both of these organs. *L.canicola* primarily leads to renal disease with about 15 per cent of affected dogs having, in addition, hepatic involvement. *L.icterohaemorrhagiae* infection usually causes a more serious illness and results in 70% of clinically affected dogs showing hepatic disease of which icterus may be a major sign (Michna, 1970).

Experimental and natural infection with *L.bataviae*, *L.grippotyphosa*, *L.ballum* and *L.bratislava* have led to significant changes in hepatic and renal functions in dogs (Keenan *et al.*, 1978; Thomas, 1980; Cole *et al.*, 1982).

Following infection with either *L.canicola* or *L.icterohaemorrhagiae*, clinically affected dogs commonly have a history of depression, lethargy, inappetence, vomiting, diarrhoea and increased thirst (McIntyre and Stuart, 1949; Baldwin and Atkins, 1987). Occasionally a cough may be elicited (Hartman *et al.*, 1986). Jaundice is a common presenting sign in dogs infected with *L.icterohaemorrhagiae* (Michna, 1970).

Fever is an inconsistent finding in canine leptospirosis. In the acute renal stage of the disease caused by *L.canicola*, McIntyre and Montgomery (1952) were unable to demonstrate a rise in body temperature. Similarly, Weaver (1962) could only demonstrate a rise in body temperature in one out of 8 non-fatal cases of infection with *L.icterohaemorrhagiae*. In experimental infections with *L.icterohaemorrhagiae* and *L.canicola*, Low *et al.* (1956a; 1956b) and Anderson (1967), respectively, demonstrated pyrexia 4-6 days post-infection.

Mucous membranes may be congested and occasionally petechiae or ecchymotic haemorrhages are found (Baldwin and Atkins, 1987).

Signs of renal failure, including pain over the sublumbar area, production of an increased volume of a dilute and sometimes foamy urine (McIntyre and Montgomery, 1952) with a compensatory polydipsia may be observed. An ammoniacal odour (uraemic halitosis) may be elicited from the mouth (Hartman *et al.*, 1986). In advanced uraemia, the dorsum of the anterior tongue is brown and may be necrotic, and ulceration of the gum and cheek mucosae may occur. Uraemia is also associated with vomiting and possibly diarrhoea. Gastric ulceration leads to bleeding; vomited material may contain fresh or altered blood and faeces may be melaenic. Muscular pain has been described (Baldwin and Atkins, 1987) and in later stages, uraemic twitches and convulsions. In young pups, intestinal intussusception has been reported (Low *et al.*, 1956a; Hartman *et al.*, 1986). Severe infections are usually fatal (Low *et al.*, 1956a; Navarro *et al.*, 1981; Baldwin and Atkins, 1987).

Biochemical changes in canine leptospirosis depend on the severity of infection and the type of organ dysfunction. In dogs with acute renal failure, there is an elevation of plasma urea and creatinine levels. Although Low *et al.* (1956a) demonstrated an appreciable rise in levels of serum creatinine and urea in dogs severely affected by *L.icterohaemorrhagiae*, levels remained normal in moderately affected dogs. Arean (1962) observed the same changes in guinea pigs infected with *L.icterohaemorrhagiae*.

Hyponatraemia, hypochloraemia, and hypokalaemia have been observed in puppies at the initial stage of infection (Finco and Low, 1968a). However, hyperkalaemia and hyperphosphataemia developed during the later stages of the disease. Similar observations were reported

by Navarro *et al.* (1981). Although Keenan *et al.* (1978) demonstrated a hypoalbuminaemia in dogs experimentally infected with *L.bataviae*, Navarro *et al.* (1981) using *L.icterohaemorrhagiae*, observed hypoalbuminaemia in both infected and control dogs.

In dogs with hepatic dysfunction, increased plasma levels of alkaline phosphatase (AP), alanine aminotransferase (ALT), and total and direct (conjugated) bilirubin levels have been reported (Navarro *et al.*, 1981; Keenan *et al.*, 1978).

Haematological changes, such as leukopaenia, early in the course of the disease and a leukocytosis later on have been observed (Keenan *et al.*, 1978). Thrombocytopaenia and increased fibrinolytic products have been described in dogs experimentally infected with *L.ictero haemorrhagiae* (Navarro and Kociba, 1982), but not in dogs infected with *L.canicola* (Finco and Low, 1968b).

Urine from affected dogs may have a low specific gravity, and contain variable amounts of protein and bilirubin (McIntyre and Montgomery, 1952; Baldwin and Atkins, 1987). Using darkground microscopy, leptospiral organisms might be observed in urine from dogs during the acute stages of infection and prior to antibiotic therapy.

DIFFERENTIAL DIAGNOSIS

Other causes of hepatic or renal dysfunction must be considered in the differential diagnosis of canine leptospirosis. Depending on clinical signs the following conditions should be considered:

1. *Canine adenovirus-1 (CAV-1) infection*: Both infections can present similarly as an acute hepatic disorder, with fever, lethargy, abdominal pain and jaundice, (Timoney *et al.*, 1974; Darke, 1983).

In CAV-1 infection there is often a lymphadenopathy and subcutaneous oedema. Differential diagnosis is aided by demonstration of the infecting organism or on serology.

2. *Hypoadrenocortism*:

Chronic adrenocortical insufficiency in dogs may present with similar clinical signs of lethargy, vomiting and diarrhoea, and evidence of renal failure. Raised levels of blood urea and creatinine are common findings in both conditions. In hypoadreno-corticism, resting cortisol levels are very low, with little or no rise following ACTH stimulation (Darke, 1983). Confirmatory diagnosis of leptospirosis can be reached by demonstrating a four-fold rise to the infecting leptospiral serovar.

3. *Gastrointestinal disease*:

The classical signs of diarrhoea, vomiting, and occasionally intussusception, in canine leptospirosis might lead to an initial diagnosis of a gastroenteritis (Weaver, 1962). Many dogs with acute renal failure have been misdiagnosed as having an intestinal foreign body (W.I.M.McIntyre, 1973, unpublished observation). The presence of other signs of a hepatorenal disorder, such as jaundice or uraemic signs, and the demonstration of a four-fold rise in antibody level to leptospiral organism will aid confirmation of the diagnosis.

4. *Porto-systemic shunt*:

Young dogs with a history of polyuria, polydipsia, intermittent inappetence and vomiting due to porto-systemic shunts often have palpably enlarged kidneys. However, the latter are unassociated with pain, and blood urea levels remain very low (Darke, 1983).

OTHER EPIZOOTIOLOGICAL FACTORS IN CANINE LEPTOSPIROSIS

Rats in canine leptospiral infection

L.canicola has been found to be the most prevalent serotype affecting dogs in various parts of the world (Ryu, 1976). However in Australia, a higher proportion of dogs were shown to be serologically positive to the *Icterohaemorrhagiae* group (Watson *et al.*, 1976). Earlier, Emanuel *et al.* (1964) reported rats to be the principal maintenance hosts for leptospirae in that part of the world. Similarly, Thiermann (1977; 1980) in Detroit, USA, related the high level of *L.icterohaemorrhagiae* infection in Norway rats to a large number of stray dogs seropositive to *L.icterohaemorrhagiae*.

In the U.K., *L.canicola* had previously been identified as the major cause of canine leptospirosis (McIntyre and Stuart, 1949; McIntyre and Montgomery, 1952; Michna and Ellis, 1973), but the more recent report by Pritchard (1986) indicated that more dogs are now seropositive to *L.icterohaemorrhagiae*. The brown rat (*Rattus norvegicus*) has been identified as the main maintenance host of *L.icterohaemorrhagiae* in the U.K. (Twigg, 1973). The significance of this observation in relation to canine leptospirosis in the UK is unknown.

Age

Canine leptospirosis has been observed in dogs ranging from 9 weeks of age to 8 years (Hartman *et al.*, 1986), although Broom and Joshua (1949) did not observe the disease in dogs under 6 months of age.

Experimental infections have been successfully induced in young animals. Low *et al.* (1956a; 1956b) and Gleiser (1957) were able to establish infection with *L.icterohaemorrhagiae* in young dogs aged 2-6

months. Anderson (1967) used 10 week old mongrels in an attempt to induce experimental interstitial nephritis with *L.canicola*. The puppies were immunosuppressed with corticosteroids but overall, the results were disappointing. Navarro and Kociba (1982) used 14 week old puppies and successfully established infection with *L.icterohaemorrhagiae*.

Sex

A consistently higher prevalence of male infections in canine leptospirosis has been reported by several workers (Newman, 1950; McIntyre and Montgomery, 1952; Weaver, 1962; Ryu, 1976). The reason for this is unknown but it may be connected with the sniffing behaviour in male dogs (Michna, 1970).

Seasonal variation

No consistent seasonal prevalence in canine leptospirosis has been reported. Outbreaks have been monitored in both winter (Weaver, 1962) and summer (Reis *et al.*, 1973). Alston and Broom (1958) did not find any seasonal prevalence.

Carrier state in canine leptospirosis

A large number of dogs shedding leptospires in their urine may not show any sign of disease but are capable of transmitting infection to other susceptible hosts (Alston and Broom, 1958). Hubbert and Shotts (1966) observed that 9 out of 10 dogs actively shedding leptospires in their urine showed no clinical sign of disease. Similarly, Weaver (1962), described an outbreak of clinical disease in a greyhound kennel and stated that serological examination of 121 apparently normal dogs revealed 30 with significant titres to leptospiral antigens. Healthy unvaccinated dogs have

been reported by several workers as a source of human infections (Clegg and Heath, 1975; Wong *et al.*, 1977). Nash (1976-77, unpublished observations) showed that experimental dogs infected with *L.canicola* could excrete viable organisms for up to 11 months.

The mechanism responsible for maintaining the carrier or disease state in canine leptospirosis is unknown but may be related to the immune status of the dog.

PROPHYLAXIS

A canine canicola-icterohaemorrhagiae bacterin is available in the U.K. and many other parts of the world. Its efficacy against leptospirosis is usually determined by a hamster protection test (Hartman *et al.*, 1984a). Immunity provided by this bivalent bacterin is said to be limited and lasts for about 6 months to one year (Stoenner, 1976). To improve the immunogenicity of vaccines, use of leptospirae or living attenuated cultures has been proposed (Glosser *et al.*, 1974). Recently, Bey and Johnson (1982) found no difference in the humoral immune response of dogs vaccinated with whole cell and outer envelop bacterins. Hartman *et al.* (1986) using the ELISA test observed that the humoral immune response of vaccinated dogs drops rapidly after the 5th week post primary and booster vaccination. An annual revaccination was required to maintain a longer lasting IgG response.

In the U.K., the recommended immunization programme consists of initial vaccination of young puppies with a bacterin containing *L.canicola* and *L.icterohaemorrhagiae*, repeated 2-3 weeks later and followed by annual revaccination. A similar immunization programme operates in the U.S.A. Stoenner (1976) observed that few dogs in the

U.S.A. receive the annual revaccination doses to maintain adequate protection.

Reports of capability to produce disease by other serovars such as *L.grippotyphosa* and *L.ballum* (Cole *et al.*, 1982), *L.bataviae* (Keenan *et al.*, 1978) and *L.bratislava* in a vaccinated dog (Thomas, 1980) call into question the rationale for vaccinating dogs with a bivalent bacterin. In addition, recent serological and bacteriological evidence of infection with other serovars in the U.K., (Pritchard, 1986), suggests that vaccines containing additional serovars may be necessary. However, leptospirosis seldom occurs in appropriately vaccinated dogs, although Everard *et al.* (1987), did report the deaths in Barbados of 7 vaccinated dogs following infection with the Autumnalis serogroup.

Alston and Broom (1958) referred to several workers who had earlier used immune serum in preventing disease in experimental dogs.

PUBLIC HEALTH ASPECTS OF CANINE LEPTOSPIROSIS

Man is an incidental host to leptospiral infections and prevention of the disease depends primarily on the control of the disease in domestic and wild animals (Pritchard, 1986). The infection in man varies from an inapparent infection to a fatal illness. Alston and Broom (1958) described the major clinical signs of the disease in man as an aseptic meningitis, jaundice and renal failure. Human leptospirosis in the U.K. is a notifiable disease. Cattle and dogs have been implicated as sources of infection for man (Waitkins, 1986). Leptospirosis is also known to be an occupational hazard for farmers and veterinary surgeons. Rats are thought to play an increasing role in the transmission of infection (Twigg *et al.*, 1968).

Healthy vaccinated dogs have been implicated as source of infection in man (Feign *et al.*, 1973). Moreover, Kaufmann (1976)

demonstrated leptospiruria in both vaccinated and unvaccinated dogs following challenge with *L.canicola*. However, Broughton and Scarnell (1985) reported no leptospiruria in vaccinated dogs challenged with *L.canicola* and *L.icterohaemorrhagiae* 2 and 4 weeks, respectively, after their second dose of vaccination. Hartman *et al.* (1984a) had earlier observed a rapid fall in IgG levels in dogs 5 weeks post-primary vaccination. The IgG level had fallen to prevaccination levels by the 19th week post-vaccination in dogs vaccinated against *L.canicola*. It is not known what the response would be if dogs were challenged at this stage.

Cases of human disease acquired from unvaccinated dogs are well documented (Clegg and Heath, 1975; Wong *et al.*, 1977).

Recently Waitkins (1986) reported 90 confirmed cases of human leptospirosis in the U.K. Thirty-seven had *L.icterohaemorrhagiae* infection. Five of the 6 patients with *L.canicola* infection had been in contact with dogs.

DIAGNOSIS OF CANINE LEPTOSPIROSIS

Clinical aspects of canine leptospirosis have been described earlier (p.48-52). Laboratory methods for the identification of leptospiral infection have been included in Chapter 1, pages 13-17.

TREATMENT OF CANINE LEPTOSPIROSIS

There are few reports on controlled studies into treatment of canine leptospiral infection. By comparison, extensive studies on various broad spectrum antibiotics have been carried out in man (Stoenner, 1976), although varying criteria had been used in evaluating responses.

Hubbert and Shotts (1966) showed that dihydrostreptomycin was effective in eliminating leptospiruria from dogs. In a review of canine

leptospiral infection, Baldwin and Atkins (1987) recommended the use of procaine penicillin G, 40,000-80,000 units/kg body weight, given intramuscularly once daily combined with dihydrostreptomycin sulphate at 10-15 mg/kg body weight intramuscularly twice daily to eliminate leptospiruria.

Doxycycline has been used effectively in a double blind study of human patients and has been shown to shorten the course of the disease and prevent leptospiruria (McClain, 1984). There is need to investigate the use of this tetracycline in canine leptospirosis because of its apparent safety in dogs in renal failure (Shaw and Rubin 1986). Earlier, Hubbert and Shotts (1966) reported the inability of oxytetracycline given at a dose of 25 mg/kg body weight to eliminate canine shedding of leptospires.

Appropriate supportive therapy dependent on the presenting clinical signs is essential in the management of severe cases of clinical leptospirosis (Weaver, 1962).

2.3. MATERIALS AND METHODS

Animals

The dogs used in this study were all referred to the Small Animal Clinic of the University of Glasgow Veterinary School between September 1987 and October 1988. Records of breed, age, sex, residential address, previous and immediate medical history were obtained and recorded. Owners were closely questioned about the leptospiral vaccination history of their dog. Based on this information the dogs in this survey were broadly classified into the following groups:

Group A - Dogs with up-to-date vaccination records, which had been revaccinated within the previous 12 month period.

Group B - Dogs previously vaccinated, usually during puppyhood but not revaccinated within the last 12 months.

Group C - Dogs whose previous vaccination history was unknown but definitely vaccinated within the last one month.

Group D - Unvaccinated dogs or those whose vaccination history was unknown.

Each animal was given a thorough clinical examination and the clinical findings recorded.

Blood collection and processing

Following clinical examination, dogs were bled by jugular venipuncture, using the method of Kirk and Bistner (1985). For ease of access, smaller dogs were lifted on to the examination table, while larger

dogs were kept on the floor and backed into a corner of the room. With an assistant or the owner raising the dog's head, the jugular vein was palpated with the minimum of hair clipping and 15-20ml of blood collected using a 20 gauge needle fitted to a 20ml disposable syringe.

Blood was transferred to EDTA tubes for haematology, lithium heparin tubes for biochemistry and sterile 10ml plain tubes for serology. Sample handling, storage and testing was thereafter carried out as described for cat blood (pages 18-19).

A second serum sample was obtained if the dog was re-presented for examination.

Urine sampling and testing

Urine was collected from male dogs by catheterization using 6 or 8 FG nylon catheters (Portex^{R}) and from female dogs during natural urination into a clean, dry, stainless steel bowl. Urine samples were transferred into sterile detergent free bottles. Routine urine analysis was carried out as described for cat urine (pages 19-20).

Aids to diagnosis

When deemed necessary, dogs underwent radiography, electrocardiography and ultrasonography, and those requiring further assessment or specialist treatment were admitted to the canine wards of the Department of Veterinary Medicine, Glasgow University Veterinary Hospital.

Leptospiral diagnosis

The microscopic agglutination test was carried out on dog serum samples by the author at the Veterinary Research Laboratories, Stormont, Belfast, using the methods previously described for cat sera (pages 20-23).

2.4. RESULTS

DOGS

One hundred and fifty dogs were examined for clinical signs of leptospiral infection and serum samples from these dogs tested for antibodies to 11 leptospiral serovars. The majority of the dogs had been previously vaccinated using one of several commercially available bivalent bacterin preparations containing *L.canicola* and *L.icterohaemorrhagiae*. The dogs were classified according to their vaccination status as previously described in Chapter 2.3 (page 59).

One hundred and thirty eight of the dogs were purebred and 12 were crossbred. Forty two breeds of dogs were represented in the purebred population and breeds are detailed in Table 9. Ages ranged from 8 weeks to 14 years. The distribution of age and sex is shown in Table 10.

DOGS WITH POSITIVE LEPTOSPIRAL TITRES

Twenty nine dogs were positive to 5 out of the 11 leptospiral serovars used and at a titre of 1/30 and above. The results are summarised in Tables 11A-D. Eighteen of the dogs were positive to *L.icterohaemorrhagiae* (48.6%), 9 to *L.bratislava* (24.3%) and 8 to *L.canicola* (21.6%). One was positive to *L.hardjo* and another to *L.ballum*, possibly as a cross reaction to *L.canicola* and *L.icterohaemorrhagiae*. Seven dogs showed a dual or multiple reaction.

Sixteen of the dogs were male and 13 female. The age range of seropositive dogs was 24 weeks to 12 years and details are presented in Tables 11A-D.

TABLE 9. Breeds represented in 138 purebred dogs sampled.

Breed Type	No. of animals
Alsatian	14
Labrador	14
Border terrier	8
Yorkshire terrier	7
Golden retriever	6 each
Dobermann	
Boxer	5 each
West highland white terrier	
Greyhound	
Great Dane	
Rottweiler	
Cavalier King Charles spaniel	3 each
Shetland sheepdog	
Springer spaniel	
Cocker spaniel	
Beagle	
Pinscher	
Corgi	
Old English sheepdog	
Standard poodle	
Border collie	
Rough collie	2 each
Gordon setter	
Irish setter	
Scottish terrier	
Skye terrier	
Bedlington terrier	
Bull terrier	1 each
Cairn terrier	
Dandie dinmont	
Irish wolfhound	
Pomeranian	
Weimaraner	
Bull mastiff	
Saluki	
Newfoundland	
Sharpei	
Pyrenean mountain dog	
Bouvier des Flanders	
Standard schnauzer	
Foxhound	

TABLE 10. Age and sex of dogs sampled.

Age in years	Male	Female (spayed)	Total
0.1 - 0.5	4	4	8
0.6 - 1.0	9	3	12
1.1 - 2.0	12	15 (4)	27
2.1 - 3.0	5	8 (3)	13
3.1 - 4.0	6	8 (2)	14
4.1 - 5.0	3	4	7
5.1 - 6.0	3	9 (8)	12
6.1 - 7.0	10	6 (1)	16
7.1 - 8.0	6	8 (5)	14
8.1 - 9.0	4	5 (4)	9
9.1 - 10	1	3 (2)	4
> 10 years	9	5 (4)	14
TOTAL	72	78 (33)	150

Twenty six (89.7%) of the seropositive dogs had been previously vaccinated and 15 (51.7%) had been revaccinated within the previous 12 months.

Group A ("Fully" vaccinated): 72 of the 150 (48.0%) dogs sampled had been initially vaccinated as puppies and subsequently revaccinated against leptospirosis within the previous 12 months. Of these, 15 (20.8%) had an MAT titre of >1:30 to leptospiral antigens. One of these dogs showed a multiple reaction to *L.canicola*, *L.icterohaemorrhagiae* and *L.ballum*. Eight dogs (47.1%) had a positive reaction to *L.icterohaemorrhagiae*, 5 (29.4%) were seropositive to *L.canicola*, and 2 had antibodies to *L.bratislava* (11.8%). One was positive to *L.hardjo* and another to *L.ballum* possibly a cross reaction. Ages of seropositive dogs in this Group ranged from 6 months to 12 years. The individual details of each dog and titres to the reacting serovars are summarised in Table 11A.

Group B (previously vaccinated but not recently revaccinated): 56 of the 150 dogs (37.3%) had been initially vaccinated against leptospirosis but had not been revaccinated within the previous 12 months. Seven (12.5%) had positive leptospiral titres to various leptospiral serovars and 2 of these dogs showed a dual reaction. Three each (33.3%) were positive to *L.icterohaemorrhagiae*, *L.canicola* and *L.bratislava*, respectively. Ages of seropositive dogs in this Group ranged from 15 months to 9 years. The individual details of each dog and titres to the reacting serovars are presented in Table 11B.

TABLE 11A. Seropositive dogs in Group A.

Dog number	Hospital number	Breed	Age	Sex	Reciprocal of Titre	Reacting Serovar
1	81403	Border terrier	8 yrs	M	30 300	*L.icterohaemorrhagiae* *L.canicola*
2	102837	Border terrier	12 yrs	M	300 100	*L.icterohaemorrhagiae* *L.ballum*
3	104196	Yorkshire terrier	8 yrs	M	100	*L.icterohaemorrhagiae*
4	106154	Beagle	9 yrs	M	1,000	*L.bratislava*
5	106360	Standard poodle	2 yrs	FS	100	*L.icterohaemorrhagiae*
6	106776	Cavalier King Charles spaniel	5 yrs	M	100	*L.icterohaemorrhagiae*
7	106880	Border collie	2 yrs	F	300	*L.hardjo*
8	106936	Golden retriever	2.5 yrs	M	30	*L.icterohaemorrhagiae*
9	107001	Cocker spaniel	9 yrs	M	100	*L.canicola*
10	107057	Old English sheepdog	0.5 yrs	M	100	*L.icterohaemorrhagiae*
11	107497	Poodle	3.5 yrs	M	100	*L.canicola*
12	107704	Standard schnauzer	2 yrs	F	100	*L.canicola*
13	107813	Shetland collie	6.5 yrs	FS	30	*L.icterohaemorrhagiae*
14	108023	Boxer	3 yrs	F	100	*L.bratislava*
15	108154	Bull terrier	0.7 yrs	M	30	*L.canicola*

TABLE 11B. Seropositive dogs in Group B.

Dog number	Hospital number	Breed	Age	Sex	Reciprocal of Titre	Reacting Serovar
16	102623	Labrador	2 yrs	M	30	*L.icterohaemorrhagiae*
17	105676	Beagle	9 yrs	M	1,000	*L.bratislava*
18	108866	Dobermann	4 yrs	F	10,000	*L.icterohaemorrhagiae*
19	106875	Cavalier King Charles spaniel	6 yrs	FS	100 30	*L.icterohaemorrhagiae* *L.canicola*
20	107143	Cross bred	7 yrs	M	100	*L.canicola*
21	107674	Beagle	3.5 yrs	F	100	*L.bratislava*
22	108157	Old English sheepdog	1.2 yrs	M	100 30	*L.bratislava* *L.canicola*

Group C (recently vaccinated): 6 of the 150 dogs (4%) in the sampled population had an unknown previous vaccination history but had been vaccinated within the last one month. Four of the 6 dogs (66.7%) had positive leptospiral titres: 3 dogs showed a dual reaction to *L.icterohaemorrhagiae* and *L.bratislava*, and the other was positive to *L.icterohaemorrhagiae*. All 4 dogs came from the same household while the 2 negative Group C dogs came from different households. Ages of seropositive dogs in this Group ranged from 1.5 to 2 years. The individual details of each dog and titres to the reacting serovars are presented in Table 11C.

Group D (unvaccinated or vaccination status unknown): 16 dogs (10.7%) of the sampled population had never been vaccinated against leptospirosis or had an unknown vaccination history. Three of these 16 dogs (18.8%) had a positive leptospiral titre. This constitutes 10.3 per cent of the total number of seropositive dogs (29) in this work. Two were positive to *L.icterohaemorrhagiae*, the other one reacted to *L.bratislava* antigen. Ages of seropositive dogs in this Group ranged from 5 months to 3 years. The individual details of each dog and reacting serovars are summarised in Table 11D.

CLINICAL RESULTS AND DIAGNOSES IN SEROPOSITIVE DOGS IN GROUP A

The major clinical features present at the initial clinical examination and the diagnosis for each dog are summarised in Table 12A. In no case was disease due to leptospiral infection considered either as a primary diagnosis, or secondary to another condition. Twelve of the dogs were seropositive to the two vaccine serovars and only one of these (dog no.6)

TABLE 11C. **Seropositive dogs in Group C.**

Dog number	Hospital number	Breed	Age	Sex	Reciprocal of Titre	Reacting Serovar
23	106378	Greyhound	1.5 yrs	M	100	*L.icterohaemorrhagiae*
24	106384	Greyhound	1.5 yrs	F	1,000 300	*L.bratislava* *L.icterohaemorrhagiae*
25	106385	Greyhound	2 yrs	F	300 100	*L.bratislava* *L.icterohaemorrhagiae*
26	106386	Greyhound	2 yrs	M	300 100	*L.bratislava* *L.icterohaemorrhagiae*

TABLE 11D. Seropositive dogs in Group D.

Dog number	Hospital number	Breed	Age	Sex	Reciprocal of Titre	Reacting Serovar
27	106881	Cocker spaniel	0.5 yrs	F	100	*L.icterohaemorrhagiae*
28	107721	Boxer	3 yrs	FS	100	*L.icterohaemorrhagiae*
29	108028	Crossbred	1.5 yrs	F	30	*L.bratislava*

TABLE 12A. Clinical signs and diagnosis in seropositive dogs in Group A.

Dog number	Anorexia	Weight loss	Dullness	Vomiting	Polydipsia	Other	Diagnosis
1			+		+	polyphagia	hyperadrenocorticism
2		+			+	polyuria; polyphagia	hyperadrenocorticism
3					+	polyuria; polyphagia	hyperadrenocorticism
4*		+	+	+	+	diarrhoea; dyspnoea	widespread haemangiosarcoma
5		+		+		diarrhoea	chronic diarrhoea
6		+	+	+		uraemia signs; non-regenerative anaemia	chronic renal failure
7*	+	+	+	+		pyrexia;dyspnoea	septicaemia
8	+	+	+				chronic idiopathic weight loss
9	+	+	+		+	obstructive jaundice	pancreatic adenocarcinoma
10				+		dyspnoea	congenital diaphragmatic - pericardial hernia
11				+		peripheral lymphadenopathy	multicentric lymphosarcoma
12				+	+		megaoesophagus
13				+	+		chronic pyoderma
14*	+	+	+	+	+	mild anaemia	hypothyroidism
15	+	+	+		+		generalised muscular atrophy

* Seropositive to non-vaccine serovars

might have had a condition, chronic renal failure, resulting from earlier leptospiral infection.

Of the 3 dogs (nos.4, 7 and 14), which were seropositive to non vaccine serovars, dog no.7, positive to *L.hardjo*, is of greatest interest. This dog came from a farm and was suffering from generalised septicaemia. She was euthanased soon after admission to the Hospital but no detailed post-mortem examination was carried out.

Haematology and biochemistry results together with radiographic and ultrasonographic findings supported the clinical diagnosis in each case and have not been included in these results.

CLINICAL RESULTS AND DIAGNOSES IN SEROPOSITIVE DOGS IN GROUPS B, C AND D

The major clinical features present at the initial clinical examination and the diagnosis for each dog are summarised in Table 12B.

Group B: (Dog nos. 16-22)

Of particular interest in this Group are dog nos. 17 and 18.

Dog no. 17, which was positive to *L.bratislava* at 1/1000, had a pyrexia of unknown origin and biochemical evidence (raised AST and ALT levels) of liver damage. This dog recovered with antibiotic (ampicillin) therapy and was discharged. No further serum samples were taken.

Dog no. 18 developed classical signs of hepatic leptospirosis caused by infection with *L.icterohaemorrhagiae*. She had an initial titre to *L.icterohaemorrhagiae* of 1/10000 and this had fallen to 1/1000 5 weeks later. The essential details concerning the history, clinical signs, laboratory findings and management are presented in Table 13.

TABLE 12B. Clinical signs and diagnosis in seropositive dogs in Groups B, C and D.

Dog number	Anorexia	Weight loss	Dullness	Vomiting	Polydipsia	Other	Diagnosis
Group B							
16		+		+			eosinophilic gastritis
17*				+	+	pyrexia; hepatic damage	pyrexia of unknown origin
18	+	+	+	+		pyrexia; jaundice; uveitis	leptospirosis icterohaemorrhagiae
19						nervous disposition	behavioural problem
20					+	polyuria; polyphagia	hyperadrenocorticism
21*					+	polyuria; polyphagia	hyperadrenocorticism
22*	+	+	+	+		diarrhoea	chronic diarrhoea
Group C							
23			+			scleral congestion; pyrexia; CNS signs	vaccine induced distemper encephalitis
24*			+			scleral congestion; pyrexia	vaccine induced distemper encephalitis
25*							normal dog
26*							normal dog
Group D							
27			+		+	polyuria; intermittent hepatic encephalopathy	porto-systemic shunt
28		+			+	polyuria	chronic renal failure - juvenile nephropathy
29*	+	+		+		diarrhoea	? leptospirosis

* Seropositive to non-vaccine serovars

TABLE 13. **Summary of clinical and laboratory findings in dog number 18 (case number 108866).**

Subject: Dobermann, 4 years, female.

History: Born in Germany August 1984; lived in Morocco and Spain. Entered UK in November 1987 and in quarantine for 8 months.
Vaccinated against leptospirosis at 3 months and revaccinated at 15 and 27 months. Good health record.
Two weeks after leaving quarantine had back pain and inappetence. Partial improvement with antibiotic therapy. Two weeks later was dull, anorexic, vomiting, diarrhoeic, and 48 hours later was jaundiced. Antibiotic therapy reinstituted and dog referred to GUVH. Owner stated that dog played in burn and she had caught a rat 10 days earlier.

Clinical findings: Well grown, lean, dull. Temperature 103°F.
Heart rate 150/min. Respiratory rate 35/min.
Moderately dehydrated. Marked jaundice.
Slight peripheral lymph node enlargement.
Bilateral uveitis. Resented anterior abdominal palpation.

Diagnosis: Suspected leptospirosis icterohaemorrhagiae.

Dog admitted to GUVH; placed on 24 hours i/v N-saline and i/m oxytetracycline. Improved rapidly over next 48 hours; bright and eating.
Discharged with 3 weeks oral oxytetracycline.
Improvement maintained and dog returned to normal.

Biochemistry findings:	**23/8/88**	**2/9/88**	**27/9/88**	**Normal value**
urea (mmol/l)	6.7	3.1	5.5	<7
bilirubin (µmol/l)	64	5	0	0
alk.phos (iu/L)	2688	588	106	<230
AST (iu/L)	349	26	18	<40
ALT (iu/L)	1001	402	25	<40
Serology:				
L.icterohaemorrhagiae	1/10000	ND	1/1000	0-1/10

The clinical and laboratory findings in relation to the other 5 dogs in Group B aided diagnosis but did not arouse suspicion that leptospiral infection was either present or contributing to the diseases in these dogs.

Group C: (Dog nos. 23-26)

This Group consisted of 4 greyhounds, all from the same kennel, of which 2 (dog nos. 23 and 24) were suffering from canine distemper, possibly as a result of inoculation with virulent canine distemper vaccine. Both dogs (nos. 23 and 24) recovered after 10 and 14 days, respectively. Dog no.24 showed a 4-fold rise in antibody to canine distemper. The other 2 dogs (nos. 25 and 26) remained well and were examined and sampled only for comparative purposes.

Group D: (Dog nos. 27-29)

Of these 3 dogs, no.29 had a non-specific illness of 8 days duration prior to referral. Routine examination of urine by dark ground microscopy revealed a few actively motile organisms resembling leptospires. Prolonged culture was attempted but proved unsuccessful. Plasma biochemistry indicated moderate rises in urea (18mmol/l) and creatinine (176umol/l) levels, but other parameters tested were normal. Oral oxytetracycline therapy was instituted and the dog improved. She was discharged after 5 days with a further 14 days supply of oxytetracycline. Apart from a report that progress had been maintained, the dog was lost to further follow-up.

Dog no.28 had been seen originally as a 5 month old puppy and diagnosed as having a juvenile nephropathy. She was maintained on a

low protein diet and sampled at 3 years old when presented for a check examination.

STATISTICAL ANALYSIS

To discover if there was any significant difference in the number of dogs seropositive to leptospiral antigens in Groups A and B, results were subjected to the chi square test with Yates's correction.

$$X^2 = 0.11.$$

At one degree of freedom

$$P > 0.5.$$

Thus there is no significant difference between the two groups.

2.5. DISCUSSION

Twenty nine (19.3%) of the 150 dogs in this survey were positive to various leptospiral serovars. Seven of these dogs showed a dual or multiple reaction. Eighteen dogs (48.6%) were seropositive to *L.icterohaemorrhagiae*, 9 (24.3%) to *L.bratislava* and 8 (21.6%) to *L.canicola*. One was positive to *L.hardjo* and another to *L.ballum*, possibly as a cross reaction. Pritchard (1986) similarly observed a higher prevalence (20%) of *L.icterohaemorrhagiae* in dogs in the U.K. This may be significant, in that earlier surveys in Scotland have shown *L.canicola* to be the predominantly infecting serovar (Stuart, 1946; Cunningham *et al.*, 1957; Michna and Ellis, 1973). As the dog is the maintenance host of *L.canicola* (Pritchard, 1986), it is possible that the effect of vaccination over many years has been to reduce the pool of *L.canicola* in the dog population, thereby reducing the risk of infection. This reduction may have given greater opportunity for infection by the other serovars, which have maintenance hosts other than the dog. Moreover, this appears to be the first report of a relatively high proportion of dogs being seropositive to a non vaccine serovar, *L.bratislava*, in the U.K. In this work, 16 (55.2%) of the seropositive dogs were male. Ages of the dogs ranged from 24 weeks to 12 years.

About half (48.0%) of the sampled population had been revaccinated within the previous 12 months. Hanson (1976) similarly observed that only a small proportion of dogs in the U.S.A. are revaccinated annually to maintain an adequate level of protection. Only 15 (20.8%) of the dogs in Group A had a detectable antibody level to the vaccine serovars (using the MAT), despite the fact that they had been

vaccinated, and older dogs revaccinated annually. This is similar to the observation of various workers (Heath and Box, 1965; Negi *et al.*, 1971; Hartman *et al.*, 1984a) who concluded that vaccinated animals have a low titre or no evidence of antibody to leptospires using the MAT, despite the fact they had protective antibody. One of these groups (Hartman *et al.*, 1984a) demonstrated a rise in IgM and IgG levels using an ELISA test in dogs primarily vaccinated against *L.canicola* despite a negative MAT titre. Morris and Hussaini (1974) had earlier demonstrated agglutin activities in both IgM and IgG classes of antibody. These observations are probably due to the low sensitivity of the MAT.

Although it is virtually impossible to differentiate between a vaccinal response and active leptospiral infection using the MAT (Hartman, 1984b), in the present study there did not appear to be evidence of disease caused by leptospiral infection in Group A dogs seropositive to the bivalent bacterin serotypes (*L.canicola* and *L.icterohaemorrhagiae*) (Table 12A). However, in view of the lack of follow-up serology to demonstrate a substantial rise or fall in antibody level, the diagnosis of leptospirosis remains inconclusive. There does not appear to be any report of disease due to the bacterin serotypes in appropriately vaccinated dogs, although healthy vaccinated dogs have been reported to be leptospiruric (Feign *et al.*, 1973; Kaufmann, 1976). More recently, Broughton and Scarnell (1985) could find no evidence of leptospiruria in vaccinated dogs 4 weeks post-vaccination following challenge.

In this work, a large proportion of seropositive dogs in Group A reacted to the bivalent bacterin serotypes. However, 3 dogs showed a single antibody response to non-bacterin serovars, 2 to *L.bratislava* and one to *L.hardjo*. In 2 of these cases (dog nos. 4 and 7) active leptospiral infection could not be ruled out. Dog no. 4 had a titre of 1:1,000 to

L.bratislava and a clinical diagnosis of haemangiosarcoma was made and confirmed at post-mortem. Nevertheless, a concurrent infection with leptospires remains a possibility. Dog no. 7, a farm dog with a titre of 1:300 to *L.hardjo*, had been previously pyrexic and was negative for canine distemper and toxoplasma antibodies. No definitive diagnosis was reached in this case, so leptospirosis cannot be ruled out. As protection afforded by the bivalent bacterins are serogroup specific (Bey and Johnson, 1978), active infection was possibly present in these 2 cases. However, *L.hardjo* has not been implicated as a cause of leptospirosis in dogs. Thomas (1980) demonstrated a 4-fold rise in antibody level to *L.bratislava* in a jaundiced dog.

Seven (12.5%) of the 56 dogs in Group B had antibody titres to various leptospiral serovars with 2 of the dogs showing a dual reaction. A higher proportion of dogs in this group were seropositive to *L.bratislava* than Group A dogs. In view of the fact that these dogs had been vaccinated but not revaccinated within the previous 12 months, the possibility that those seropositive to bacterin serotypes were as a result of vaccination cannot be excluded. In addition, there is no significant difference ($p>0.5$) in the number of dogs seropositive in Groups A and B.

The only evidence of classical leptospiral disease in Group B dogs seropositive to the bacterin serovars was in dog no. 18. Based on her recent history and clinical signs present at initial examination, hepatic leptospirosis was strongly suspected. The MAT titre of 1/10000 to *L.icterohaemorrhagiae* confirmed recent active infection (Baldwin and Atkins, 1987). Active leptospirosis following infection with the Autumnalis serogroup has been reported in vaccinated dogs (Everard *et al.*, 1987) but disease caused by natural infection with the vaccine serovars is very rare in dogs which have been regularly vaccinated (A.S.Nash, 1988, personal

communication). It would appear that, in dog no. 18, a primary vaccination course, followed by 2 annual boosters, the last at 27 months, provided insufficient protection when the dog was challenged naturally some 21 months later, reinforcing the recommendation that dogs receive annual revaccination against leptospirosis (Stoenner, 1976). A combination of 3 factors may have combined to produce illness in dog no. 18: no known revaccination in the previous 12 months; an 8 month period of quarantine, during which time natural immunity was unlikely to have been boosted; and a recent history of access to rats. Of particular clinical interest in dog no. 18 was the development of a transient bilateral inflammatory uveitis. This has been reported in horses infected with the Pomona serogroup (Twigg *et al.*, 1971), but not in dogs with leptospirosis, although Michna (1970) noted a transient vascular congestion of the conjunctiva in dogs with leptospirosis.

Dog no. 17, with a titre of 1:1000 to *L.bratislava* had a pyrexia of unknown origin. Urine cultures were negative for leptospires. A negative urine culture however does not rule out active leptospiral infection (Turner, 1970b). In addition, this dog had shown biochemical evidence of liver damage and had been reported to have "drinking bouts". Two other dogs in Group B (nos. 21 and 22) had low titres to *L.bratislava* and the serological response may well have been as a result of previous exposure.

The 4 seropositive dogs (out of 6) in Group C were from the same household. The reason for separating this group of dogs from Group A dogs is based on the possibility that the Group C dogs had been exposed to infection prior to vaccination. All the dogs were seropositive to *L.icterohaemorrhagiae* which could have been due either to previous infection or vaccination. Three of the dogs were also positive to

L.bratislava although 2 were clinically normal. Dog no. 23 showed signs of central nervous system disease 12 days after vaccination, and had more than a 4-fold rise in distemper antibody levels. Dog no. 24, a little-mate, had a similar but milder illness and was not tested for canine distemper antibody. All 4 dogs had been recently brought in from Ireland and were boarded in the same kennel. Weaver (1962) had reported an outbreak of leptospirosis in a kennel of racing greyhounds. He suggested that the close proximity in kennels may be a predisposing factor to the spread of infection.

The possibility of natural infection cannot be excluded in vaccinated dogs seropositive to the components of the commercial bivalent bacterin, *L.icterohaemorrhagiae* and *L.canicola*. However, it is virtually impossible to differentiate antibody response due to vaccination and that due to natural infection using the MAT (Hartman *et al.*, 1984b), unless there is overwhelming evidence of clinical disease, as was present in dog no.18.

The 3 dogs in Group D seropositive to various leptospiral serovars are most likely to have acquired natural infection. The fact that a porto-systemic shunt was the reason for illness in dog no. 27 does not exclude the possibility of concurrent active infection. In one of these cases (dog no. 29), a few motile organisms thought to be leptospires were seen in urine under the darkground microscopy but prolonged culture was unsuccessful. The clinical signs and blood chemistry were compatible with a diagnosis of renal leptospirosis although the titre to *L.bratislava* (1/30) was rather low. Active leptospiral infection in this dog and in dog no. 28 cannot be ruled out, even though they had low titres. Hartman *et al.* (1984b) demonstrated active infection due to leptospires in MAT negative dogs using ELISA techniques.

The high percentage of dogs seropositive to *L.icterohaemorrhagiae* in this study may be the result of vaccination, although natural infection was suspected in some cases and confirmed in dog no. 18. Rats have been implicated as the maintenance host of *L.icterohaemorrhagiae* in the U.K. (Twigg, 1973), while unvaccinated dogs still remain the major source of infection for *L.canicola* (Pritchard, 1986). The sources of infection for suspected cases in this work remain unknown, but there was strong circumstantial evidence of close contact with rats in dog no.18.

The relatively high proportion of dogs seropositive to *L.bratislava* in this work gives cause for concern as *L.bratislava* is known to be pathogenic in dogs (Thomas, 1980). However, dogs are not routinely protected against this serovar. The maintenance host of *L.bratislava* and *L.muenchen* (another Australis group) in the U.K. is unknown but infection is widespread among pigs, horses and small rodents (Pritchard, 1986). The source of infection with this serovar in the present work is unknown but a dog to dog transmission was possible in the 4 Group C dogs which shared a common environment. Dog no. 7, seropositive to *L.hardjo* was a farm dog and infection could have been acquired from cattle or sheep.

From these observations, it is concluded that vaccinated dogs can become infected with both bacterin and non-bacterin serotypes. A history of vaccination should therefore not rule out investigation into the possibility of infection with vaccine or other serotypes where the clinical signs and laboratory findings may suggest a tentative diagnosis of leptospirosis. Bey and Johnson (1978) previously observed that protection provided by bivalent bacterins are serovar specific. There are also reports of dogs developing clinical disease as a result of infection with non-bacterin serotypes (Keenan *et al.*, 1978; Thomas, 1980; Cole *et al.*, 1982; Everard *et al.*, 1987). A routine check for *L.bratislava* in

addition to *L.canicola* and *L.icterohaemorrhagiae* serovars would appear to be useful in the diagnosis of leptospirosis in dogs.

CHAPTER III

CONCLUSIONS

3. CONCLUSIONS

This study has fulfilled many of the objectives outlined earlier (pages 1 and 37).

In cats, the serological survey demonstrated that there is a low but significant level of leptospiral infection due to *L.hardjo*, *L.autumnalis* and *L.icterohaemorrhagiae*, in cats from the Glasgow area. Further studies with samples from a larger number of cats from over a wider area would give a more accurate estimate of the infection rate.

The question of whether exposure to leptospiral infection in cats leads to clinical disease remains unanswered. Of the seropositive cats examined, only one might have had a leptospira-related illness. However, this remains in doubt, as he only showed a transient titre to *L.hardjo*, despite ongoing clinical signs. Clearly, a larger survey, with stricter follow up, both in life and at post-mortem, would be beneficial.

The epizootiological factors involved in feline leptospiral infections and possible diseases also remain obscure. The fact that 5 out of 8 seropositive cats showed evidence of *L.hardjo* infection and that 4 of the 5 were rural cats, deserves further attention, especially as cattle are recognised as the maintenance host of *L.hardjo* in Britain. The presence of neoplasia and immunosuppression in 5 of the 8 cats is also of interest and worth closer scrutiny.

In dogs, it was confirmed that there is still a significant level of leptospiral infection in the Glasgow area, whether or not the dog is vaccinated. For the first time in a Scottish serological survey, *L.icterohaemorrhagiae* was the predominant serovar, followed by the non-bacterin serovar, *L.bratislava*, and then *L.canicola*.

The presence of a significant number of dogs seropositive to *L.bratislava* and one animal possibly clinically affected is important, as dogs are not at present protected by vaccination against this serovar. Further studies to define any canine diseases resulting from infection with *L.bratislava* should be carried out, and consideration given to the possible incorporation of this serovar into the vaccination combination.

Clinical canine leptospiral disease does still occur, although there was no evidence of this in dogs deemed to be adequately vaccinated. The high number of dogs seropositive to *L.icterohaemorrhagiae* and confirmed hepatic leptospirosis in one dog, suggest that this is likely to be the currently predominant leptospiral disease of dogs and owners should be strongly advised to ensure that annual leptospirosis vaccination is continued throughout a dog's life.

Further work is required on the epizootiology of all the identified leptospiral infections in the dog.

REFERENCES

REFERENCES

Alexander, A.D. (1976) Immunity in leptospirosis. In: The Biology of Parasitic Spirochetes. Publ.Academy, 339-349.

Alston, J.M., Broom, J.C. (1958) In: Leptospirosis in Man and Animals. Publ. E & S Livingstone, Edinburgh and London, p.367.

Anderson, L.J. (1967) Experimental reproduction of canine interstitial nephritis. J.Comp.Path. **77**, 413-418.

Andreani, E., Farina, R., Tolari, F. (1986) Spread and epidemiology of leptospirosis among animals in Italy. In: The present state of diagnosis and control of leptospirosis. Ed. Ellis, W.A., Little, T.W.A. Publ. Martinus Nijhoff, 191-196.

Arean, V.M. (1962) Studies on the pathogenesis of leptospirosis. II: A clinico pathologic evaluation of hepatic and renal function in experimental leptospiral infections. Lab.Invest. **11** (4), 273-288.

Arean, V.M., Sarasin, G., Green, J. (1964) The pathogenesis of leptospirosis: Toxin production by *Leptospira icterohaemorrhagiae*. Am.J.Vet.Res., **25** (106), 836-842.

Babudieri, B. (1961) Laboratory diagnosis of leptospirosis. Bull.Wld. Hlth.Org. **24**, 45-58.

Baldwin, C.J., Atkins, C.E. (1987) Leptospirosis in dogs. Compend. Contin.Educ.Pract.Vet. **9** (5), 499-5O7.

Baker, J.A., Little, R.B. (1948) Leptospirosis in cattle. J.exp.Med. **88**, 295-296.

Bey, R.F., Johnson, J.C. (1978) Humoral immune response of dogs vaccinated with leptospiral pentavalent outer envelope and whole culture vaccines. Am.J.Vet.Res. **39**, 831-836.

Bey, R.F., Johnson, J.C. (1982) Immunogenicity and humoral immune responses to leptospiral whole cell, outer envelope, and protoplasmic cylinder vaccines in hamsters and dogs. Am.J.Vet. Res. **43**, 835-838.

Bishop, L., Stranberg, J.D., Adams, R.J., Browstein, D.G., Patterson, R. (1979) Chronic active hepatitis in dogs associated with leptospires. Am.J.Vet.Res. **40** (6), 839-844.

Broom, J.C., Coghlan, J.D. (1958) quoted by Pritchard (1986) National status of leptospirosis in the U.K. In: The present state of leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff, 221-234.

Broom, J.C., Joshua, J.O. (1949) Leptospirosis. Vet.Rec. **6**, 711-713.

Broom, J.C., McIntyre, A.B. (1948) A survey of canine leptospirosis in England. Vet.Rec. **60**, 487-488.

Broughton, E.S., Scarnell, J. (1985) Prevention of renal carriage of leptospirosis in dogs by vaccination. Vet.Rec. **117**, 307-311.

Bryson, D.G., Ellis, W.A. (1976) Leptospirosis in a British domestic cat. J.Small.Anim.Pract. **17**, 459-465.

Carlos, E.R., Kundin, W.D., Watten, M.D., Tsai, C.C., Irving, G.S., Carlos, E.T., Director, A.C. (1971) Leptospirosis in the Philippines: Feline studies. Am.J.Vet.Res. **32** (9), 1455-1456.

Churcher, R.B., Lawton, J.H. (1987) Predation by domestic cats in an English village. J.Zool.Res. London **212**, 439-455.

Clegg, F.G., Heath, P.J. (1975) Subclinical *Leptospira ictero-haemorrhagiae* in dogs associated with a case of human leptospirosis. Vet.Rec. **96** (17), 385.

Coghlan, J.D., Bain, A.D. (1969) Leptospirosis in human pregnancy followed by death of the foetus. Br.med.J. **1**, **258**, 228-230.

Cole, J.E., Sangster, L.T., Sulzer, P.S., Pursell, A.R. (1982) Infections with *Encephalitozoon cuniculi* and *Leptospira interrogans* serovar *grippotyphosa* and *ballum* in a kennel of Foxhounds. J.Am.Vet. Med.Ass. **180** (2), 435-437.

Cunningham, M.P., McIntyre, W.I.M., Ives, J.C.J. (1957) The incidence of canine leptospirosis in Glasgow. Vet.Rec. **96**, 903-905.

Dabalis, T. (1986) Leptospirosis in Greece. A review. In: The present state of Leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff. 175-178.

Darke, P.G.G. (1983) In: Notes on Canine Internal Medicine. Publ. Wright, P.S.G. Bristol. 131; 132-136; 194-196.

Desmecht, M. (1986) Importance of leptospirosis in Belgium. In: The present state of Leptospirosis diagnosis and control. Ed. W.A.Ellis and T.W.A.Little. Publ. Martinus Nijhoff. 197-204.

Elliott, K.G. (1988) A simple step to enhance the demonstration of leptospira in Young's Warthin-Starry technique. Stain Technol. **63** (2), 122-123.

Ellis, T.M., Robertson, G.M., Husters, L. and Kirby, M. (1983) Detection of leptospires in tissue using an immunoperoxidase staining procedure. Aust.Vet.J. **60** (12), 364-367.

Ellis, W.A. (1986a) Leptospirosis. J.Small Anim.Pract. **27**, 683-691.

Ellis, W.A. 1986b) The diagnosis of leptospirosis in farm animals. In: The present state of Leptospirosis diagnosis and control. Ed. W.A. Ellis and T.W.A.Little. Publ. Martinus Nijhoff. 13-32.

Emanuel, M.L. Mackerras, I.M., Smith, D.J.W. (1964) The epidemiology of leptospirosis in North Queensland. 1. General survey of animal hosts. J.Hyg.Camb. **62**, 451-484.

Esseveld, H., Collier, W.A., Mochtar, A. (1940) quoted by Murphy *et al.* (1955) Prevalence of leptospiral agglutinin in sera of the domestic cat. Cornell Vet. **48**, 3-10.

Everard, C.O.R., Cazabon, E.P.I., Dreesen, D.W., Sulzer, C.R. (1979) Leptospirosis in dogs and cats on the Island of Trinidad, West Indies. Int.J.Zoonoses. **6** (1), 33-40.

Everard, C.O.R., Jones, C.J., Inniss, V.A., Carrington, D.G., Vaughan, A.W. (1987) Leptospirosis in dogs on Barbados. Israel J.Vet.Med. **43** (4), 288-295.

Faine, S., Stallman, N.D. (1982) Amended description of the genus *Leptospira* (Noguchi 1917) and the species *L.interrogans* (Stimson 1907; Wenyon 1926) and *L.biflexa* (Wolbach and Binger, 1914; Moguchi 1918). Int.J.Syst.Bact. **32**, 461-463.

Feign, R.D., Lobes, L.A.Jn., Anderson, D., Pickering, L. (1973) Human Leptospirosis from immunized dogs. Ann.Int.Med. **79** (6), 777-785.

Fennell, C. (1975) Some demographic characteristics of the domestic cat population in Great Britain with particular reference to feeding habits and the incidence of Feline Urological Syndrome. J.Small Anim.Pract. **16**, 775-785.

Fennestad, K.L. (1956) Nord.Vet.Med. **8**, 325. Quoted by Lucke and Crowther (1965). The incidence of leptospiral agglutinin titre in the domestic cat. Vet.Rec. **77** (23), 647-648.

Ferris, H., Andrews, R.D. (1965) *Leptospiral pomona* in the feral cat. Am.J.Vet.Res. **26** (11), 373-375.

Fessler, J.F., Morter, R.L. (1964) Experimental feline leptospirosis. Cornell Vet. **54**, 176-190.

Finco, D.R., Low, D.G. (1968a) Water, electrolyte and acid-base alterations in experimental canine leptospirosis. Am.J.Vet.Res. **29**, 1799-1807.

Finco, D.R., Low, D.G. (1968b) Fibrinogen content and fibrinolytic activity of plasma from dogs infected with *Leptospira canicola*. Am. J.Vet.Res. **29**, 2037-2040.

Fletcher, W. (1928) Recent work on leptospirosis *tsutsugamushi* disease and tropical typhus in the Federated Malay states. Trans.Roy.Soc. Trop.Med.Hyg. **21**, 265-288.

Gaumont, R., Trap, D. (1986) Leptospirosis in domestic animals in France: Current situation. In: The present state of Leptospirosis diagnosis and control. Ed. Ellis, W.A. and Little, T.W.A. Publ. Martinus Nijhoff, 179-184.

Gleiser, C.A. (1957) Experimental canine leptospirosis. III. Histopathologic changes. J.infect.Dis. **100**, 249-256.

Glosser, J.W., Johnson, R.C., Sulzer, C.R., Auran, N.E. (1974) Immunogenic properties of a leptospiral outer envelope (OE) bacterin in hamsters and foxes. Am.J.Vet.Res. **35** (5), 681-684.

Hanson, L.E. (1973) Immunologic problems in bovine leptospirosis. Am.J.Vet.Res. 7, 919-921.

Hanson, L.E. (1976) The pathogenesis of leptospirosis. In: The biology of parasitic spirochetes. Ed. R.C.Johnson, Publ. Academy Press. 295-305.

Hartman, E.G., Van Houten, M., Frik, J.F., Van der Donk, J.A. (1984a) Humoral immune response of dogs after vaccination against leptospirosis measured by an IgM and IgG specific ELISA. Vet. Immunol.Immunopath. 7, 33-42.

Hartman, E.G., Van Houten, M., Van der Donk, J.A., Frik, J.F. (1984b) Serodiagnosis of canine leptospirosis by solid phase ELISA. Vet. Immunol.Immunopath. 7, 33-42.

Hartman, E.G., Van den Ingh, T.S.G.A.M., Rothuizen, J. (1986) Clinical, pathological and serologic features of spontaneous canine leptospirosis: An evaluation of the IgM and IgG specific ELISA. Vet.Immunol.Immunopath. **13**, 261-271.

Hathaway, S.C., Little, T.W.A., Stevens, A.E. (1982a) Failure to demonstrate the maintenance of leptospires by house mice (Mus musculus) in the south east of England. Res.Vet.Sci. **32**, 387-388.

Hathaway, S.C., Little, T.W.A., Stevens, A.E. (1982b) Serological survey of leptospiral antibody in sheep from England and Wales. Vet.Rec. **110**, 99-101.

Hathaway, S.C., Little, T.W.A., Headlam, S.A., Stevens, A.E. (1983a) Infection of free living carnivores with leptospires of the *Australis* serogroup. Vet.Rec. **113**, 233-235.

Hathaway, S.C., Little, T.W.A., Stevens, A.E. (1983b) Serovar identification of leptospires of the *Australis* serogroup isolated from free living and domestic species in the U.K. Res.Vet.Sci. **35**, 64-68.

Heath, K.R., Box, P.G. (1965) Immunity to leptospirosis. Antibody in vaccinated and infected dogs. J.Comp.Path. **75**, 127-136.

Hemsley, L.A. (1956) *Leptospira canicola* and chronic nephritis in cats. Vet.Rec. **68**, 300-301.

Hovind-Hougen, K. (1986) Morphology of leptospires: Electron microscopic studies in relation to the classification of Leptospira. In: The present state of leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff. 1-12.

Hubbert, T.W., Shotts, B.E. (1966) Leptospirosis in kennel dogs. J.Am. Vet.Med.Ass., **148**, 1152-1159.

Huhn, R.G., Baldwin, C.D., Cardella, M.A. (1975) Immunity to leptospirosis: Bacterin in dogs and hamsters. Am.J.Vet.Res. **36** (1), 71-74.

Johnson, R.C., Harris, V.G. (1967) Differentiation of pathogenic and saprophytic leptospires: Growth at low temperatures. J.Bact. **94**, 27-31.

Johnson and Rodgers (1964) Ibid 85, 983-985 quoted by Turner, L.H. (1970) Leptospirosis III: Maintainance, isolation and demonstration of leptospires. Roy.Soc.Trop.Med.Hyg. **64** (4), 623-646.

Jones, C.K. (1964) Response of *Felis domestica* to leptospiral infections. South west Vet. **12** (3), 109-113.

Jones, T.C., Hunt, D.R. (1983) Veterinary Pathology. 4th Edn. Publ. Lea and Febiger, Philadelphia. p.1790.

Jungheer, E. (1944) Bovine leptospirosis. J.Am.Vet.Med.Ass. **105**, 276-281.

Kadis, S., Pugh, W.L. (1974) Urea utilization by leptospires. Infect. Immun. **10**, 793-801.

Kaufmann, A.F. (1976) Epidemiologic trends of leptospirosis in the United States, 1965-1974. In: The biology of parasitic spirochetes. 177-189.

Keenan, K.P., Alexander, A.D., Montgomery, C.A. (1978) Pathogenesis of experimental *Leptospira interrogans* serovar *bataviae* infection in the dog: Microbiological, clinical, haematologic and biochemical studies. Am.J.Vet.Res. **39** (1), 449-454.

Kirk, R.W., Bistner, (1985) Handbook of veterinary procedures and emergency treatment. Section 4: Clinical Procedures. 4th Edn. W.B.Saunders, Philadelphia, USA. p.490-494.

Knight, L.L., Miller, N.G., White, R.J. (1973) Cytotoxic factor in the blood and plasma of animals during leptospirosis. Infect.Immun. **8** (3), 401-405.

Krumbein, R., Frieling, B. (1916) Dtsch.Med.Wschr. **42**, 564, quoted by Alston and Broom (1958) In: Leptospirosis in man and animals. Publ. E.& S. Livingstone, London and Edinburgh. p.368.

Larsson, C.E., Santa Rosa, C.A., Larsson, M.A., Birgel, E.H., Fernandes, W.R., Paim, G.V. (1985) Laboratory and clinical features of feline leptospirosis. Int.J.Zool. **12**, 111-119.

Little, T.W.A. and Salt, G.F.H. (1975) Isolation of a leptospira of the *pomona* serogroup from a field vole. Vet.Rec. **97**, 56.

Little, T.W.A., Stevens, A.E., Hathaway, S.C. (1987) Serological studies of British leptospiral isolates of the *Serjroe* serogroup. III. The distribution of leptospires of the *Sejroe* serogroup in the British Isles. Epidem.Infect. **99**, 117-126.

Low, D.G., Hiatt, C.W., Gleiser, C.A., Bergman, E.N. (1956a) Experimental canine leptospirosis: *Leptospira icterohaemorrhagiae* infections in immature dogs. J.Infect.Dis. **98**, 249-259.

Low, D.G., Berman, E.N., Hiatt, C.W., Gleiser, A. (1956b) Experimental canine leptospirosis. II. Renal function studies. J.Infect.Dis. **98**, 260-265.

Low, D.G., Mather, G.W., Finco, D.R., Anderson, V.N. (1967) Long-term studies of renal functions in canine leptospirosis. Amm. J. Vet.Res. **28**, 731-739.

Lucke, V.M., Crowther, S.T. (1965) The incidence of leptospiral agglutination titres in the domestic cat. Vet.Rec. **77** (23), 647-648

Lucke, V.M., Davies, J.D. (1984) Progressive lymphocytic cholangitis in the cat. J.Small Anim.Pract. **25**, 249-260.

Lucke, V.M., Hunt, A.C. (1965) Interstitial nephropathy and papillary necrosis in the domestic cat. J.Path.Bact. **89**, 723-728.

McClain, J.B., Ballon, N.R., Harrison, S.M., Steinway, D.L. (1984) Doxycycline therapy for leptospirosis. Ann.Intern.Med. **100**, 696-698.

Mackintosh, C.G., Blackmore, D.K., Marshall, R.B. (1980) Isolation of *Leptospira interrogans* serovars *tarassovi* and *pomona* from dogs. New Zeal.Vet.J. **28** (5), 100.

Mason, R.W., King, S.J., McLahan, N.M. (1972) Suspected leptospirosis in two cats. Aust.Vet.J. **48** , 622-623.

McIntyre, W.I.M., Montgomery, G.L. (1952) Renal lesions in *Leptospira canicola* infection in dogs. J.Path.Bact. **64**, 145-160.

McIntyre, W.I.M., Stuart, R.D. (1949) Canine leptospirosis. Vet.Rec. **61** (20), 411-418.

Mertens, W. (1938) quoted by Murphy, L.C., Cardeilhac, P.T., Carr, J.W. (1958) Prevalence of leptospiral agglutinins in sera of the domestic cat. Cornell Vet. **48**, 3-10.

Michna, S.W. (1970) Leptospirosis. Vet.Rec. **86**, 484-496.

Michna, S.W., Ellis, W.A. (1973) Incidence of antibody to leptospirosis in dogs in Glasgow: a comparison of the conventional (Schuffner) and the rapid microscopical agglutination test (RMAT). Vet.Rec. **93** (24), 633-634.

Mochmann, H. (1955) Die verbreitung de canicola leptospirose in de DDR. Zentbl.fur bact.u.Parasitent. Abt.1.Originale. **164**, 551. English Abstract. Vet.Bull (1956) Abst. no. 2809.

Modric, Z. (1978) Natural and experimental leptospirosis in the cat. English summary. Veterinarski Archiv. **48** (3), 147-156.

Morris, J.A., Hussaini, S.N. (1974) Characterization of antibodies detected by the microscopic agglutination test for bovine leptospirosis. J.Hyg.Camb. **73**, 425-432.

Morrison, W.I., Wright, N.G. (1976) Canine leptospirosis: An immuno-pathological study of interstitial nephritis due to *L.canicola*. J.Pathol. **120**, 83-89.

Murphy, L.C., Cardeilhac, P.T., Carr, J.W. (1958) The prevalence of leptospiral agglutinins in the sera of the domestic cat. Cornell Vet. **48**, 3-10.

Navarro, C.E.K., Kociba, G.J. (1982) Hemostatic changes in dogs with experimental *Leptospira interrogans* serovar *icterohaemorrhagiae* infection. Am.J.Vet.REs. **43** (5), 904-906.

Navarro, C.E.K., Kociba, G.J., Kowalski, J.J. (1981) Serum biochemical changes in dogs with experimental *leptospira interrogans* serovar *icterohaemorrhagiae* infection. Am.J.Vet.Res. **42**, 1125-1129.

Negi, S.K., Myers, W.L., Segre, D. (1971) Antibody response of cattle to *Leptospira pomona*: Response measured by haemagglutination, microscopic agglutination and hamster protection tests. Am.J.Vet. Res. **32** (12) 1915-1920.

Newman, J.P. (1950) Studies of canine leptospirosis. I. Evaluation of laboratory diagnostic procedures. II. Serological determination of the incidence of latent infections in the Lansing, Michigan area. Am.J.Vet.REs. **11**, 405-411.

Okell, C.C., Dalling, T., Pugh, L.P. (1925) Leptospiral jaundice in dogs (yellows). Vet.J. **81**, 3.

Palmer, M.F. (1988) Laboratory diagnosis of leptospirosis. Med.Lab.Sci. **45**, 174-178.

Plesko, I. (1974) Cross immunity relations among lipase positive and negative leptospiral strains. Biologia (bratislava) **29** (9), 705-710.

Povey, R.C. (1985) In: Infectious Diseases of Cats: A clinical handbook. Centaur Press, Guelph, Ontario. p.1A1-1A4.

Pritchard, D.G. (1986) National situation of leptospirosis in the United Kingdom. In: The present state of leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff. 221-234.

Rees, H.G. (1964) Leptospirosis in a cat. New Zeal.Vet.J. **12**, 64.

Reis, R., Ryu, E., Motta, J.G., Correa, O., Wiest, J.M. (1973) Investigation of dogs for leptospiral agglutinins by the rapid micro-agglutination tests. Arquivos da Escola de Veterinaria Minas Gerais, **25** (1), 15-25. English summary. Vet.Bull. Vol.44. Abst. No. 465.

Ryu, E. (1976) An international survey of leptospiral agglutinin of dogs by the Rapid Macroscopic Agglutination Test (RMAT). Int.J. Zoonoses, **3**, 33-60.

Schonberg, A., Brem, S., Staak, C., Kampe, U. (1986) Leptospirosis in the Federal Republic of Germany - Preliminary results of a 1987 animal investigation program. In: The present state of leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff. 205-210.

Schuffner (1934) Recent work on leptospirosis. Trans.Roy.Soc.Trop. Med.Hyg. **28**, 7.

Shaw, D.H., Rubin, S.I. (1986) Pharmacologic activity of Doxycycline. J.Am.Vet.Med.Ass. **189**, 808-810.

Shophet, R. (1979) quoted by Shophet, R. (1980) A serological survey of leptospirosis in cats. New Zeal.Vet.J. **27**, 236-246.

Shophet, R., Marshall, R.B. (1980) An experimentally induced predator chain transmission of *Leptospira ballum* from mice to cats. Br.Vet. J. **136**, 265-270.

Sleight, S.D., Williams, J.A. (1961) Transmission of canine leptospirosis by coition and artificial insemination. A preliminary report. J.Am. Vet.Med.Ass. **138**, 152-152.

Spencer, A.J., Wright, N.G. (1981) Chronic interstitial nephritis in the dog: an immunofluorescence and elution study. Res.Vet.Sci. **30** (2), 226-232.

Stoenner, H.G. (1976) Treatment and control of leptospirosis. In: The biology of parasitic spirochetes. Publ. Academy Press. 375-388.

Stuart, R.D. (1946) Canine leptospirosis in Glasgow. Vet.Rec. **58**, 131-132.

Taylor, P.L., Hanson, L.E., Simon, J. (1970) Serologic, pathologic and immunologic features of experimentally induced leptospiral nephritis in dogs. Am.J.Vet.Res. **31** (6), 1033-1046.

Terpstra, W.J., Korver, H., Van Leeuwen, J., Kolk, A.H.J. (1986) Alternative methods for the classification of leptospiral serovars. In: The present state of leptospirosis diagnosis and control. Ed. W.A.Ellis, T.W.A.Little. Publ. Martinus Nijhoff. 83-90.

Thiermann, A.B. (1977) Incidence of leptospirosis in the Detroit rat population. Am.J.Trop.Med.Hyg. **26**, 970-974.

Thiermann, A.B. (1980) Canine leptospirosis in Detroit. Am.J.Vet.Res. **41** (10), 1659-1661.

Thomas, S. (1980) Leptospirosis apparently due to *L.bratislava* in a dog. Vet.Rec. **106** (8), 178-179.

Thompson, J.C., Manktelow, B.W. (1986) Pathogenesis and red blood cell destruction in haemoglobinaemic leptospirosis. J.Comp.Path., **96**, 529-539.

Timoney, J.F., Sheahan, B.J., Timoney, P.J. (1974) Leptospiral and infectious canine hepatitis (ICH) virus antibodies and nephritis in Dublin dogs. Vet.Rec. **94** (14), 316-319.

Torten, M., Ben Efraim, S., Shenberg, E., Van der Hoeden, J. (1967) The detection of autoantibodies to kidney tissue in a dog experimentally infected with *Leptospira canicola*. Refuah Vet. **24**, 185-187.

Torten, M., Kaplan, A., Shenberg, E. (1971) A survey of leptospirosis in stray dogs. Jap.J.Microbiol. **28** (3), 98-101. English abstract. Vet.Bull. (1972) Vol.42. Abstract No. 1775.

Trifunovic, Z., Nesic, D. (1986) Leptospirosis in cats. (*Felis domestica*) on a dairy farm. Acta Veterinaria. Yugoslav. **36** (5/6), 301-306.

Turner, L.H. (1970a) Leptospirosis II. Serology. Tran.Roy.Soc.Trop. Med.Hyg. **62** (6), 880-889.

Turner, L.H. (1970b) Leptospirosis III. Maintainance, isolation and demonstration of leptospires. Trans.Roy.Soc.Trop.Med.Hyg. **64**, 623-646.

Twigg, G.I. (1973) Rat-borne leptospirosis in wildlife and on the farm. Mammal.Rev. **3**, 37-42.

Twigg, G.I., Cuerden, C.M., Hughes, D.M. (1968) Leptospirosis in British wild mammals. Sym.Zool.Soc.Lond. **24**, 75-98.

Twigg, G.I., Hughes, D.M., McDiarmid, A. (1971) Occurrence of leptospirosis in thoroughbred horses. Equine Vet.J. **3**, 52-55.

Waitkins, S.A. (1986) Leptospirosis in man, British Isles, 1984. Brit. Med.J. **292**, 1324.

Watson, A.D.J., Wannan, J.S. (1973) The incidence of leptospiral agglutinins in domestic cats in Sydney. Aust.Vet.J. **49**, 545.

Watson, A.D.J., Wannan, J.S., Porges, W.L., Testoni, F.J. (1976) Leptospiral agglutinin in dogs in Sydney. Aust.Vet.J. **52**, 970-974.

Watt, G., Alquiza, L.M., Padre, L.P., Tuazon, W.L., Laughlin, L.W. (1988) A rapid diagnosis of leptospirosis. A prospective comparison of the Dot Enzyme-Linked Immunosorbent Assay (Dot ELISA) and the genus specific Microscopic Agglutination Test (MAT) at different stages of illness. J.Infect.Dis. **157** (4), 840-842.

Weaver, A.D. (1962) Leptospirosis in greyhounds. Vet.Rec. **74**, 1457-1463.

WHO (1982) Guidelines for the control of leptospirosis. WHO offset Publication No.67. Ed. S.Faine. p.171.

Wolff, J.W. (1954) The laboratory diagnosis of leptospirosis. Publ. Charles C. Thomas. p.129.

Wong, M.L., Kaplan, S., Dunkle, L.M., Stechenberg, B.W., Feigin, R.D. (1977) Leptospirosis : A childhood disease. J.Pediatr. **90**, 532-537.

Young, B.J. (1969) A reliable method for demonstrating spirochetes in tissue sections. J.Med.Lab.Technol. **26**, 248-252.