PREVALENCE OF LEPTOSPIROSIS AMONG DOGS IN THRISSUR

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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Department of Preventive Medicine
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DECLARATION

I hereby declare that this thesis entitled **"PREVALENCE OF LEPTOSPIROSIS AMONG DOGS IN THRISSUR"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy
1-12-1997

INDU, S.

CERTIFICATE

Certified that this thesis, entitled "**PREVALENCE OF LEPTOSPIROSIS AMONG DOGS IN THRISSUR**" is a record of research work done independently by **Miss Indu, S.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Miss Indu, S.,** a candidate for the degree of Master of Veterinary Science in Preventive Medicine, agree that the thesis entitled **"PREVALENCE OF LEPTOSPIROSIS AMONG DOGS IN THRISSUR"** may be submitted by Miss Indu, S., in partial fulfilment of the requirement for the degree.

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INDU, S.

Dedicated to

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CHAPTER-I

INTRODUCTION

Leptospirosis is a zoonosis of world wide distribution being responsible for heavy economic loss in the livestock industry due to loss of milk production, still birth, abortion and infertility (Thiermann, 1984). It is caused by several pathogenic species of leptospira having broad spectrum of hosts (Parma *et al.*, 1997).

Every animal which is known to be infected by leptospirosis is considered a possible carrier and disseminator of leptospirosis not only to the same species but also to others, including man (Torten, 1979). Serovars of leptospires that infect reservoir hosts have become the most prevalent serovars in the world, resulting in endemic infection (Heath and Johnson, 1994).

Among domestic animals, the dogs play an important role in the transmission of leptospirosis to man and other animals. The distribution of leptospires among dogs is very high and their infection is obviously facilitated by their habit of sniffing where other dogs have urinated (Babudieri, 1958).

Serologic surveys have revealed that leptospiral agglutinating antibodies can be found in upto 60 per cent of

the canine populations throughout the world (Everard *et al.*, 1987; Venkataraman and Nedunchelliyan, 1993 and Brihuega *et al.*, 1995).

Dogs acquire *L. icterohaemorrhagiae* infection from carrier rats but transmission of *L. canicola* is direct from dog to dog via contaminated urine. The most common mode of transmission is by direct contact with infected urine or ingestion of water or food contaminated with infected urine. Outbreaks have occurred in man and dog after flood or swimming in or drinking water contaminated by urine of rodents or other animals (Clegg and Heath, 1975; Venkataraman and Nedunchelliyan, 1992).

Leptospira serovars *icterohaemorrhagiae* and *canicola* were reported as the primary pathogen in canine leptospirosis till 1984. However in 1990 *L.* serovar *grippotyphosa* was noted as an important pathogen in dogs and evidence now suggests that *L.* serovar *pomona* may also be a common canine pathogen (Rentko *et al.*, 1992). Dogs are incidental host for infection with *L.* serovars *autumnalis*, *australis*, *tarassovi*, *ballum*, *bataviae* and *bratislava* (Greene and Shotts, 1990).

Subclinical or latent infections are of great importance in the epidemiology of leptospirosis as clinically recovered animals are often carriers for long periods (Taylor *et al.*,

1970). The average duration of carriership of leptospirosis in dogs is 120 days (Ratnam, 1994).

Infection in man is incidental and with rare exception it represents a dead end in the chain of transmission. Man gets infected through occupational exposures and by close contact with infected pets (Hanson, 1982).

The prevalence of leptospira serovars in human population, generally, is a reflection of the prevalence in animal population with which those people have contact. Infection with *L. icterohaemorrhagiae* is associated with exposure to infected dogs and is the most commonly diagnosed leptospiral infection in man (Heath and Johnson, 1994). It is likely that canine urine is the most probable source of *canicola* infection for dog or man. The isolation of serovar *pomona* from the urine of dogs indicates that the dogs should be considered as a possible source of this serovar. This further indicates the role of dogs in the transmission of pomona to man and livestock causing in-apparent leptospirosis (Morter *et al.*, 1959).

The mobility of animals and warm wet conditions of the environment facilitate the dissemination of infection from one area to another and from one reservoir to another.

In Kerala, leptospirosis was surveyed in cattle and pig farms with the history of abortion (Sivadas *et al.*, 1970). The murine reservoir problem was focussed by Adinarayanan and James (1980a) and they could isolate a total of 28 strains.

It is noted that though the leptospira reservoir status in Kerala was assessed among cattle, pig, goat, sheep and rodents, dogs with so close an association with man have not been studied. Dogs as companion animals and watch dogs are maintained by a large section of people. The population of stray dogs is rising, unchecked. From the zoonotic perspective and incidence of leptospirosis in livestock, knowledge of the status of leptospirosis among dogs is very important. This can help in the effective control of leptospirosis in dogs and in turn in man and other animals.

Hence, the present study was undertaken with the following objectives.

1. To assess the prevalence and magnitude of leptospirosis among dogs in and around Thrissur by serological and/or cultural screening of dogs.

2. To find out the alterations of haematological and biochemical values, consequent to leptospiral infection.

Review of Literature

CHAPTER-II

REVIEW OF LITERATURE

2.1 History

Leptospirosis, an acute illness associated with febrile jaundice and nephritis, was first recognised by Adolf Weil in 1886 as a clinical entity distinct from other icteric fevers (Gutman, 1976).

The term Weil's disease was first used by Goldsmidt, 1887 to denote the severe febrile illness with jaundice and renal abnormalities that had been described by Adolf Weil (Pritchard, 1990). A canine disease similar to the human infection became an epidemic in Germany in 1898 when it was first called as Stuttgart disease (Kenzy and Ringen, 1967).

The causative agent of Weil's disease *Leptospira icterohaemorrhagiae* was discovered in 1914 by Inada and Ito in Japan and was also found in Germany the following year by Hubener and Reiter and by Uhlenhuth and Fromme. It was called *Spirochaeta icterogenes* by the German workers (Burrows, 1968).

In 1917, Noguchi isolated the organism from a patient with jaundice and haemorrhage and suggested the generic name "Leptospira" under spirochaetes (Ratnam, 1994).

Courmont and Durand in 1917 found that puppies could be infected with the spirochaetes which produced typical jaundice in man (Kenzy and Ringen, 1967).

In 1916 Uhlenhuth and Fromme found typical leptospires in the liver and kidneys of a jaundiced wolf hound

The organism *Spirochaetosis icterohaemorrhagiae* was found by Kirkwood and Horning (1923) and the disease was termed "sore mouth of dogs". In 1924 Lukes and Drivacek independently observed organisms which were morphologically identical to Leptospires in the tissues of dogs which had died of Stuttgart disease. In the following year Okell and others found leptospiral organisms in epizootic jaundice in tissues of hunting dogs in England. The canine strain of the leptospires was studied by Klarenbeek (1928) who referred to it as *Spirochaeta ictero-uraemia canis*. The organism was subsequently admitted to be the classical organism Leptospira *interrogans* serotype *icterohaemorrhagiae* (Kenzy and Ringen, 1967).

Klarenbeek and Schuffner in 1933 isolated a type of leptospira distinct from *L. icterohaemorrhagiae* from dogs and named this organism *L. canicola* (Stuart, 1946).

2.2 Incidence of leptospirosis in dogs

2.2.1 Abroad

In consequence of the findings of leptospirosis in dogs, serological investigations were carried out in dogs in various parts of the world as early as 1936 (Stuart, 1946).

Uhlenhuth and Zimmermann (1936) found 15 to 20 per cent of the dogs examined to be infected with leptospirosis, of which two-thirds reacted with *Leptospira canicola*.

Babudieri and Castagnoli (1940) in an examination of 159 sera samples from dogs in Rome found 54 sera positive for *L. icterohaemorrhagiae* and two for *L. canicola*.

Stuart (1946) carried out serological test for leptospiral antibodies in two groups of dogs in Glasgow. In the general group of house dogs about 40 per cent had been infected with *L. canicola* and only six per cent with *L. icterohaemorrhagiae*. In the kennel dogs, 28 per cent had been infected with *L. icterohaemorrhagiae* and none with *L. canicola*.

An investigation of 416 dogs brought to the Royal Dick Veterinary College in U.K. pointed to leptospiral infection in 116 (89.2 per cent) of 130 dogs with detectable renal

disease, and 33 (23.6 per cent) of 140 other dogs (McIntyre, 1949).

Murphy *et al.* (1958) recorded 16.2 per cent prevalence of leptospirosis by microscopic agglutination test (MAT) among 357 dogs from Pennsylvania. Among the reactors, 41 per cent were seropositive to *L. autumnalis*, 24 per cent to *L. canicola*, 12 per cent to *L. icterohaemorrhagiae*, eight per cent to *L. grippotyphosa* and three per cent to *L. ballum* and ten per cent of the dogs showed agglutinins of the same order against multiple serotypes.

Schnurrenberger *et al.* (1962) demonstrated leptospiral agglutinins in 12 (7.3 per cent) of 164 pet dogs in Pennsylvania. Of these two had antibodies to *L. pomona*, four to *L. icterohaemorrhagiae*, three to *L. canicola* and three to antibodies to both serotypes. In the 51 stray dogs examined, six (11.3 per cent) were positive of which four were positive to *L. canicola* and two to *L. pomona*.

Serological survey of 150 apparently normal dogs in Brisbane revealed the presence of leptospiral antibodies in eleven sera (7.3 per cent) tested. Reactions were noted against serotypes *L. esposito*, *L. icterohaemorrhagiae*, *L. pomona* and *L. zanoni* (Spradbrow, 1962).

Carlos *et al.* (1971) surveying 507 dogs in Manila by macroscopic and microscopic agglutination tests reported significant titres to leptospiral antigens in 66 (13 per cent) cases. The predominant reactions observed were against *L. bataviae* (twelve), *L. javanica* (eleven), *L. icterohaemorrhagiae* (eight), *L. pyrogenes* (six), *L. grippotyphosa* (six), *L. canicola* (three), *L. cynopteri* (three), *L. autumnalis* (one) and *L. hebdomadis* (one).

Michna and Ellis (1973) recorded leptospiral antibodies in 43 (39 per cent) of 120 sera samples from dogs in Glasgow by Schuffner's technique. Of these 37 (31 per cent) reacted with *L. canicola* serotype, five (four per cent) with *L. icterohaemorrhagiae* and the remaining five had the same titre to both antigens.

In Berlin among the 7750 dogs tested for antibodies to Leptospira species, 640 (8.26 per cent) were positive. Of these 377 (five per cent) were positive to *L. grippotyphosa*, 216 (three per cent) to *L. icterohaemorrhagiae* and 47 (0.6 per cent) to *L. canicola* (Horsch and Kutschmann, 1974).

Rosa *et al.* (1974) from Brazil reported that of the serum samples from 136 dogs tested by the microscopic agglutination test (MAT) eight (5.9 per cent) were positive for leptospirosis.

Sera from 446 dogs in the Dublin area were examined for antibodies to leptospirae and found 152 (34.1 per cent) seropositive. Of these 17.0 and 15.5 per cent had microscopic serum agglutinin titres to *L. canicola* and *L. icterohaemorrhagiae* respectively and 1.6 per cent of sera had the same titre to both serotypes (Timoney *et al.*, 1974).

Topacia *et al.* (1974) reported that 41 (12.7 per cent) of 321 dogs from Philippines studied were positive to the agglutination lysis test using 14 leptospiral antigens. The principal serotypes involved were *L. pyrogenes* (15 reactors), *L. canicola* (14) and *L. grippotyphosa* (five). A few reacted to *L. javanica*, *L. manilae* and *L. autumnalis*.

Of the 600 sera of dogs in Sydney, 41 (6.8 per cent) had significant titres against one or more serotypes. Thirty samples (five per cent) reacted against *L. copenhageni*, six (one per cent) against *L. pomona*, while a few samples had significant titres against *L. hardjo*, *L. tarassovi*, *L. australis*, *L. grippotyphosa* and *L. pyrogenes* serotypes (Watson *et al.*, 1976).

Droge and Vick (1977) reported that from among 167 dogs with suspected leptospirosis in Germany, 36 per cent had positive agglutination lysis titres. The serotype involved most frequently was *L. grippotyphosa*, followed by *L. canicola*, *L. icterohaemorrhagiae* and *L. bovis*. There were only

occasional reactions for *L. pomona, L. sejro, L. australis* and *L. autumnalis.*

Epidemiological studies in animals in Dageston, Moscow by microagglutination test revealed 25 per cent positive reaction to *Leptospira* serogroup *hebdomadis* and 25 per cent to *pyrogenes* among the dogs (Akhmedov *et al.*, 1979).

In Bulawayo, Banks and Pigott (1979) detected complement fixing antibody to *Leptospira canicola* in two dogs in which leptospirosis was diagnosed clinically and in 19 out of the 146 dogs (13.7 per cent).

Serological tests of dogs in Trinidad showed that 55 per cent of stray dogs had been exposed to leptospirosis. Serogroups *L. canicola, L. icterohaemorrhagiae* and *L. hebdomadis* were found most frequently (Everard *et al.*, 1979).

Higgins *et al.* (1980) examined eight canine sera from suspected cases of leptospirosis. Of these one (12.5) per cent) reacted with *L. icterohaemorrhagiae.*

Following an outbreak of leptospirosis in a pack of 35 hounds in South Auckland, 26 (74.28 per cent) were seropositive to serogroup *L. icterohaemorrhagiae* (Mackintosh *et al.*, 1980).

Thiermann (1980) found significant titres to one or more leptospiral serotypes in 164 (37.8 per cent) of the 433 urban stray dogs and 23 (18.7 per cent) of the 123 suburban stray dogs in Detroit. Among the urban stray dogs, serotype reaction was *L. icterohaemorrhagiae* (103), *L. canicola* (5), *L. pomona* (7), *L. grippotyphosa* (12), *L. icterohaemorrhagiae* and *L. canicola* (11), *L. icterohaemorrhagiae* and *L. pomona* (2), *L. icterohaemorrhagiae* and *L. grippotyphosa* (4), *L. canicola* and *L. pomona* (6), *L. icterohaemorrhagiae*, *L. canicola* and *L. pomona* (14). In the suburban stray dogs, reaction was *L. icterohaemorrhagiae* (16), *L. canicola* (3), *L. icterohaemorrhagiae* and *L. canicola* (2), *L. icterohaemorrhagiae* and *L. pomona* (2).

Yasuda *et al.* (1980) reported 21.6 per cent of leptospirosis prevalence after screening 1428 samples of stray dogs in Brazil. Reaction was mainly against *L. canicola* (51 per cent) and *L. icterohaemorrhagiae* (25.5 per cent) with four to nine per cent reacting to *L. grippotyphosa*, *L. pomona* and *L. ballum* antigens.

Sera from 582 dogs in Missouri subjected to microscopic agglutination test for leptospirosis revealed significant titres to one or more leptospiral antigens *L. canicola* (18), *L. pomona* (two) and *L. icterohaemorrhagiae* (10) (Marx *et al.*, 1981).

Cornide *et al.* (1985) conducted the micro-agglutination test using 14 leptospiral serovars on 424 sera samples from dogs in Cuba. Out of which 120 (28.3 per cent) gave positive reaction for 11 serotypes and the highest reactor rate was for *copenhageni*, followed by *butembo*, *pyrogenes*, *canicola* and *grippotyphosa*.

Umeki *et al.* (1985) reported that out of the 457 pet dogs, 6.8 per cent reacted to *L.* serovar *icterohaemorrhagiae* and 6.6 per cent to *L.* serovar *canicola*. Among 89 stray dogs, reaction to *L.* serovars *icterohaemorrhagiae* and *canicola* were 25 per cent and 7.9 per cent respectively.

In serological surveys of leptospirosis carried out between 1982 and 1983 in Italy, 157 (31.52 per cent) of 498 dogs were positive. The percentage of animals positive to the different leptospiral serotypes were 73.88 per cent to *icterohaemorrhagiae*, 22.92 per cent *bratislava*, 1.27 to *canicola* and 1.27 to *hardjo* (Andreani *et al.*, 1986).

During 1979 to 1983, 1368 canine sera from suspected leptospirosis cases in England and Wales reacted to serovars *L. canicola* and *L. icterohaemorrhagiae* at a level of one per cent and six per cent respectively (Pritchard, 1986).

Prokopcakova *et al.* (1986) reported prevalence of leptospirosis at 6.8 per cent among 350 dogs of Slovakia. Out

of 24 positive samples, 10 samples gave positive reaction to *L. grippotyphosa*, six to *L. pomona*, three to *L. icterohaemorrhagiae*, three to *L. bratislava* and two to *L. sejroe*.

Everard *et al.* (1987) examined sera for leptospirosis from 122 urban dogs of Barbados. From dogs of the island, 50 (41 per cent) were positive and 76 per cent reacted to antigens in the *Leptospira* serogroup *icterohaemorrhagiae* and/or *autumnalis*. Of 166 suburban dogs, 70 (42 per cent) were seropositive with *L. icterohaemorrhagiae*, *L. autumnalis* and *L. australis*.

From sera of dogs in Illinois evaluated for leptospiral antibodies, five per cent reaction was to *Leptospira* serovar *bratislava*, 2.3 per cent to *canicola*, 1.2 per cent to *grippotyphosa* and 2.3 per cent to *icterohaemorrhagiae* (Nielsen *et al.*, 1991).

Prescott *et al.* (1991) examined 474 sera samples from dogs of Ontario with the microscopic agglutination test for leptospiral serovars. Of the 39.2 per cent 26.2 per cent reacted at low titres to *L. canicola* or *L. icterohaemorrhagiae* or both, 8.2 per cent to *L.* serovar *bratislava*, 3.8 per cent to *L. autumnalis*, 3.2 per cent to *L. pomona* 3.0 per cent to *L. hardjo* and 1.9 per cent to *L. grippotyphosa*.

Rentko *et al.* (1992) reported on 17 clinical cases of leptospirosis in dogs, all of which had serological evidence of infection with *L. pomona* and *L. grippotyphosa*.

Myburgh *et al.* (1993) tested serum samples from 400 stray dogs from Pretoria area for leptospiral antibodies using the microscopic agglutination test. The antibody prevalence was 1.5 per cent against *L. serovars, tarassovi* and *pyrogenes*.

Brihuega *et al.* (1995) analysed 260 serum samples from dogs in Argentina for leptospiral antibodies, 58.07 per cent were positive. Serovars identified were *L.* serovar *canicola* (49.66 per cent), *copenhageni* (33.11 per cent), *castellonis* (9.93 per cent), *pyrogenes* (6.62 per cent) and *pomona* (0.66 per cent).

Microscopic agglutination test with antigens of various *Leptospira* serovars - *bratislava, castellonis, canicola, grippotyphosa, copenhageni, pomona, hardjo* and *tarassovi* were carried out on blood samples from 31059 domestic and 1092 wild animals in Italy, of which 197 (29 per cent) of the 680 dogs tested were seropositive (Tagliabue and Farina, 1995).

2.2.2 India

Ayyar (1932) recorded an outbreak of leptospiral jaundice among the Madras hounds in which 29 out of 54 dogs succumbed.

Leptospires were detected by dark ground illumination in ground up liver and kidney from a hound and in two guinea-pigs experimentally inoculated. Sera from four hounds reacted to *L. icterohaemorrhagiae*.

Dasgupta and Sen (1945) similarly described leptospiral jaundice attributable to *L.* serovar *icterohaemorrhagiae* in dogs of Calcutta.

Ball and Sheikh (1958) surveyed leptospirosis among man, dog, sheep and horse in Bombay. Out of the 120 dogs examined, 11 were sero positive (9.2 per cent) of which seven reacted with only one serotype - *L. icterohaemorrhagiae* (2), *L. pyrogenes* (4) and *L. pomona* (1). The multiple reaction to serotypes were against *L. canicola* and *L. sejroe*, *L. pyrogenes* and *L. grippotyphosa*. Two other sera agglutinated *L. icterohaemorrhagiae*, *L. canicola* and *L. pyrogenes* and in addition one of these reacted with *L. pomona* and the other with *L. grippotyphosa*.

Rajasekhar and Nanjiah (1971) in a serological survey of leptospirosis in dog, cattle, sheep, goats, pigs and horses in Bombay reported that ten per cent of the 40 dogs reacted with *L. canicola*, and one showed dual reaction with *L. pyrogenes*. Reaction to *L. pomona* was significant in pig, goat and sheep sera.

Jawad Hussain (1973) reported 9.59 prevalence to *L. canicola* and *L. icterohaemorrhagiae* serotypes among dogs in Madras.

Microagglutination test on sera of 63 ailing dogs at Madras indicated 26 (39 per cent) to be seropositives for leptospirosis. Seventeen cases reacted to *L. icterohaemorrhagiae* and nine to *L. canicola* (Basha *et al.*, 1982).

Overall prevalence of 34.3 per cent was detected among 137 dogs tested for leptospirosis by macroscopic agglutination test, in Punjab. Serotype analysis showed 15.3 per cent reaction to *L. canicola*, 12.4 per cent to *L. icterohaemorrhagiae* 5.1 per cent to *L. pomona* and 1.4 per cent to serotype *L. hardjo* (Verma, 1982).

Ratnam *et al.* (1983) recorded 54 per cent incidence of leptospirosis among 50 sick dogs presented at Madras Veterinary College Hospital. Among the 27 positive cases, 18 reacted to *L. icterohaemorrhagiae*, 17 to *L. canicola*, 9 to *L. pomona* and 6 to *L. hebdomadis*.

Venkataraman and Nedunchelliyan (1992) in an epidemiological study of an outbreak of leptopirosis among dog and human being in Madras, recorded 21.27 per cent reactors among 94 dogs. Of the 20 positive sera, ten reacted to

serovar *L. icterohaemorrhagiae*, nine to *L. canicola* and one to serovar *L. pomona*.

From 474 sera from dogs with the history of vomition, diarrhoea, jaundice and nephritis, 49 cases (10.34 per cent) were positive in the microscopic agglutination test for leptospirosis. Thirty one reacted to *Leptospira* serovar *canicola*, fifteen to *icterohaemorrhagiae*, and three to *pomona* (Venkataraman and Nedunchelliyan, 1993).

Thirunavukkarasu *et al.* (1995) in a study on leptospirosis in dogs, revealed 11 (17.10 per cent) of the 64 dogs examined to be seroreactive, 45.45 per cent reacted to *L. icterohaemorrhagiae* and 54.44 per cent to *L. canicola*.

2.2.3 Kerala

Adinarayanan and James (1970) detected leptospiral organisms in the bladder tap collection of urine from an adult mongrel dog, without any clinical symptoms.

Incidence of abortion in pigs and cattle associated with leptospirosis was recorded. Nine of the 47 pigs and 15 of 25 cows showed serum titres of 1:30 to 1:100 to *Leptospira* serotypes *australis*, *hebdomadis*, *tarassovi* (hyos) and *grippotyphosa* (Sivadas *et al.*, 1970).

Adinarayanan and James (1980a) isolated 28 strains of leptospires in Kerala, comprising 14 isolates from pigs, eight from bandicoots, one each from an aborted bovine foetus, a sheep, goat, rat and mongoose and a solitary isolate from effluents in a piggery.

Adinarayanan and James (1980b) reported isolation of *L.* serogroups Autumnalis from rat and bandicoot, Hebdomadis and Javanica from bandicoot, and Tarassovi from pig which are the earliest recoveries of those species in India. Strain *kerali*, serovar *menoni* in *Javanica* serogroup and strain *Mannuthi*, serovar *dikkeni* in Hebdomadis serogroup were new biotypes recorded for the first time.

2.3 Age

In a serological survey among the dogs in Glasgow the greater percentage of reaction was in the upper age group of above five years probably associated with the higher survival rate of the older animals exposed to leptospiral infection (Stuart, 1946).

Based on a clinical and serological study in dogs, invasive phase of the disease was identified mostly in young dogs by positive blood culture. Severe form of the primary renal disease was encountered in dogs under four years,

whereas severe form of the secondary renal disease was seen both in young and old dogs (McIntyre, 1949).

Morter *et al.* (1959) reported that six dogs, six months to two years of age, of a farm were serologically positive for *Leptospira pomona*. An eight month old German shephered dog had a serum titre of 1:10,000 for *L. pomona*. There was no clinical history of leptospiral infection. Some of the young dogs had been slightly depressed with a concurrent mild febrile state.

A gradual increase in the incidence of leptospiral infection with maturity has been reported by Borg-Petersen and Fennestad (1962) and Thomas and Evans (1967).

Dogs of one to two years showed the highest percentage of leptospiral antibodies in a prevalence study in Pennsylvania (Schnurrenberger *et al.*, 1962).

Acute febrile illness due to *L. canicola* had been observed in the different age groups - a five month old pup (Hubbert and Shotts, 1966); 14 month old dog (Dwivedi *et al* 1988) and in a bitch aged 2½ years (Venkataraman and Nedunchelliyan, 1990b).

Ryu (1975) observed a higher prevalence of leptospirosis in the age group of less than four years among dogs in Japan.

Dogs of younger age group of 14-22 weeks (Navarro *et al.*, 1981); three months (Arimitsu *et al.*, 1989); six months (Venkataraman *et al.*, 1994) were found to be susceptible to experimental infection with leptospirosis.

Verma (1982) opined that older dogs were more affected with leptospirosis than the younger ones in a prevalence study in Punjab. In the young dogs the antibody titre was minimal. Antibodies were detected in pups of about 3 months of age, suggesting that these might be maternal antibodies.

In a serological study in Madras city, 54 per cent incidence was detected in dogs above six months of age (Ratnam *et al.*, 1983).

Arimitsu *et al.* (1989) reported a fatal case of acute leptospirosis in a six year old Beagle dog with serological reaction to *L. icterohaemorrhagiae* and *L. canicola*.

Based on an incidence study in Madras, dogs of one to four years of age were the mostly affected group, reacting to serovars *L. canicola*, *L. icterohaemorrhagiae* and *L. pomona* (Venkataraman and Nedunchelliyan, 1990a).

Nielsen *et al.* (1991) observed in two dogs, aged five month and three years, serological and cultural evidence of *bratislava* infection.

During an outbreak of leptospirosis in dogs in Madras, among the age group of less than one year, 22.58 per cent were positive to *L. canicola* and *L. icterohaemorrhagiae*, whereas in those older than one year of age, 20.63 per cent were positive to serovar *L. canicola*, serovar *L. icterohaemorrhagiae* and serovar *L. pomona* (Venkataraman and Nedunchelliyan, 1992).

2.4 Sex

Among dogs, an increased incidence of Leptospirosis was reported in males than in females. (Meyer *et al.*, 1939; Pearson, 1964; Torten *et al.*, 1971; Noda and Ryu, 1973; Ryu, 1975).

Stuart (1946) documented a higher incidence of leptospirosis in male dogs, more due to serovar *canicola* (47%) than *icterohaemorrhagiae* (7%).

Among the dogs of a farm screened for leptospiral antibodies, serological reaction of *L. pomona* was more (Morter *et al* 1959).

The percentage of reactors to canine leptospirosis was slightly higher in the females (Borg-Peterson and Fennestad, 1962).

Schnurrenberger *et al.* (1962) perceived no significant difference in the incidence of leptospirosis between male and female.

The sex difference in susceptibility to leptospira serovars was found to be inconclusive by Thomas and Evans (1967).

Venkataraman and Nedunchelliyan (1992) have concluded from a serological study of an outbreak in Madras, that incidence of leptospirosis is more among the male dogs (24%) compared to female (16%). Male dogs reacted more to *L. icterohaemorrhagiae* (9), than *L. canicola* (6).

In a seroepidemiological study of 474 serum samples from dogs, Venkataraman and Nedunchelliyan (1993) have noticed a higher prevalence in male dogs (10.94%) than in females (8.08%).

Brihuega *et al.* (1995) studying on leptospirosis in dogs in Argentina have shown 65.56 per cent of the positive reactors to be male dogs and 34.43 per cent were females.

2.5 Breed

Meyer *et al.* (1939) and Moreira Caldas *et al.* (1977) observed that dogs of all breeds were susceptible to leptospirosis.

Outbreaks of *L. icterohaemorrhagiae* have been recorded among packs of hounds (Ayyar, 1932; Corbould, 1968 and Mackintosh *et al.*, 1980).

From a Beagle dog with acute febrile illness which turned fatal leptospiral agglutinins were detected in the serum. *Leptospira canicola* was isolated from other Beagle dogs of the same kennel (Hubbert and Shotts, 1966).

Mackintosh *et al.* (1980) had given an account of isolation of *L. tarassovi* from the urine of four hounds in South Auckland. On experimental inoculation of the tarassovi isolate, the dogs became infected without showing any clinical signs and developed leptospiruria which persisted for over seven months.

Beagles were found susceptible to *L.* serovar *icterohaemorrhagiae* and the clinical findings were divided as subclinical with fever for two to three days, mild with fever, mild icterus and congested sclera; severe disease with fever, icterus, dehydration, anorexia and weakness. Necropsy lesions

and serum biochemical constituents correlated with the clinical disease in infected Beagles (Navarro *et al.*, 1981).

Hartman (1984) reported a higher incidence of leptospirosis among the guard dogs and sporting dogs.

Stuttgart disease had been reported in a Doberman dog and a Pomeranian bitch (Dwivedi et al., 1988; Venkataraman and Nedunchelliyan, 1990b).

From a study in Italy, 14 dogs were reported to have died of acute leptospirosis with signs of icterus, anorexia and severe depression (Scanziani *et al.*, 1994). Of these dogs, German Shepherd, Boxer, English setter and Crossbred dogs showed a high titre to *L. bratislava* and *L. grippotyphosa*. A Chow Chow and English Setter dog with acute infection had a high titre to *L. bratislava* and *L. grippotyphosa* survived after antibiotic and symptomatic treatment.

Venkataraman et al. (1994b) has described an experimental infection in non-descript dogs. The clinical signs were consistent with those of acute natural infections.

2.6 Transmission

In male dogs, infection with *Leptospira canicola* is more common and is associated with the male dogs habit of smelling

and licking the genitals of other dogs and their urine. It is also recognised that infection could occur at coitus (Alston and Broom, 1958).

Lactating animals shed leptospires in the milk, but whole milk is leptospiricidal after a few hours.

Dogs acquire *Leptospira icterohaemorrhagiae* from carrier rats, but transmission of *Leptospira canicola* is direct from dog to dog via contaminated urine (Sullivan, 1974).

Transplacental infection with foetal death and abortion had been reported in human beings (Faine *et al.*, 1984; Songer and Thiermann, 1988; Aker et al., 1996). Transplancetal spread was common in rodents and farm animals and might result in abortion and the birth of dead or weak offspring (Pritchard, 1990).

Ellis (1986) stated that indirect transmission plays a much greater role in the transmission of incidental infection through exposure to an environment contaminated with infectious material.

Kingscote (1986) opined that from the zoonotic perspective leptospirosis is transmitted by contact with infected tissues, body fluids, urine or virulent laboratory culture or indirectly through contaminated moist environment.

Leptospires can penetrate the intact mucous membrane, abraded skin (Kingscote, 1986; Rentko and Ross, 1992) and also water softened skin (Ellis, 1986).

Greene and Shotts (1990) mentioned that leptospires are transmitted between animals by direct contact, venereal and placental transfer, bite wounds or ingestion of infected meat, and spread of infection is enhanced by crowding of animals. Recovered dogs excrete the organisms in urine intermittently for months to years following infection.

Mode of infection of leptospirosis is mainly by eating food or fluids contaminated by infected rats.

Pritchard (1990) opined that leptospiral infection in man followed the bites of animals such as dogs, rats and ferrets. Leptospires are not excreted in the saliva of infected animals. In case of dog bite, the teeth contaminated with the infected viscera of rats or rodents may act as a source of infection and the bite wounds allow the ready entry of leptospires into the body.

Rentko and Ross (1992) observed that rats are important in transmitting serovar *L. icterohaemmorhagiae* and *L. canicola* among the canine population. Raccoons, skunks and opossums are the common wildlife reservoirs and their migration

provides a source of exposure of serovars *L. grippotyphosa* and *L. pomona* for dogs.

About 10^5 organisms/ml of urine may be shed during the first few weeks of leptospirosis. Heath and Johnson (1994) have reasoned out that because of the high concentration of leptospires, splashing of urine plays an important role in the transmission of leptospirosis.

2.7 Pathogenesis

Based on a study on renal disease in *Leptospira canicola* infection, the whole disease process is divided into three stages - Invasive, primary renal and secondary renal phase (McIntyre, 1949).

Dwight *et al.* (1962) observed that leptospiral infection in the dog may induce both an acute disease or a mild subclinical form. In young dogs, the acute form is more common. With fever, jaundice, congestion of the mucous membrane and haemoglobinuria. It is frequently fatal with death occurring one to four days after disease onset.

The basic mechanism of the pathological changes appear to be related to capillary damage, but production of an endotoxin like substance has been demonstrated in some serotype (Sullivan, 1974).

Thiermann (1984) found that during bacteraemia, leptospires invade the internal organs and the extent of damage will depend on the susceptibility of the host and the virulence of the organism. Localised infections in tissues and fluids such as proximal kidney tubules, aqueous humor in the eye, cerebrospinal fluid, and portions of the genital tract will persist while antibodies were detected in circulation.

The virulent leptospires attach more readily in vitro to components of the extra cellular matrix than the non-virulent strains (Ito and Yanagawa, 1987).

It has been concluded that there are only two forms of leptospirosis - anicteric leptospirosis and icteric leptospirosis which are not specifically related to serotype (Penn and Pritchard, 1990).

2.8 Clinical symptoms

Ayyar (1932) had described the symptoms of leptospirosis as sudden off feed, sanguineous discharge from the nostrils, conjunctivitis, passing of faeces with blood and death in a few hours in Madras hounds. At a later stage of outbreak, mouth, lips and region of abdomen showed icterus associated with haemorrhages in the lips and under the subcutis in the region of the sternum and the flank. Vomiting was also

recorded in a few cases. Pyrexia was recorded only in few cases where it went upto 105°F or over and dropped before death.

Stuart (1946) observed nephrotic symptoms in dogs affected with *L. canicola*.

Spradbrow (1962) reported icterus, haemorrhagic gastritis and hepatitis in a nine year old male German Shepherd infected with *L. pomona* and *L. esposito* and another of a four year old female dog, which had a course of illness of three weeks with symptoms of jaundice, frequent vomiting, soft blood stained faeces, in which the serum reacted to *L. esposito* at 1:1000 and *L. icterohaemorrhagiae* at 1:300. Both cases ended fatally.

Dogs with abortion histories were found positive for leptospirosis with predominant reaction to serotypes *L. canicola*, *L. pyrogenes* and *L. ballum* (Jelambi *et al.*, 1976).

In an outbreak of leptospirosis in a pack of hounds, four had signs of anorexia, jaundice and depression of which two died subsequently and serum reacted to *L. icterohaemorrhagiae* and *L. tarassovi* (Mackintosh *et al.*, 1980).

Thomas (1980) reported, a clinical case of leptospirosis due to *L. bratislava* in a ten year old German Shepherd bitch which showed symptoms of increased thirst, variable appetite, weakness of the hindquarters, temperature of 106°F and abdominal pain; progressing to jaundice, disinclination to move, vomiting and dysentery. On exploratory laparotomy bladder was full of dark coloured urine.

Basha *et al.* (1982) examined 63 dogs for leptospirosis, of which 26 were positive and reacting significantly to serotype *L. icterohaemorrhagiae* (17) and *L. canicola* (8). Symptom-var analysis had revealed 33 per cent of cases of pyrexia, 37.5 per cent of stomatitis alone, 50 per cent of hepatomegaly with bile salts in urine, 60 per cent of acute nephritis and 44 per cent of hepatomegaly with ascites.

Verma (1982) in serological examination for leptospirosis among sick and healthy dogs presented to the hospitals found 47 cases to be positive. Of these 15 cases were suffering from fever, 16 of skin disease, two with bronchitis and three had hepatitis with jaundice. Rest of the seropositive dogs were clinically healthy. Higher prevalence was among the aged dogs.

Ratnam *et al.* (1983) reported that positive titres were seen mostly among dogs with pyrexia of unknown origin (PUO) -

70 per cent, followed by haemorrhagic enteritis - 45 per cent and those with other ailments - 39 per cent.

Weihe (1984) reported leptospirosis in a dog with symptom of febrile illness characterised by icterus, apathy and anorexia. The dog was seroreactive to *L. icterohaemorrhagiae* at 1:1600 and to *L. canicola* at 1:1800, rising a week later to 1:3200 and 1:4000 to the respective serotypes.

Kogika *et al.* (1987) classified cases of leptospirosis in dogs into three groups based on the symptoms. Group I showed signs of icterus and uraemia and reacted to *Leptospira* serovars *icterohaemorrhagiae*. Group II dogs were uraemic but non-icteric and mostly reacted to serovar *L. canicola*. Group III dogs which had been in contact with the former but were either asymptomatic or with vague symptoms of fever, anorexia and prostration.

In an experimental infection of puppies of three month old with *Leptospira icterohaemorrhagiae* and with *L. canicola*, symptoms noted were anorexia, listlessness and depression. Six had fever but none showed jaundice and all recovered clinically by 14 days after infection (Arimitsu *et al.*, 1989).

Meningeal involvement has been reported in leptospirosis in dogs, resulting in nervous symptoms (Hungerford, 1990).

Venkataraman and Nedunchelliyan (1990b) reported a case of Stuttgart disease in a dog with history of anorexia, vomiting and dysentry. The animal was dull, depressed and dehydrated with temperature of 39.8°C, ulcers in oral cavity and bad odour, pain on palpation of abdomen, increased thirst and scanty urine output which was positive for albumin. Paired sera reacted to *L. canicola* at 1:640 and 1:2560 .respectively.

Nielsen *et al.* (1991) had reported symptoms of depression, and poydypsia in a three year old dog seropositive to *L. serovar canicola* at 1:80. Another household dog of five months age was irritable and listless and reacted to *L.* serovar *bratislava* at 1:640 and *L.* serovar *canicola* at 1:80.

Prescott *et al.* (1991) reported a case of leptospirosis in an eight year old Cairn Terrier in acute renal failure with hepatic insufficiency, which became jaundiced as hepatic insufficiency worsened. *L.* serovar *pomona* was demonstrated by immunofluorescent staining of urinary sediment.

Rentko *et al.* (1992) had suggested that canine leptospirosis should be considered in the differential diagnosis of dogs with acute or subacute renal failure.

Venkataraman and Nedunchelliyan (1993) had reported leptospirosis in 49 (10.34 per cent) of 474 dogs with

vomiting, diarrhoea, jaundice and nephritis. Predominant reaction was to serovar *canicola* (31), followed by *icterohaemorrhagiae* (15) and *pomona* (three).

Acute leptospirosis with symptoms of febrile reaction, icterus, anorexia and severe depression which turned fatal was reported in 14 dogs in the age group of two month to eight years. Serological reaction was predominantly to *Leptospira* serovar *bratislava*, followed by *grippotyphosa*, *copenhageni* and *pomona* (Scanziani *et al.*, 1994).

Dogs experimentally infected with *L. icterohaemorrhagiae* developed acute to moderate disease. Clinical signs observed were pyrexia, anorexia, depression, congested conjunctiva, diarrhoea and dehydration (Venkataraman *et al.*, 1994b).

Ocular changes of congested mucous membrane (72.72 per cent cases), ocular discharge (54.54 per cent), scleral congestion (36.36 per cent) and icteric mucous membrane (27.27 per cent cases) were observed in dogs with leptospirosis, reacting more to *L. canicola* (54.55 per cent) than *L. icterohaemorrhagiae* (45.45 per cent) (Thirunavukkarasu *et al.*, 1995).

2.9 Clinical pathology

2.9.1 Haematology

Kogika *et al.* (1987) noted leucocytosis due to neutrophilia and monocytosis in two groups of dogs with signs of icterus and anaemia (*L. icterohaemorrhagiae*) and in uraemic but non icteric cases (*L. canicola*). Neutropaenia was seen initially in a few cases. No change in the red cell picture was detected.

Dwivedi *et al.* (1988) recorded normocytic, normochromic anaemia and leucocytosis with neutrophilia in acute *L. canicola* infection in a dog.

Haematologic findings in typical cases of canine leptospirosis include leucocytosis and thrombocytopaenia. Leucopaenia common in the leptospiraemic phase develops to leucocytosis with a left shift. A marked increase in erythrocyte sedimentation rate correspond to hyper-fibrinogenaemia and hyperglobulinaemia (Greene and Shotts, 1990).

Haemogram revealed anaemia, leucocytosis with neutrophilia and Erythrocyte sedimentation rate (ESR) of 30 mm per hour in Stuttgart disease in a dog (Venkataraman and Nedunchelliyan, 1990b).

Venkataraman and Nedunchelliyan (1992) reported increase in Erythrocyte sedimentation rate (ESR), in the two weeks after experimental infection with *L. canicola* in dogs.

2.9.2 Biochemical changes in leptospirosis

McIntyre (1949) reported increase in blood urea levels from 40 mg per cent to 200 mg per cent in mild to severe cases due to *L. canicola*. Prognosis were grave in renal infections with blood urea above 150 mg per cent.

Elevated serum urea levels (52 mg per cent) were reported in dogs with subacute and chronic renal lesions due to leptospirosis (Timoney *et al.*, 1974).

Thomas (1980) observed blood urea level of 18 m mol per litre on tenth day of *Leptospira bratislava* infection in a patient, which reduced to nine m mol per litre on 19th day.

Navarro *et al.* (1981) reported that serum biochemical changes correlated with the severity of clinical disease, following experimental infection of dogs with serovar *L. icterohaemorrhagiae*. Hepatic damage was evident from increased bilirubin, ALT and ALP values. Bilirubin was predominantly of direct-reacting type and highest value of 22 mg per dl was recorded in a dog on eighth day post infection. The ALT and AST values were increased in severe form of the disease. Renal dysfunction was reflected by

increased serum urea nitrogen (SUN) and serum creatinine values, which increased on fourth day post infection. Creatinine values were markedly increased in severe disease. Hyperphosphataemia, hypochloraemia, hypokalaemia and hyperglycaemia were noticed in severely affected dogs.

Verma (1982) correlated increased Blood urea nitrogen (BUN) values in dogs with higher titre to *L. canicola*. The BUN value varied from 28 to 48 mg per cent in a case of advanced nephritis. SGPT level was high in one case of acute hepatitis with jaundice, seropositive to *L. icterohaemorrhagiae*.

Levels of blood urea, glutamic oxalacetic transaminase and aspartate transaminase were elevated on sixth day of infection in *L.* serovar *pomona* infection in a human patient. Serum protein value was normal. In another case, serum level of enzymes, creatinine, urea, uric acid and total bilirubin were markedly elevated during the first three days of hospitalization (Kingscote, 1986).

Greene and Shotts (1990) had reported a rise in BUN and creatinine levels in dogs, with varying severity of renal failure in leptospirosis. Hyponatraemia, hypochloraemia, hypokalaemia and hyperphosphataemia were also found in most cases, whereas hyperkalaemia and hyperglycaemia develop in terminal renal failure. Mild hypocalcaemia results from

hypoalbuminaemia. Liver damage is demonstrated by increased serum ALT, AST, serum lactic dehydrogenase and serum ALP activities and bilirubin concentration.

Venkataraman and Nedunchelliyan (1990b) reported rise in creatinine (3.4 mg per dL) and Blood urea nitrogen (130 mg per dL) in a case of acute infection due to serovar *canicola*.

In experimental infection of dogs with serovar *canicola*, an increase in serum creatinine, alkaline phosphatase and blood urea nitrogen (BUN) was noted in the two weeks after inoculation (Venkataraman and Nedunchelliyan, 1992).

2.10 Diagnosis

2.10.1 Antigen detection

2.10.1.1 Dark-field microscopy (DFM)

Alexander *et al*. (1957) on direct examination of urine sample under dark-field microscope detected leptospires in eight of the 76 *L. canicola* seropositive reactors and none among *L. icterohaemorrhagiae* reactors.

Leptospires could not be detected by DFM in urine of 31 seropositive dogs, although leptospires were isolated from one dog (Murphy *et al*., 1958).

Thiermann (1980) recorded that leptospires were seen only in the urine with pH value higher than 6.5 under DFM, on screening urine samples of 40 dogs.

ThillaiKoothan *et al.* (1987) observed that DFM was better than immunoperoxidase test (IPT) in demonstration of leptospires. On DFM examination leptospires were detected in blood on fourth, fifth and sixth day of experimental infection in calves. From tenth day onwards leptospires could be seen in the urine sample after double centrifugation.

Venkataraman and Nedunchelliyan (1992) examined urine sample from 94 dogs by DFM and could detect leptospires in only eight of the 20 seropositive cases.

2.10.1.2 Staining

Baskerville (1986) reported that comparison of the different method using organ from experimentally infected animals confirmed that the Levaditi and Warthin-Starry techniques and Faine's and Young's modification gave the most consistent results.

Zamora *et al.* (1995) on comparing microscopic techniques-Levaditi silver staining, dark ground microscopy in wet smear, immunofluorescence and immunoperoxidase techniques on kidney sections of wild rodents found Levaditis technique detecting

the highest number of positive samples (67.5 per cent) and DFM the lowest (32.5 per cent).

2.10.1.3 Isolation

Murphy *et al.* (1958) could isolate *L. pomona* in Fletcher's semisolid medium by direct culture of bladder urine from a seropositive dog.

The culture of leptospires from clinical materials is the most reliable method for definitive diagnosis and also for epidemiological purposes (Adler *et al.*, 1986).

In an attempt to isolate *L. hardjo* from the kidney of 10 seropositive cattle, four different media - Ellinghausen and McCullough (1965), EMJH, EMJH plus one per cent rabbit serum, Tween 80/40/LH medium were compared. No isolates were obtained using the first two media, whereas seven isolates were made from the third and eight isolates from the fourth medium (Ellis, 1986b).

Venkataraman and Nedunchelliyan (1992) from an outbreak of leptospirosis in man and dog in Madras isolated serovar *L. canicola* from the urine of a dog and serovar *L. icterohaemorrhagiae* from the urine sample of a seropositive human patient.

Ratnam (1994) reported that leptospires can be isolated from blood, urine, dialysate fluid, liver, kidney and aborted materials.

Venkataraman *et al.* (1994a) isolated *L. canicola* in EMJH semisolid medium from urine of a dog.

2.10.1.4 Radio Immuno Assay (RIA)

Bahaman *et al.* (1986) reported that a double sandwich RIA was able to detect leptospiral antigens at $\geq 10^7$ pg per ml of urine sample.

2.10.2 Antibody detection

2.10.2.1 Agglutination tests

2.10.2.1.1 Microscopic agglutination test (MAT)

Stuart (1946) by Schuffners method of agglutination test detected 40 per cent infection among 101 Glasgow house dogs.

Murphy *et al.* (1958) employed MAT in a serological survey and detected significant titre of 1:100 or greater in 16.2 per cent of the dogs tested.

Carlos *et al.* (1971) screened 507 serum samples from dogs by macroscopic agglutination test using five antigenic pools, and positive sera were titrated against 23 live leptospiral

antigens using MAT and found that 13 per cent of the dogs had significant titres of 1:100 to leptospiral antigens.

Michna and Ellis (1973) compared Schuffner's agglutination test with rapid microscopic agglutination test (RMAT) and observed higher reactors by Schuffner's test (39 per cent) as against 18 per cent by RMAT. RMAT only detects antibodies present at high titres and misses many low titre positive animals.

Thiermann (1980) in a survey of 556 stray dogs, tested serum by MAT using four leptosira antigens - *L. icterohaemorrhagiae, L. canicola, L. pomona* and *L. grippotyphosa* and further treated the positive samples by 2-mercapto ethanol (2 ME) to determine IgG. In total 33.63 per cent were positive. Use of 2 ME eliminated titres of 1:320 and lower and detected convalescent titres. Sera showing reaction to more than one serotype, became negative or reacted to only one serotype after treatment with 2 ME.

By microscocpic agglutination test (MAT) 39 per cent positive cases were detected from 63 dogs presented to the hospital (Basha *et al.*, 1982).

Microagglutination test was performed on 50 canine sera in Madras and 54 per cent were found positive to *Leptospira*

serovars *icterohaemorrhagiae*, *canicola*, *hebdomadis* and *pomona* at titres of 1:80 to 1:320.(Ratnam et al., 1983).

MAT is the most widely used serological test for leptospirosis. In hardjo infection in cattle using an antibody titre of 1:10 the sensitivity of the test was 0.67 and specificity 0.86. When antibody titre of 1:100 was used sensitivity dropped to 0.41 but specificity (1.0) was good (Ellis, 1986b).

Srivastava *et al.* (1989) reported a higher reaction to MAT (12.5 per cent) compared to 4.0 per cent by Latex agglutination test (LAT) on sera of 473 animals.

Venkataraman and Nedunchelliyan (1990a) employing MAT detected 10.3 per cent incidence among 474 dogs tested in Madras.

A four fold increase in antibody titre to *L. canicola* was detected by MAT from an acute infection in a dog (Venkataraman and Nedunchelliyan, 1990b).

Serological testing is the most widely used means for the diagnosis of leptospirosis and the microscopic agglutination test (MAT) is the standard serological test. It is the reference test against which all other serological tests are

evaluated and is the test specified for import and export testing (OIE, 1992).

Venkataraman and Nedunchelliyan (1992) conducted MAT using nine live leptospiral antigens on 94 canine sera and observed 20 samples (21.27 per cent) seroreactive.

Venkataraman *et al.* (1994b) detected significant MAT titres on post infection day (PID) 14 and persistence of high titres for one to three weeks, then declining below significant levels by PID 84 in experimental infection of dogs with *L. canicola*

Leptospiral antibody titres of 1:100 to 1:6400 against *L.* serovar *hardjo* were detected by MAT in the serum of 19 dogs with interstitial nephritis, but without any clinical abnormality (Scanziani *et al.*, 1994).

2.10.2.1.2 Macroscopic agglutination test

Watson *et al.* (1976) tested sera from 600 dogs by the rapid slide agglutination method against 12 leptospiral serotypes and reported that 6.8 per cent had significant titre.

Verma (1982) by the use of macroscopic agglutination test with serotypes *L. icterohaemorrhagiae*, *L. canicola*, *L. pomona*,

L. grippotyphosa and *L. hardjo* detected prevalence rate of 34.3 per cent in dogs in Punjab.

2.10.2.1.3 Passive haemagglutination test (PHA)

Nair (1980) compared the sensitivity of MAT and PHA and recorded that titres by PHA test were far below that of MAT. The PHA titres after reaching the maximum level remained detectable for longer period when compared to MAT titres.

2.10.2.1.4 Latex agglutination test (LAT)

Srivastava *et al.* (1989) standardised LAT using partially purified antigen of *Leptospira biflexa* serovar *patoc*, and results were compared with MAT. Out of 473 animal sera tested 71.7 per cent were positive to MAT and LAT of which 12.5 per cent were positive to MAT and four per cent to LAT only. The sensitivity and specificity of LAT was 85.5 per cent and 76.3 per cent respectively.

2.10.2.1.5 Microcapsule agglutination test (MCAT)

The immune response of six puppies infected with *L. icterohaemorrhagiae* and six with *L. canicola* were compared by the MCAT and MAT. Antibody was detected by MCAT as early as the fifth day after infection at a titre of 80 and above. Whereas MAT detected at the seventh day of infection at low titre of 10-30. In another case serum from a dog which died

of acute leptospirosis was tested by MCAT and MAT. MAT titre of 8 and 2 were detected against *L. icterohaemorrhagiae* and *L. canicola*, respectively. The titres of MCAT were 1280 and 160 to *L. canicola* and *L. icterohaemorrhagiae* respectively (Arimitsu *et al.*, 1989).

Arimitsu *et al.* (1994) reported on the sensitivity of MCAT during the early stage in that the one-point MCAT detected antibodies in 64.8 per cent human serum samples compared with 37 per cent by MAT and 38.9 per cent by ELISA IgM.

2.10.2.1.6 Skin tests

Skin test for leptospirosis using an allergen (leptospirin) was evaluated in man and animals including 12 dogs. The detection rate of the seropositive animals was low by the skin test. But animals which did not react serologically were negative in the skin test (Schonberg, 1986).

2.10.2.1.7 Enzyme linked immunosorbent assay (ELISA)

Hartman (1986) compared ELISA with the MAT and showed that during the first two weeks after an experimental infection of dogs with serotype *L. canicola* the ELISA detected antibody at higher dilutions than did the MAT. The ELISA

became positive on an average five days earlier in the course of infection than MAT. Following the second week after infection both tests detected antibody at almost equal titres.

Venugopal and Ratnam (1989) observed that ELISA was much more sensitive than MAT in serodiagnosis of *L. pomona* in cattle. Twenty one MAT negative samples, were detected positive by ELISA.

In a comparative study of serologic test of leptospirosis using sera from domestic animals including dogs ELISA was more sensitive than the MAT and immunofluorescence test (Sting and Dura, 1994).

Venkataraman *et al.* (1994b) using ELISA and MAT assessed the humoral immune response from day five to 84 post infection of dogs with *L. icterohaemorrhagiae*. ELISA IgM was detected as early as day five and titres were higher than those in MAT recording. Maximum titre on day 14 and were detected till day 70. ELISA IgG appeared late, the increase was less rapid and titres were not significantly different from IgM titre from day 77 post infection.

Balasubramanian and Ramakrishna (1996) reported high ELISA titre compared to MAT titre in the first two weeks post infection in lambs.

2.10.2.1.8 Polymerase chain reaction (PCR)

Ramadass *et al.* (1997) reported 65 to 81 per cent positive reaction with PCR, compared to 27 to 37 per cent with DFM, in serum and urine samples of man, dog and cattle.

PCR helps early detection of leptospires in blood, urine or cerebrospinal fluid (CSF) in the period between the first appearance of clinical symptoms and the time when antibodies become detectable (Rodriguez, 1997).

Materials and Methods

CHAPTER-III

MATERIALS AND METHODS

The study was conducted at the Department of Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during 1995-1997.

3.1 Preparation of glasswares and reagents

Borosilicate (BOROSIL) glasswares and analytical or guaranteed reagent grade of chemicals only were used for the study. The glasswares were processed using standard methods (Hoskins, 1967) and sterilised either in hot air oven or in autoclave depending upon the material to be sterilized.

3.2 Collection of samples

One hundred and thirty-eight dogs presented at the outpatient units of the University Veterinary Hospital, Kokkalai and Mannuthy and twelve stray dogs brought for practicals in the Department of Surgery constituted the materials for study.

Details of each case namely age, breed, sex, vaccination status and history were noted, except those of the stray dogs where the details were not available.

From each case, three ml of blood was collected in heparinised vials for haematology and five ml of wholeblood for serum separation. Serum was separated and stored at -20°C in two aliquotes until used for biochemical and serological studies.

Microscopic agglutination test (MAT) was carried out on the 150 sera samples. Haematology and biochemical studies were conducted in 120 cases. Among the 138 cases presented at the Hospital, twenty three dogs were showing symptoms suspicious of leptospirosis viz., high temperature, anorexia, vomiting, blood stained faeces, dark-brown urine, jaundice, lumbar pain, ascites and weakness of hindlimbs. These 23 cases and 12 cases of stray dogs were subjected to detailed clinical study including examination of blood sample by Dark-field microscope (DFM), culture, haematology and biochemical studies. Urine samples were collected from eight suspected cases of leptospirosis, were examined under DFM and cultured. From six sacrificed stray dogs, kidney were removed aseptically, the capsule was cut and a portion of the cortex was taken as the inoculum.

3.3 Isolation procedure

3.3.1 Culture media

The media employed for culture and isolation were Fletcher's semisolid medium (HI-Media, Mumbai) and Ellinghausen-McCullough-Johnson-Harris (EMJH) media (DIFCO, USA).

3.3.2 Rabbit sera

Three healthy rabbits six to eight weeks of age, free from leptospiral antibodies were procured from the Small Animal Breeding Station (SABS), College of Veterinary and Animal Sciences, Mannuthy and were maintained under disease free condition in the laboratory for harvesting rabbit serum.

By cardiac puncture, twenty millilitre of whole blood was collected from each rabbit. The serum was separated, pooled and centrifuged at 1000 rpm for five minutes.

The clear serum was inactivated at 56°C in a waterbath for 30 minutes. The heat inactivated sera were filtered using millipore filter 0.45 μ (Laxbro).

3.3.3 Fletcher's semisolid medium

3.3.3.1 Composition of the base

Peptone	-	0.3 g
Beef extract	-	0.2 g
Sodium chloride	-	0.5 g
Agar	-	1.5 g
Triple distilled water	-	900 ml

3.3.3.2 Preparation

Dissolved the base in 900 ml of triple distilled water by heating. Autoclaved at 121°C for 20 minute and added heat inactivated (56°C, 30 min.) sterile rabbit serum to a final concentration of 10 per cent by volume/volume and 0.1 per cent sodium pyruvate. The pH of the medium was adjusted to 7.2-7.6.

5-Fluorouracil was added to this medium in a concentration of 200 µg/ml, especially for medium used for culturing urine.

3.3.4 EMJH medium (DIFCO, USA)

This medium is supplied as a base and enrichment.

3.3.4.1 Composition of the base

Sodium phosphate dibasic	-	1.0 g
Potassium phosphate monobasic	-	0.3 g
Sodium chloride	-	1.0 g
Ammonium chloride	-	0.25 g
Thiamine	-	0.005 g

3.3.4.2 Preparation of medium

Liquid EMJH medium was prepared by dissolving 2.3 g of EMJH base in 900 ml of triple distilled water. The pH was adjusted to 7.5. After autoclaving at 15 lbs/inch^2 for 15 min, the medium was added aseptically with enrichment at 10 per cent level.

To prepare the semisolid EMJH medium, 0.2 per cent bacteriological agar was added with EMJH base and the complete medium was prepared as above.

3.3.5 Testing and storage of media

Five ml of prepared medium was transferred to each screwcapped test tubes (BOROSIL, 15 mm x 125 mm) under sterile conditions. These tubes were incubated at 37°C for atleast 3 days and an additional 3 days at room temperature to check fungal contaminants. These were stored at 4°C and used within 2 months.

3.4 Microscopic agglutination test (MAT)

3.4.1 Serovars used as antigen for MAT

Serovars	Reference strain
L. canicola	- Hond Utrecht (IV)
L. icterohaemorrhagiae	- RGA
L. autumnalis	- Akiyami A
L. australis	- Ballico
L. ballum	- Mus 127
L. bataviae	- Swart
L. hebdomadis	- Hebdomadis
L. pomona	- Pomona
L. pyrogenes	- Salinem
L. grippotyphosa	- Moskva V

These reference strains were maintained in EMJH medium.

3.4.2 Culture technique

Under sterile conditions, 0.5 ml of the culture of the reference strain was added to Flectcher's Semisolid and EMJH liquid media tubes in triplicate and incubated at 30°C. These culture media were examined under darkfield microscope for the presence of leptospires at weekly intervals for upto 4 weeks.

In semisolid medium, samples showing growth were subcultured at 6 to 8 weeks interval.

A 4-10 day old live culture of leptospires grown in EMJH liquid medium incubated at 30°C was employed for the test. Only these cultures without clumps and a density of $2x10^8$ leptospires/ml was selected and used. A density of $2x10^8$ - leptospires/ml of culture was ensured, following the procedure of Alston and Broom (1958) by checking for atleast 200 organisms for 250x of dark field microscope.

3.4.3 Procedure

MAT was carried out as per the procedure of Faine (1982) using a battery of live *Leptospira* serovars (*canicola, pomona, icterohaemorrhagiae, autumnalis, grippotyphosa, australis, ballum, bataviae, hebdomadis, pyrogenes*). Test was carried out in Laxbro microtitre plates with round bottom. A volume of 25 µl of sterile phosphate buffer saline (PBS) pH 7.2 was added to each well of the plate except the first row. The sera samples were diluted to 1:50 in PBS in test tubes. Transferred 50 µl of 1:50 diluted serum sample to the first well, making a dilution of 1:50 and then continued serial dilution to 1:3 200 by transferring 25 µl. To these wells 25 µl of liquid culture was added. The final dilution after addition of the antigen ranged from 1:100 to 1:6400. Known positive and negative control sera were included. The samples

were mixed and incubated at 30°C for 4 hours. At the end of incubation, 10 μl of each dilution was taken on a clean glass slide and examined under low power (10x) of dark field microscope, without using a coverslip.

The end point of agglutination reaction was taken as the highest dilution in which 50 per cent of the leptospires had agglutinated. The reciprocal of end point was taken as the titre. A titre of 100 and above is considered positive (Faine, 1982).

3.5 Examination of leptospirosis suspected cases

3.5.1 Darkfield microscope examination (DFM)

3.5.1.1 Blood

Blood samples from 23 suspected cases of leptospirosis and 12 stray dogs were subjected to DFM examination (Faine, 1982). The samples were first centrifuged at 1000 g for 10 minutes to remove the gross particles and then at high speed of 3000-4000 g for 20-30 minutes to concentrate leptospires.

3.5.1.2 Urine

Urine samples were collected from eight suspected cases into equal quantity of sterile phosphate buffer saline (PBS)

pH 8 by catheterization. It was then subjected to double centrifugation (Faine, 1982). The samples were centrifuged at 1000 g for 10 minutes to remove the gross particles and then at a high speed of 3000-4000 g for 20-30 minutes to concentrate leptospires and then examined by DFM to identify motile leptospires.

3.5.2 isolation methods

3.5.2.1 Blood

Blood from 23 suspected cases of leptospirosis and 12 stray dogs were collected aseptically and two drop of blood was inoculated into five ml of Fletcher's semisolid media and EMJH liquid media in 35 cases. Serial dilutions of ten-fold were made in three more tubes in each case. The tubes with media were incubated at 30°C for 6 weeks. Examination for the leptospiral organisms was done under DFM at 3 days interval.

3.5.2.2 Urine

Urine samples were collected from eight suspected cases in PBS (pH 8). Later it was diluted to 10 fold with PBS (pH 8). A drop of this diluted urine was inoculated to the Fletchers semisolid medium. Further triplicate serial dilutions were made from the first tube and the culture media

was incubated at 30°C for 6 weeks and examined for the organisms under DFM at 3 day interval.

3.5.2.3 Kidneys

Portions of cortex both kidneys from six sacrificed stray dogs were removed and placed in a sterile petridish. A rat toothed forceps holding tissue material of kidney was inserted into a medium tube and the tissue was left in the tube; and the tissue free rat toothed forceps was inserted into second media tube and further serial dilution in five more test tubes were made. The tubes were incubated at 30°C for 6 weeks and examined at 3 days interval for the growth of organisms.

3.6 Haematology

3.6.1 Erythrocyte sedimentation rate (ESR) and Packed cell volume (PCV)

ESR and PCV were estimated as per the method described by Wintrobe (1981).

3.6.2 Haemoglobin

Haemoglobin content was determined by the acid haematin method, using Sahli's haemoglobinometer (Benjamin, 1985).

3.6.3 Total leucocyte count (TLC) and Absolute differential count

TLC and DC were carried out by the method of Schalm et al (1975).

3.7 Biochemical analysis

3.7.1 Serum total bilirubin

For determining serum total bilirubin, the method of Jendrassik and Grof (1938) was followed making use of Merck kit and absorbance read at 578 nm.

3.7.2 Serum creatinine

Serum creatinine was analysed following Alkaline picrate method (Bonses and Taussky, 1945). as per the Qualigens Diagnostic kit and absorbance read at 520 nm.

3.7.3 Serum alanine amino transferase (ALT)

Serum ALT was estimated by the DNPH method of Reitman and Frankel (1957) using Qualigens Diagnostic Kit and read at 505 nm.

3.7.4 Serum Total Protein, Albumin and Globulin

Serum total protein was estimated by the Biuret method (Varley, 1988) using Qualigens Diagnostic kit and absorbance read on spectrophotometer (Digispec 110) at 555 nm.

Serum albumin was determined by the Bromocresol green (BCG) dye binding method (Doumas, 1978) using Qualigens Diagnostic Kit and read at 630 nm.

Serum globulin was calculated from the difference of serum total protein and albumin (Benjamin, 1985).

Albumin/globulin ratio (A/G ratio) was calculated from the albumin and globulin values (Benjamin, 1985).

Results

CHAPTER-IV

RESULTS

The seroprevalence of leptospirosis in one hundred and fifty dogs, including twelve stray dogs was studied and the results are presented in Table 1. Haematology and biochemical analysis were also done in one hundred and twenty dogs and are presented in Tables 3, 4 and Fig.3, 4. Blood samples collected from twentythree suspected cases of leptospirosis and twelve stray dogs, urine samples from eight suspected cases of leptospirosis and kidney tissue from six sacrificed stray dogs were examined under dark field microscope and cultured for isolation of leptospires. The results are presented.

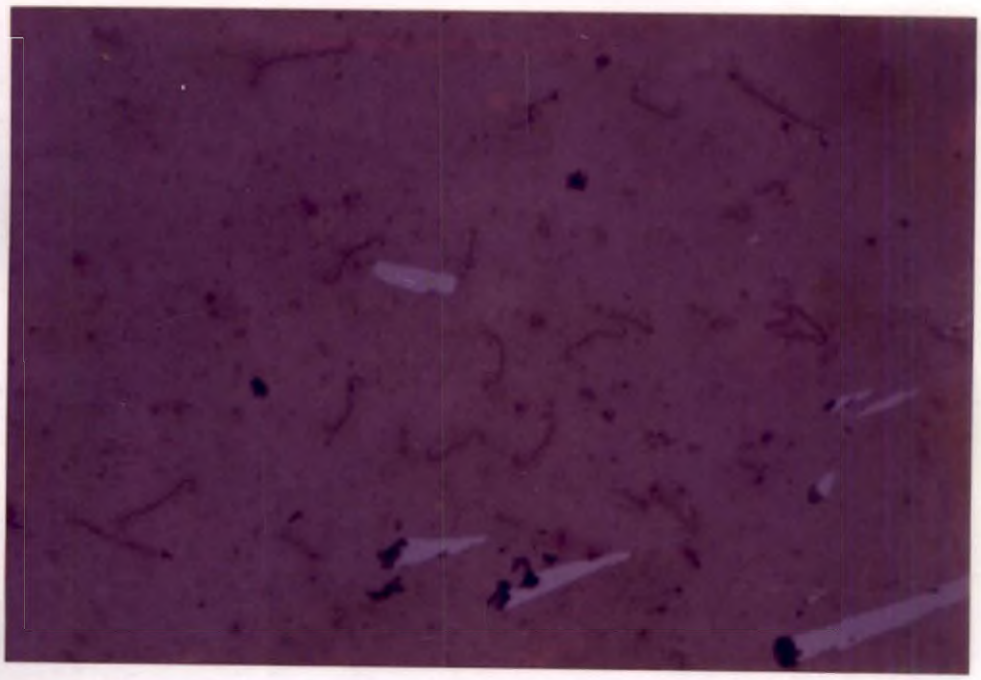
4.1 Microscopic agglutination test (MAT)

The results of MAT are presented in Table 1 & 2 and Fig.1 and 2.

Out of the 150 sera samples tested, 49 (32.67 per cent) were positive for antibodies to leptospira serovars.

Predominant sero reaction were to *L.* serovar *pomona* 26 cases (53.06 per cent), and *L.* serovar *canicola* 16 cases

Plate 1. Suspension of Leptospires used in MAT x 1200



(32.65 per cent) followed by *L.* serovar *icterohaemorrhagiae*, 7 cases (14.28 per cent).

Among the twelve stray dogs tested, ten (83.33 per cent) showed the presence of leptospiral agglutinins and *L.* serovar reaction were against *pomona*, 9 cases (90 per cent) and *canicola* 1 case (10 per cent).

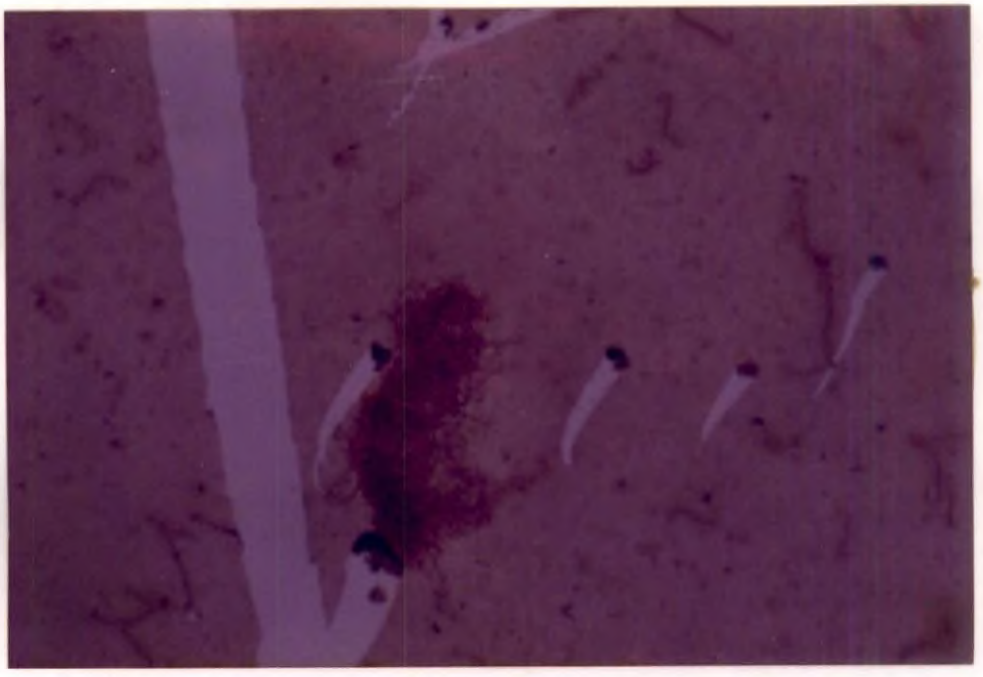
On analysing the clinical cases, C 261 with jaundice and haemoglobinuria reacted to *L. icterohaemorrhagiae* at 1:1600, C 11556 with symptoms of gastritis and haemoglobinuria reacted to *L. pomona* at 1:1600. The two cases with ascites C 4006 and C 5136 reacted to *L. pomona* at 1:800 and 1:1600 respectively.

The highest titre of 1:3200 was noted in two cases, C 16066 with symptoms of pyrexia (104°F), and gastroenteritis reacting to *Leptospira*. serovar *pomona* and C 6793 with pyrexia (104°F), haemorrhagic gartroenteritis, deep yellow urine, positive reaction for bile salt and bile pigment reacted to *L. canicola*.

MAT titre to different serovars are presented in Table 2, Fig.1 and 2. Among the seropositive dogs, 30 (61.22 per cent) reacted at a higher titre of 1:800 or more, whereas 19 cases (38.77 per cent) reacted at low titre of 1:200-1:400.

Plate 2. Agglutination of Leptospires in MAT (50 per cent reaction) x 1200

Plate 3. Agglutination of Leptospires in MAT (100 per cent reaction) x 1200



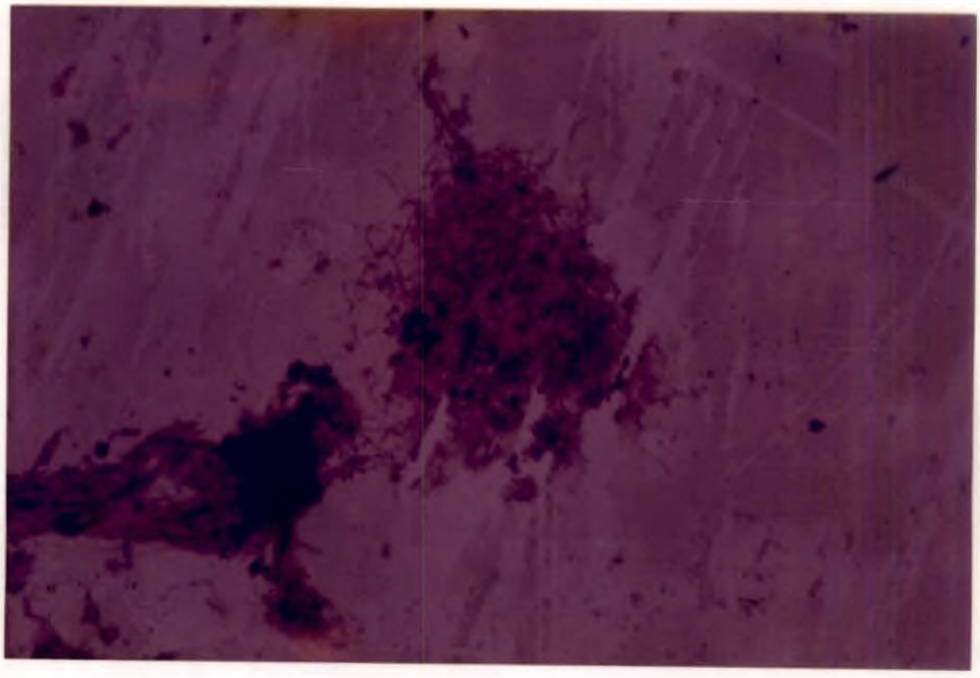


Table 1. MAT titre

Sl. No.	Case No.	Positive titre to serovars		
		L.canicola	*L.icterohaem-orrhagiae*	*L.pomona*
1.	C 2259	200	-	-
2.	C 2826	400	-	-
3.	C 3905	-	-	800
4.	C 3982	-	-	200
5.	C 3875	-	-	800
6.	C 4221	-	-	1600
7.	C 2069	-	-	800
8.	C 1225	1600	-	-
9.	C 6851	-	-	400
10.	C 11303	800	-	-
11.	C 14728	-	-	1600
12.	C 15613	-	-	400
13.	C 15136	-	-	800
14.	C 15197	-	-	400
15.	C 16066	-	-	3200
16.	C 11623	200	-	-
17.	C 261	-	1600	-
18.	C 11556	-	-	1600
19.	C 533	-	-	800
20.	C 5136	-	-	1600
21.	C 4789	-	-	1600
22.	C 4006	-	-	800
23.	C 6462	-	-	1600
24.	C 6793	3200	-	-
25.	C 5373	1600	-	-
26.	C 7644	800	-	-
27.	C 154	-	200	-

Contd.

Table 1 (Contd.)

Sl. No.	Case No.	Positive titre to serovars		
		L.canicola	*L.icterohaem-orrhagiae*	*L.pomona*
28.	C 7012	-	400	-
29.	C 8260	800	-	-
30.	C 4007	-	1600	-
31.	C 9517	-	400	-
32.	C 9549	-	1600	-
33.	C 10228	800	-	-
34.	C 10584	-	400	-
35.	C 9279	400	-	-
36.	C 10824	1600	-	-
37.	C 11291	800	-	-
38.	C 11813	800	-	-
39.	C 6841	800	-	-
40.	C S-1	-	-	400
41.	C S-3	-	-	400
42.	C S-5	-	-	400
43.	C S-6	400	-	-
44.	C S-7	-	-	400
45.	C S-8	-	-	800
46.	C S-9	-	-	200
47.	C S-10	-	-	200
48.	C S-11	-	-	400
49.	C S-12	-	-	800

Table 2. MAT titre to different Leptospira serovars, among the seropositive dogs

Serovars	MAT titre					Total	Percent
	1:200	1:400	1:800	1:1600	1:3200		
L. canicola	2	3	7	3	1	16	32.65
L. ictero-haemorrhagiae	1	2	1	3	-	7	14.28
L. pomona	3	8	8	6	1	26	53.06
Total	6	13	16	12	2	49	
Percent	12.24	26.53	32.65	24.49	4.08		
	(38.77)		(61.22)				

FIG.1 SEROREACTION TO DIFFERENT LEPTOSPIRA SEROVARS AMONG SEROPOSITIVE DOGS

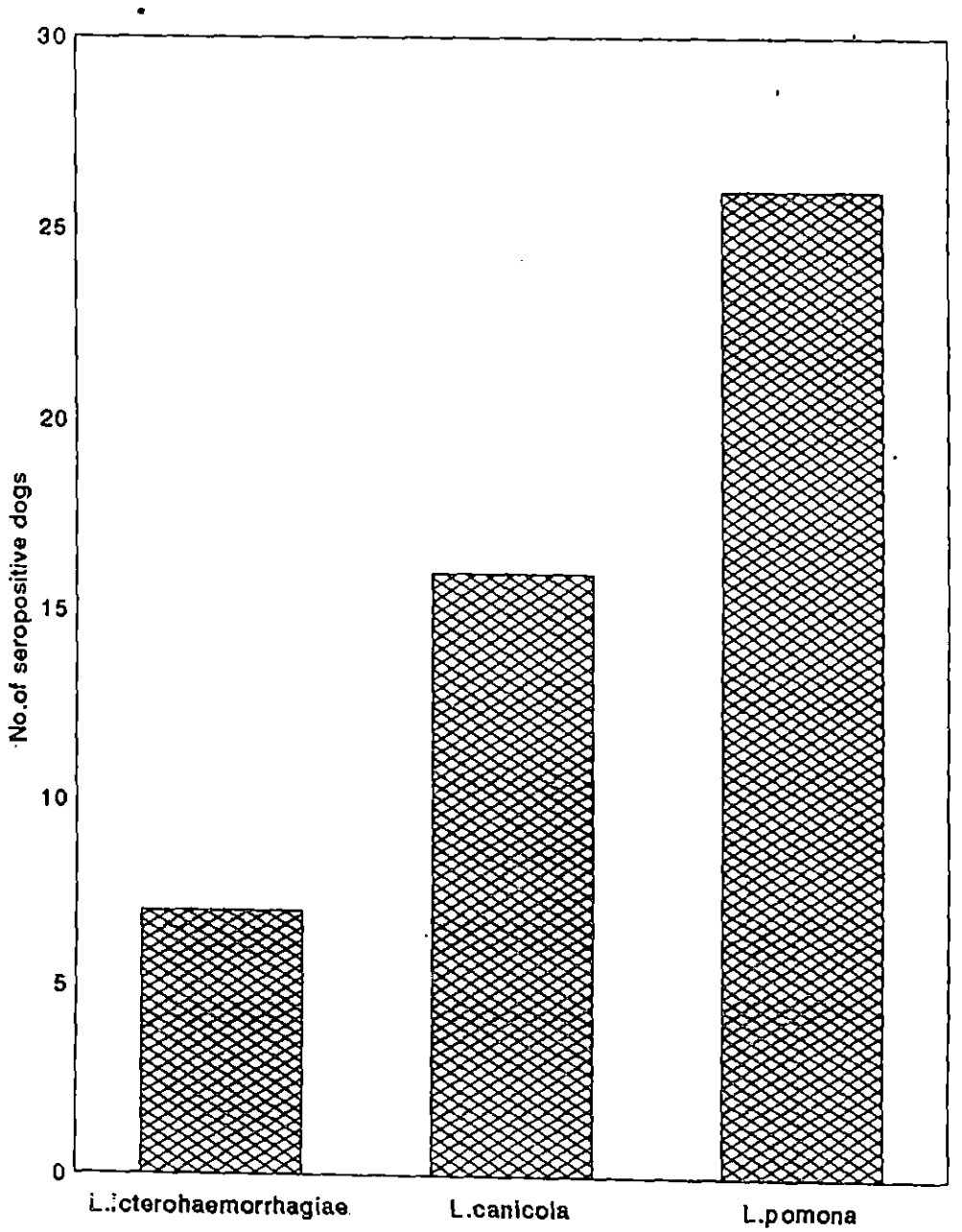
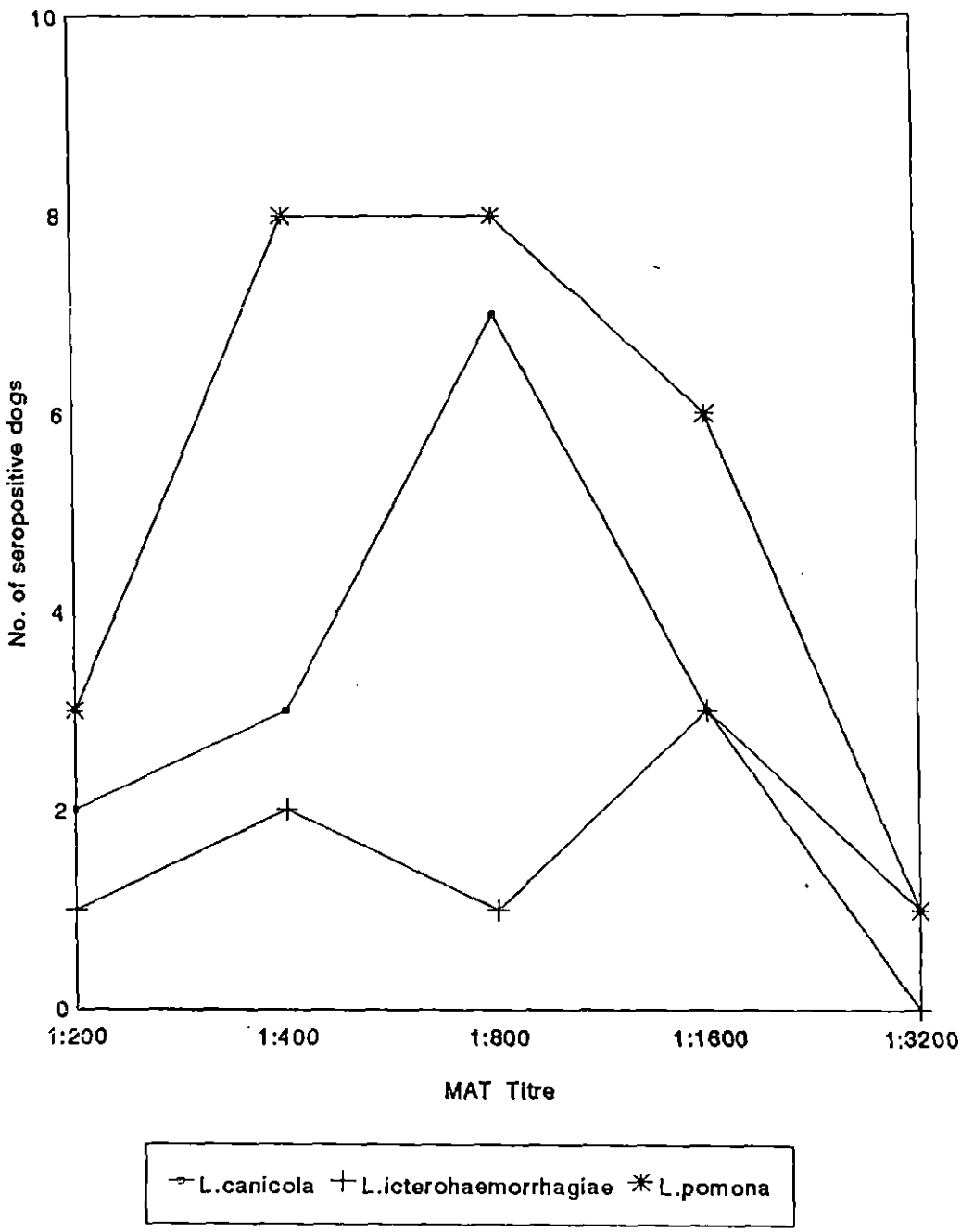


FIG.2 MAT TITRE TO DIFFERENT LEPTOSPIRA SEROVARS AMONG THE SEROPOSITIVE DOGS



4.2 Examination of leptospirosis suspected cases

4.2.1 Dark field microscope examination (DFM)

4.2.1.1 Blood

Blood samples from twenty three dogs showing symptoms suggestive of leptospirosis and from the twelve stray dogs were examined by DFM after double centrifugation. Out of the 23 leptospirosis suspected cases, blood sample from two cases (C 261 and C 11556) revealed the presence of typical, motile leptospires.

The case C 261, showed pyrexia (104.2°F), icteric mucous membrane, polydypsia, polyurea, vomiting, increased respiratory rate and pulse, abdominal thread, enlargement of superficial lymph nodes, haemoglobinuria and dehydration. On palpation, renal and hepatic enlargement was appreciated.

The second case C 11556 was presented with symptoms of gastritis, pyrexia (102.8°F), congested mucous membrane, enlargement of the superficial lymph nodes, haemoglobinuria, dehydration and general weakness.

From among the blood samples of stray dogs, leptospires could be detected in two cases (S-6 and S-10).

4.2.1.2 Urine

Urine samples from eight suspected cases of leptospirosis did not reveal the presence of leptospires by dark field microscope examination.

4.2.2 Isolation methods

4.2.2.1 Blood

Blood sample from 23 suspected cases of leptospirosis and from twelve stray dogs were cultured on semisolid Fletcher's medium for isolation of leptospires. Blood inoculated from a stray dog S-6, showed evidence of ring like growth three cm below the surface of the culture medium after 7 days. But on dark field microscopic examination, leptospires could not be detected. The contaminated culture was further subcultured and media were incubated and examined at three days interval, but leptospires could not be observed. Attempts to purify the culture by millipore filtration and chick inoculation were not successful.

4.2.2.2 Urine

Leptospires could not be isolated from any of the eight urine samples cultured.

4.2.2.3 Kidney

Kidney tissue from the six sacrificed stray dogs were cultured for isolation of leptospires. Darkfield microscopic examination of the culture from the kidney tissue of stray dog S-6 revealed the presence of motile, leptospires after two weeks of incubation but the culture was found to be contaminated. Guinea pig inoculation and millipore filtration were tried to purify the culture from contaminants. But neither method was successful, in purifying the growth.

4.3 Haematology

The mean haematological values are presented in Table 3 and Fig.3.

4.3.1 Erythrocyte sedimentation rate (ESR) and Packed cell volume (PCV)

The mean values of ESR in the seropositive and seronegative group were 9.84 ± 2.12 and 6.42 ± 1.32 mm per hour respectively. The variation is not statistically significant.

The mean packed cell volume of the seropositive and seronegative group were 44.76 ± 1.34 and 43.17 ± 1.35 per cent respectively which are not significantly different.

4.3.2 Haemoglobin

In the leptospira seropositive and seronegative group, the mean haemoglobin levels were 14.08 ± 0.42 and 14.47 ± 0.39 g per dl respectively. Statistical analysis showed no significant difference between the two groups.

4.3.3 Total leucocyte count and absolute differential counts

Total leucocyte count

The mean total leucocyte count was apparently higher in the seropositive dogs 14.84 ± 3.37x10^3 per µl, compared to the seronegative dogs 10.24 ± 0.49x10^3 per µl. But the difference between the seropositive and seronegative cases was not statistically significant.

Absolute neutrophil count

The difference in the absolute neutrophil count between seropositive and the seronegative group was not significant, with mean values at 11.42 ± 2.71x10^3 per µl and 7.52 ± 0.37x10^3 per µl respectively.

Absolute lymphocyte count

The mean absolute lymphocyte count was 3.13 ± 0.68x10^3 per µl and 2.49 ± 0.16x10^3 per µl in the seropositive and

seronegative animals, respectively. The difference is not significant, statistically.

Absolute eosinophil count

The mean eosinophil count in the seropositive and seronegative dogs was 0.43 ± 0.08x10^3 per µl and 0.30 ± 0.07x10^3 per µl respectively. Statistical analysis showed no significant difference.

Absolute monocyte count

The mean absolute monocyte counts in seropositive and seronegative dogs was 0.42 ± 0.03x10^3 per µl and 0.03 ± 0.01x10^3 per µl respectively. The increase in the absolute monocyte count is statistically significant ($P \leq 0.01$) in the seropositive dogs.

4.4 Biochemical analysis

The results of biochemical analysis are given in Table 4 and Fig.4.

4.4.1 Serum total bilirubin

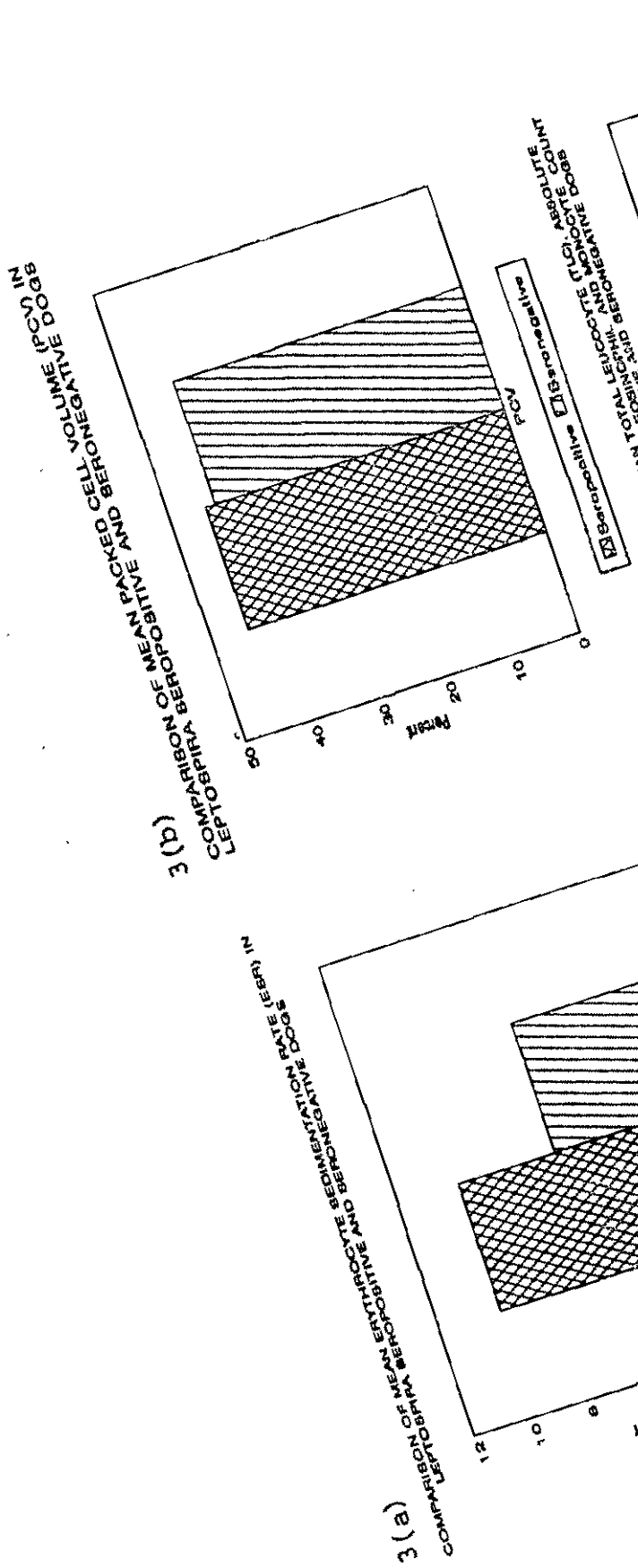
The mean serum total bilirubin values were 1.30 ± 0.92 and 0.29 ± 0.04 mg per dl in the seropositive and seronegative animals, respectively. The difference was statistically not

Table 3. Mean haematological values in Leptospira seropositive and seronegative dogs

Parameters	Mean±SE		't' value
	Seropositive	Seronegative	
Erythrocyte sedimentation rate - ESR (mm/hr)	9.84± 2.12	6.42± 1.32	1.37 NS
Packed cell volume-PCV (per cent)	44.76± 1.34	43.17± 1.35	0.80 NS
Haemoglobin (g/dl)	14.08± 0.42	14.47± 0.39	0.65 NS
Total leucocyte countx$10^3/\mu l$	14.84± 3.37	10.24± 0.49	1.35 NS
Absolute neutrophil countx$10^3/\mu l$	11.42± 2.71	7.52± 0.37	1.42 NS
Absolute lymphocyte countx$10^3/\mu l$	3.13± 0.68	2.49± 0.16	0.93 NS
Absolute eosinophil countx$10^3/\mu l$	0.43± 0.08	0.30± 0.07	1.00 NS
Absolute monocyte countx$10^3/\mu l$	0.42± 0.03	0.03± 0.01	6.41 **

NS : Non-significant

** : Highly significant ($P \leq 0.01$)


3 (a)
COMPARISON OF MEAN ERYTHROCYTE SEDIMENTATION RATE (ESR) IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS
ESR
Seropositive
Seronegative
3 (b)
COMPARISON OF MEAN PACKED CELL VOLUME (PCV) IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS
PCV
Seropositive
Seronegative
3 (c)
COMPARISON OF MEAN HAEMOGLOBIN LEVEL IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS
Haemoglobin
Seropositive
Seronegative
3 (d)
COMPARISON OF MEAN TOTAL LEUCOCYTE (TLC) ABSOLUTE NEUTROPHIL, LYMPHOCYTE, EOSINOPHIL AND MONOCYTE COUNT IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS
TLC
Neutrophil
Lymphocyte
Eosinophil
Monocyte
Seropositive
Seronegative

significant. In C 261, with jaundice a higher value of total bilirubin level, 45.25 mg per dl was recorded.

4.4.2 Serum creatinine

The mean serum creatinine in the seropositive and negative dogs was 1.68 ± 0.28 and 0.93 ± 0.06 mg per dl respectively. The rise in serum creatinine in the seropositive dogs was significant statistically ($P \leq 0.05$) from that of the seronegative dogs. In C 261, the serum creatinine level was 2.95 mg/dl and in C 6793 with haemoglobinuria, 2.41 mg/dl was recorded.

4.4.3 Serum alanine amino transferase (ALT)

The mean serum ALT activity in the seropositive and seronegative dogs was 45.39 ± 6.47 and 51.52 ± 6.94 units per ml respectively, and the difference was not significant statistically.

4.4.4 Total protein, albumin and globulin

Serum total protein

The mean serum total protein in the seropositive and seronegative group was 6.38 ± 0.30 and 6.41 ± 0.21 g per dl respectively. There was no significant difference in the total protein between the two groups.

Serum albumin

Statistical analysis showed no significant difference in the mean serum albumin between the seropositive, 3.04 ± 0.14 g per dl and seronegative group, 2.98 ± 0.11 g per dl.

Serum globulin

The mean serum globulin level in the seropositive and seronegative group was 3.30 ± 0.26 and 3.43 ± 0.19 g per dl respectively. The difference was not significant statistically.

Albumin-globulin ratio (A:G)

The mean albumin globulin (A:G) ratio in the seropositive and seronegative animals was 1.24 ± 0.11 and 1.00 ± 0.06 respectively. The change was non significant on statistical analysis.

4.5 Incidence

4.5.1 Age

The sero reactive dogs were mostly in the age group below three years with 26 cases (66.67 per cent) followed by the three to six year group with 8 cases (20.51 per cent). Statistical analysis showed the difference in seroreaction

Table 4. Mean biochemical values in Leptospira seropositive and seronegative dogs

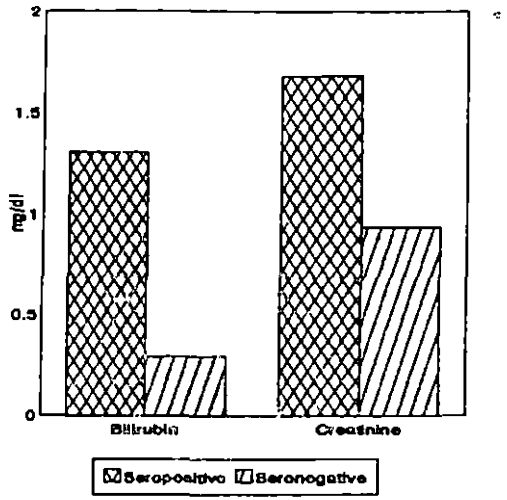
Parameters	Mean±SE		't' value
	Seropositive	Seronegative	
Serum total bilirubin (mg/dl)	1.30± 0.92	0.29± 0.04	1.10 NS
Serum creatinine (mg/dl)	1.68± 0.28	0.93± 0.06	2.58 *
ALT (units/ml)	45.39± 6.47	51.52± 6.94	0.62 NS
Serum total protein (g/dl)	6.38± 0.30	6.41± 0.21	0.07 NS
Serum albumin (g/dl)	3.04± 0.14	2.98± 0.11	0.32 NS
Serum globulin (g/dl)	3.30± 0.26	3.43± 0.19	0.39 NS
A:G ratio	1.24± 0.11	1.00± 0.06	1.85 NS

NS : Non-significant

* : Significant ($P \leq 0.05$)

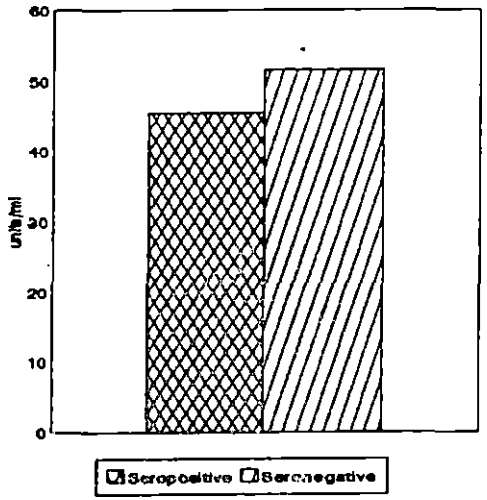
4(a)

COMPARISON OF MEAN SERUM BILIRUBIN AND CREATININE IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS



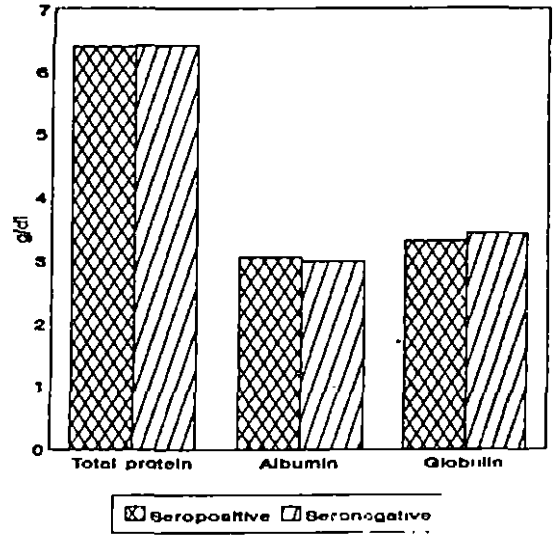
4(b)

COMPARISON OF MEAN SERUM ALANINE AMINO TRANSFERASE (ALT) IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS



4(c)

COMPARISON OF MEAN SERUM TOTAL PROTEIN, ALBUMIN AND GLOBULIN IN LEPTOSPIRA SEROPOSITIVE AND SERONEGATIVE DOGS



among the zero to three year age group to be highly significant (P≤0.01) (Table 5, Fig.5).

4.5.2 Sex

Male dogs were more seropositive 30 (61.22 per cent), compared to the females 19 (38.77 per cent). The difference in seroreaction between the male and female dogs was significant (P≤0.05) (Table 6, Fig.6).

4.5.3 Breed

More seropositive cases were noted among the nondescript dogs 16 (32.65 per cent) followed by German Shepherd dogs 15 (30.61 per cent), Doberman, 7 (14.28 per cent), Pomeranian 6 (12.45 per cent), Dachshund, 3 (6.12 per cent), Labrador and Great Dane 1 each (2.04 per cent).

On statistical analysis there was significant difference (P≤0.05) in the sero-reaction of nondescript and German Sheperd dogs, compared to Doberman and Pomeranian which was again significantly different at (P≤0.05) from the group of Dachshund, Labrador and Great Dane dogs (Table 7, Fig.7).

4.5.4 Seasonal variation

More number of seropositive cases were recorded during June with 11 cases (22.45 per cent), May - 6 cases

(12.25 per cent) and December - 6 (12.25 per cent). The difference in incidence was statistically significant at ($P \leq 0.05$) between June, May and December compared to other months (Table 8, Fig.8).

Table 5. Age distribution of Leptospira seropositive dogs

Age years	Number sero-positive	Percentage
0-3	26	66.67 **
3-6	8	20.51
6-9	2	5.13
9-12	3	7.69
Total	39	

** : Highly significant (P≤0.01)

Table 6. Sex distribution of Leptospira seropositive dogs

Sex	Number sero-positive	Percentage
Male	30	61.22 *
Female	19	38.77
Total	49	

* : Significant (P≤0.05)

FIG.5 AGE DISTRIBUTION OF LEPTOSPIRA SEROPOSITIVE DOGS

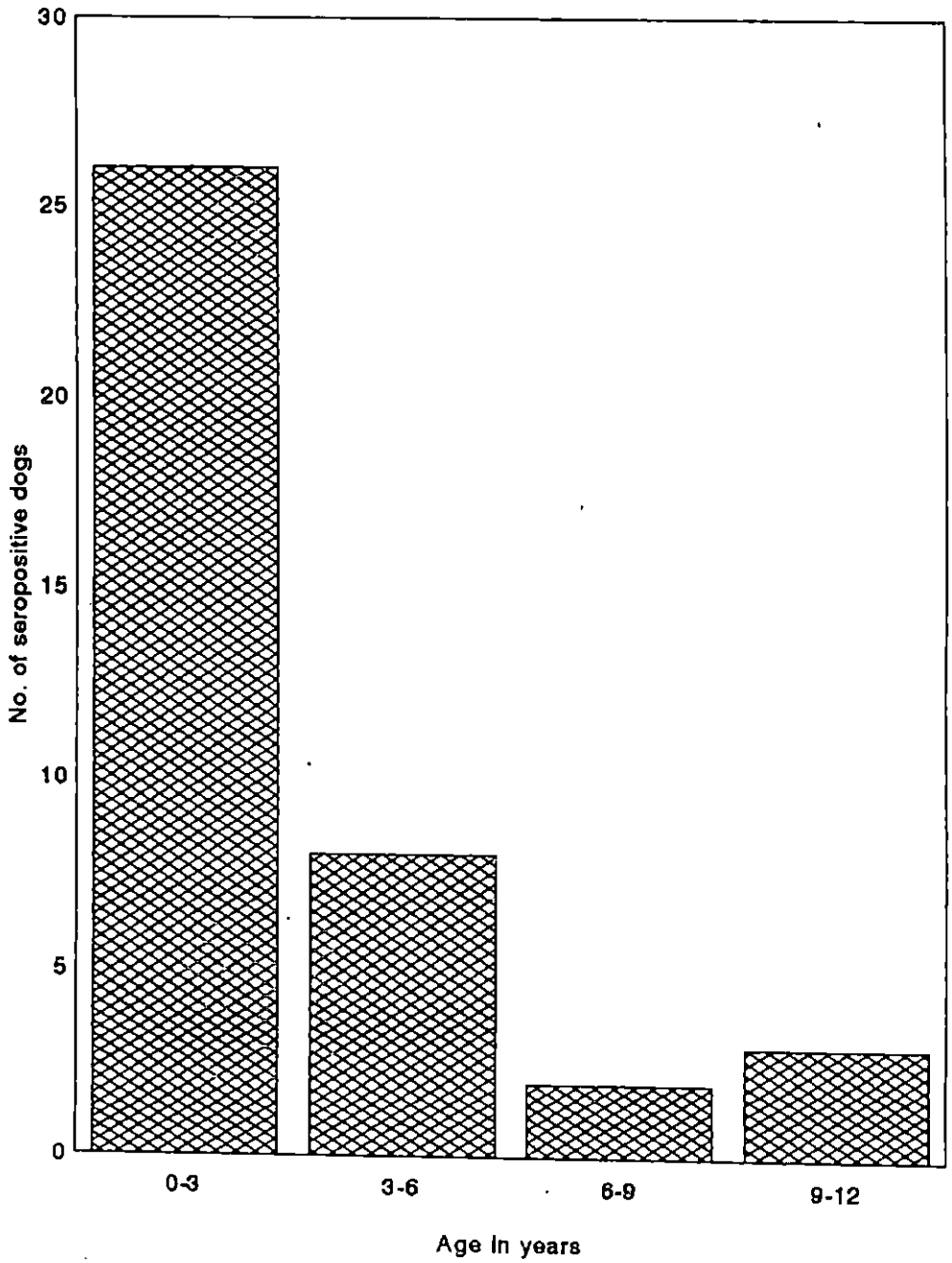


FIG.6 SEX DISTRIBUTION OF LEPTOSPIRA SEROPOSITIVE DOGS

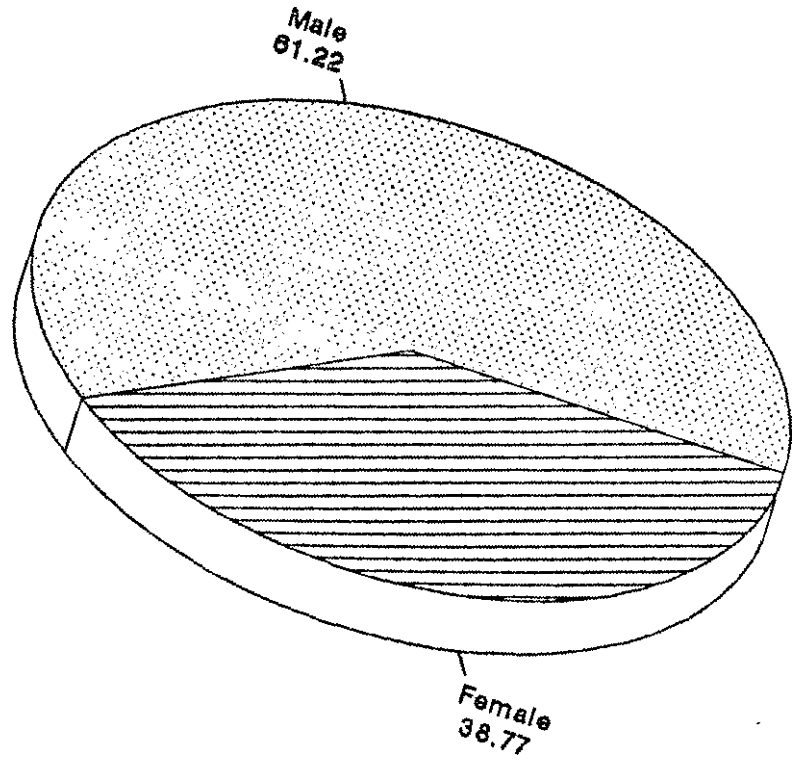


Table 7. Breed-wise distribution of Leptospira seropositive dogs

Breed	Number sero-positive	Per cent
German Shepherd	15	30.61*
Pomeranian	6	12.45*
Doberman pinscher	7	14.28*
Dachshund	3	6.12
Labrador	1	2.04
Great dane	1	2.04
Nondescript	16	32.65*
Total	49	

* : Significant (P≤0.05)

FIG. 7 BREED-WISE DISTRIBUTION OF LEPTOSPIRA SEROPOSITIVE DOGS

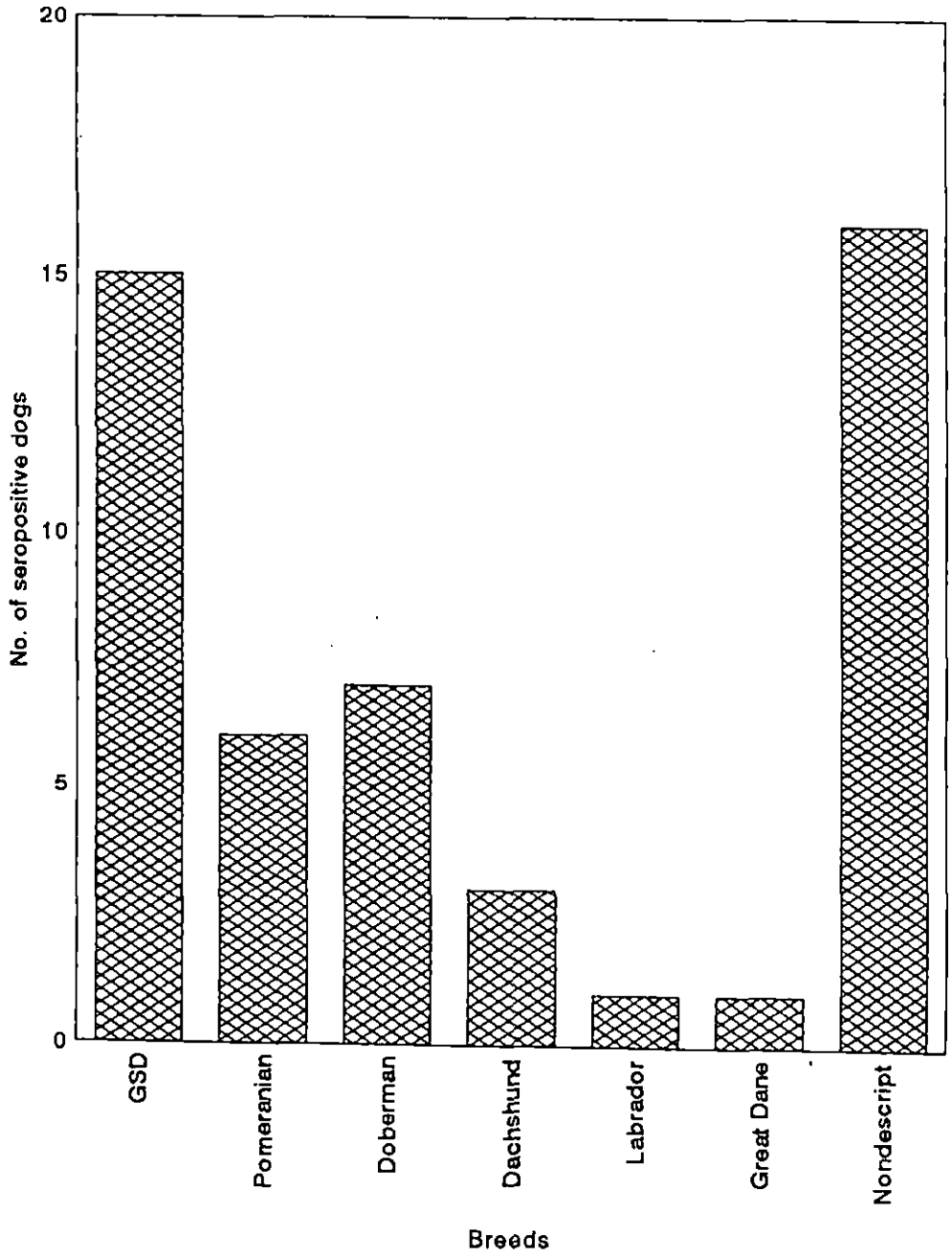


Table 8. Month-wise distribution of Leptospira seropositive dogs

Month	Number sero-positive	Per cent
January	3	6.12
February	1	2.04
March	3	6.12
April	5	10.20
May	6	12.25*
June	11	22.45*
July	5	10.20
August	4	8.16
September	2	4.08
October	2	4.08
November	1	2.04
December	6	12.25*

* : Significant ($P \leq 0.05$)

FIG.8 MONTH-WISE DISTRIBUTION OF LEPTOSPIRA SEROPOSITIVE DOGS

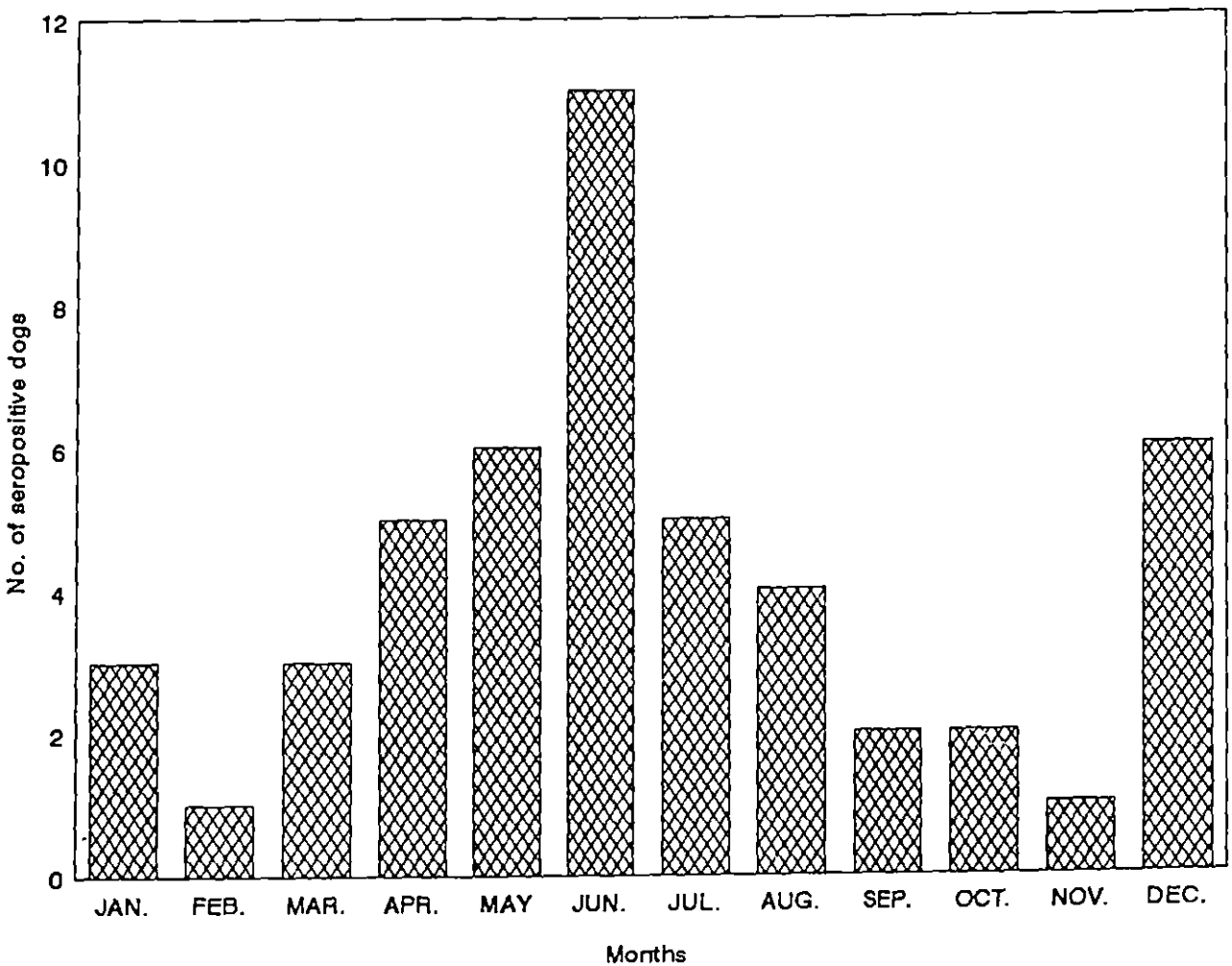


Table 9. Relationship of clinical symptom to the Leptospira serovar reacted

Clinical symptom	No. positive to			Total
	L.canicola	*L.icterohaemorrhagiae*	*L.pomona*	
Pyrexia	7	3	9	19
Anorexia	8	1	5	14
Gastro-enteritis	5	-	3	8
Haemorrhagic gastro-enteritis	2	1	5	8
Gastritis	4	1	2	7
Congested mucous membrane	8	4	7	19
Nephritis	1	1	-	2
Hepatomegaly	1	1	-	2
Yellow urine	4	2	2	8
Lumbar pain	1	-	1	2
Lymph node enlargement	2	3	1	6
Ascites	-	-	2	2
Haemoglobi-nuria	1	1	2	4
Otitis	-	-	1	1
Corneal opacity	-	1	1	2
Clinically healthy for vaccination	-	3	2	5

Discussion

CHAPTER-V

DISCUSSION

In the present study, the seroprevalence of leptospirosis in one hundred and fifty dogs from various parts of Thrissur district was assessed. The biochemical and haematological changes were studied and recorded from one hundred and twenty cases. Clinically suspected cases of leptospirosis were subjected to detailed investigations viz., dark-field microscope examination and isolation of leptospires from blood and urine sample. Kidney tissue from six sacrificed stray dogs were also subjected to isolation procedure.

5.1 Microscopic agglutination test (MAT)

Out of the 150 sera tested, 49 (32.67 per cent) were positive for antibodies to *Leptospira* serovars *pomona* (53.06 per cent), *canicola* (32.65 per cent) and *icterohaemorrhagiae* (14.28 per cent).

Seroprevalence of 31.52 per cent and 34.1 per cent among dogs were recorded by Andreani *et al.* (1986) and Timoney *et al.* (1974) respectively.

In India many workers had reported the involvement of more than one serovar in canine leptospirosis (Ball and

Sheikh, 1958; Rajasekhar and Nanjiah, 1971; Verma, 1982; Venkataraman and Nedunchelliyan, 1993) and in other parts of the world by Carlos *et al.* (1971); Thiermann (1980); Cornide *et al.* (1985); Prokopcakova *et al.* (1986); Prescott *et al.* (1991) and Brihuega *et al.* (1995).

The present study showed highest prevalence of antibody to *L. pomona*, followed by *L. canicola* and *L. icterohaemorrhagiae*. Significantly high agglutination titre to *L. pomona* among dogs was reported by Morter *et al.* (1959).

Prevalence of agglutinins to *L. canicola*, *L. icterohaemorrhagiae* and *L. pomona* among dogs were recorded by Schnurrenberger *et al.* (1962); Marx *et al.* (1981); Venkataraman and Nedunchelliyan (1992); Venkataraman and Nedunchelliyan (1993).

It has been recorded by many workers that pig and cattle act as ideal hosts for the perpetuation of *L. pomona* and hence these hosts could be an important source of *L. pomona* to dogs (Morse, 1960).

Morter *et al.* (1959) have opined about the possible role of dog in the transmission of *L. pomona* to man and livestock and that the high rate of *L. pomona* among dogs could be correlated to their close contact with the livestock. But Schnurrenberger *et al.* (1962) have expressed the view that

though dogs can be infected with and shed *L. pomona*, they are probably of minor importance as reservoirs.

Though 32.67 per cent of the dogs were seropositive to leptospires, most of these seropositive dogs were not showing specific symptoms and hence man and animals who come in contact with the symptomless seropositive groups have a high risk of getting infection. MacKintosh *et al.* (1980) have also warned about such a probability.

In the present study, 83.33 per cent of the stray dogs tested showed the presence of leptospiral agglutinins to *L. pomona* and *L. canicola*. Similar result of seroreaction to *L. canicola* and *L. pomona* among stray dogs was reported by Schnurrenberger *et al.* (1962). The seroprevalence of leptospirosis among stray dogs, in this study is higher than the reports of many other workers (Everard *et al.*, 1979; Thiermann, 1980; Yasuda *et al.*, 1980 and Myburgh *et al.*, 1993). The high incidence of leptospirosis in stray dogs could be due to their habit of wandering and also smelling and licking the genitals of other dogs and their urine; which was also reported by Alston and Broom (1958).

The serum samples collected from stray dogs in this study mainly reacted to *L. pomona* (90 per cent) and *L. canicola* (10 per cent), whereas none to *L. icterohaemorrhagiae*. Similar observations were also made by Schnurrenberger *et al.* (1962).

The probable close contact with livestock and other stray dogs could be the reason for high prevalence of *L. pomona* in stray dogs.

Dogs with fever, anorexia and gastroenteritis have often been believed to be suffering from other diseases and the possibility of leptospirosis is ignored (Carlos *et al.*, 1971). In this study also the seropositive dogs were showing pyrexia in 19 cases, anorexia in 14, gastroenteritis in 16, congested mucous membrane in 19 and ascites in 2 cases (Table 9). Similar symptoms in seropositive dogs were recorded by Basia *et al.* (1982), Ratnam *et al.* (1983) and Venkataraman and Nedunchelliyan (1992). This shows that though the dogs are not showing any specific symptoms of leptospirosis, they have to be periodically screened and treated or immunized against this zoonotic disease. This in turn will reduce the incidence of leptospirosis in animals and human beings.

The high incidence of *L. pomona* in dogs in this study warrants the inclusion of *L. pomona* bacterin in the vaccine to be used in canines in addition to the the presently available *L. canicola* and *L. icterohaemorrhagiae* bacterins. Prescott *et al.* (1991) also were of the opinion that leptospiral bacterins for use in dogs be broadened to include serovars *autumnalis* and *pomona*. Such a suggestion had also been given

by Scanziani *et al.* (1994) based on epidemiological study conducted in Italy.

The high prevalence of *L. pomona* among dogs reveals that the epidemiology of canine leptospirosis is changing with the emergence of serovars different from those commonly infecting dogs namely *L. canicola* and *L. icterohaemorrhagiae*.

5.2 Examination of leptospirosis suspected cases

5.2.1 Dark field microscope examination (DFM)

5.2.1.1 Blood

Demonstration of leptospires in blood and urine is one of the confirmatory test in the diagnosis of leptospirosis. In the present study, leptospires could be detected in the blood samples of 4 dogs out of 35 dogs screened. Of these, two were from cases (C261 and C11556) with symptoms suggestive of leptospirosis and two from stray dogs (S-6 and S-10). Demonstration of leptospires in blood by dark field microscopy has been reported by other workers (Taylor *et al.*, 1970; Keenan *et al.*, 1978).

5.2.1.2 Urine

Leptospires could not be detected from the urine samples of eight suspected cases, in agreement with the finding of Murphy *et al.* (1958). Observations as against this was

reported by Alexander *et al.* (1957); Thillaikoothan *et al.* (1987) and Venkataraman and Nedunchelliyan (1992). It is noted that leptospiruria in canines even with the best adapted serotype *L. canicola* is intermittent and transient. Also, leptospiruria was observed more frequently in dogs with relatively high titre of agglutinins (Alexander *et al.*, 1957).

In the present study, examination of urine samples under DFM were carried out from the suspected clinical cases. Repeated examinations of more than one sample were not possible from these cases and leptospiruria was not detected. Comparatively high titre of agglutinins of >1:1600 was noted in a few cases only, thus reducing the chance of detection of leptospires in urine samples.

Moreover, leptospiruria is mainly seen in convalescent and recovering dogs, than during the acute stage (Ratnam, 1994). This factor also could be the reason for the absence of leptospiral organisms in the urine of suspected cases.

5.2.2 Isolation methods

5.2.2.1 Blood

Leptospires could not be isolated from the blood sample of 35 dogs. The combination of the slow growth of leptospires and the requirement for a rich medium at a neutral pH predisposes the cultivation of leptospires to problems of

contamination particularly in the isolation of leptospirae from natural sources (Alston and Broom, 1958; Babudieri, 1961 and Johnson and Rogers, 1964).

5.2.2.2 Urine

Leptospires could not be isolated from any of the eight urine samples cultured. Absence of organism in urine during the acute stage might be the reason for the failure of isolation in the present cases. Though cultural propagation of leptospires is considered as the most reliable method of diagnosis it depends on the requirements of the fastidious leptospires and on the relative freedom of the urine sample from gross bacterial contamination (Bahaman *et al.*, 1986). The intermittent shedding of the organism in urine could be an important factor for not getting a growth in the present study.

5.2.2.3 Kidney

Kidney tissues from six sacrificed stray dogs were cultured and leptospires were identified in the kidney tissue of the stray dog (S-6), after two weeks of incubation. But the culture was contaminated and could not be purified.

A major problem in culturing leptospires, is contamination with other microorganisms, particularly when

attempting to culture from non-sterile sources; and the need for an improved selective medium for isolation of leptospires from clinical material has been expressed by Adler *et al.* (1986).

Addition of 5-Fluorouracil to the medium did not give any additional advantage in reducing the contaminants of the culture. Once the contaminating organisms start to grow in the 5-Fluorouracil medium, addition of higher concentration of 5-Fluorouracil had no effect, as has also been reported by Johnson and Rogers (1964).

Though millipore filtration, guinea-pig inoculation and dilution method were described to be effective in purification of culture (Ratnam, 1994) in the present study these techniques were not found advantageous.

5.3 Haematology

5.3.1 Erythrocyte sedimentation rate (ESR) and Packed cell volume (PCV)

In the present study, a higher ESR was recorded in the seropositive group. Similar finding was reported by several workers in dogs experimentally infected with leptospira species (Keenan et al., 1978; Greene and Shotts, 1990; Venkataraman and Nedunchelliyan, 1992).

There was little difference in the mean PCV values of seropositive and seronegative dogs as was also reported by Taylor *et al.* (1970). In the present study, apparently higher PCV was recorded in the seropositive group. This may be due to the fact that about 37 cases of the seropositive group had symptoms of anorexia, gastritis, gastroenteritis, haemorrhagic gastroenteritis with dehydration which causes haemo-concentration and increased packed cell volume. On the contrary, decrease in PCV was reported by Keenan *et al.* (1978) and Navarro *et al.* (1981).

5.3.2 Haemoglobin

Mean haemoglobin level was apparently lower but non-significant in the seropositive dogs which agrees with the findings of Taylor *et al.* (1970).

Decrease in Haemoglobin concentration in individual cases was reported by Keenan *et al.* (1978); Dwivedi *et al.* (1988) and Venkataraman and Nedunchelliyan (1990b).

5.3.3 Total leucocyte count and absolute differential count

Total leucocyte count

The mean total leucocyte count was apparently higher though non-significant in the seropositive dogs. Several workers have reported leucocytosis in experimentally infected

dogs and from individual clinical cases (Taylor *et al.*, 1970; Keenan *et al.*, 1978; Kogika *et al.*, 1987; Dwivedi *et al.*, 1988; Greene and Shotts, 1990 and Venkataraman and Nedunchelliyan, 1990b).

Absolute neutrophil count

In the present study, a higher mean value of absolute neutrophil count was recorded in the seropositive dogs, eventhough the difference was non-significant. Leucocytosis with neutrophilia in leptospirosis in dogs was also recorded by (Keenan *et al.*, 1978, Kogika *et al.*, 1987; Dwivedi *et al.*, 1988; Greene and Shotts, 1990 and Venkataraman and Nedunchelliyan, 1990b).

Absolute lymphocyte count

The difference in the mean lymphocyte count of seropositive and seronegative group was non-significant, contrary to the report of lymphopaenia by Keenan *et al.* (1978).

Absolute eosinophil count

The mean eosinophil count was apparently high but non-significant in seropositive dogs as against the finding of eosinopaenia by Keenan *et al.* (1978).

Absolute monocyte count

In the present study the mean value of absolute monocyte count was significantly higher ($P \leq 0.01$) in seropositive dogs which is in agreement with the findings of other workers (Keenan *et al.*, 1978 and Kogika *et al.* 1987).

5.4 Biochemical analysis

5.4.1 Serum total bilirubin

Though serum total bilirubin value was higher in the seropositive group the difference was non-significant. Keenan *et al.* (1978); Navarro *et al.* (1981); Kingscote (1986) and Greene and Shotts (1990) also recorded increased bilirubin level in leptospirosis.

Hepatic cholestasis and hepatic pathological changes as evidenced by increased bilirubin level is marked in the severely and moderately ill groups. The total bilirubin level of C 261 was 45 mg per dl. The animal was showing severe jaundice and reacted to *L. icterohaemorrhagiae*. Icterus appears to be the result of hepatic cell damage due to obstruction of canaliculi in serovar *icterohaemorrhagiae* of man and dog, as was reported by Navarro *et al.* (1981). In the present study, seroreaction was predominantly against *L. pomona* and *L. canicola* than *L. icterohaemorrhagiae* and this may be the reason for less hepatic pathologic changes. Also,

the clinical symptoms of hepatic changes were not severe in most cases.

5.4.2 Serum creatinine

In the present study, significant increase in the mean serum creatinine was discovered in the seropositive dogs, as was reported by several workers (Keenan *et al.*, 1978; Navarro *et al.*, 1981; Venkataraman and Nedunchelliyan 1990b, 1992).

5.4.3 Serum alanine amino transferase (ALT)

In the present study, the difference in serum ALT was non-significant, as against the observations of increased ALT value by several workers (Keenan *et al.*, 1978; Navarro *et al.*, 1981; Verma, 1982 and Greene and Shotts, 1990). Navarro *et al.* (1981) had reported increased serum ALT in the severe form, in icteric dogs. In the present study, severe form of the disease with icterus was noted in one case only. Most of the seropositive dogs showed mild to moderate symptoms of infection and hence had a lower ALT value.

5.4.4 Serum total protein

The mean serum total protein was slightly less in seropositive dogs, but the difference was non-significant. The seropositive stray dogs had very low value of serum total

protein and consequently a low mean value. Progressive increase in the serum total protein, with increase in the days of post inoculation was reported by Keenan *et al.* (1978).

Serum albumin

In the present study, no significant difference in the mean albumin value was recorded, as against the observation of lower albumin value in experimental cases of leptospirosis by Keenan *et al.* (1978) and Navarro *et al.* (1981).

Serum globulin

There was non-significant difference in the mean serum globulin, as against the change in different globulin fractions recorded by Keenan *et al.* (1978).

Albumin-globulin ratio (A:G)

There was no significant difference in the A:G ratio in seropositive dogs.

5.5 Incidence

Age

In the present study, dogs of the younger age group of upto three years were more affected, which is in agreement with reports of Schnurrenberger *et al.* (1962);

Ryu (1975); Venkataraman and Nedunchelliyan (1990a); Venkataraman and Nedunchelliyan (1992).

Sex

In the present study, male dogs were more seropositive, as has been observed by several other workers (Meyer *et al.*, 1939; Pearson, 1964; Torten *et al.*, 1971; Ryu, 1975; Stuart, 1946; Morter *et al.*, 1959; Venkataraman and Nedunchelliyan, 1992; Venkataraman and Nedunchelliyan, 1993 and Brihuega *et al.*, 1995).

Breed

In the present study, non-descript and German shepherd dogs were more seropositive compared to other breeds; as has been described by Hartman (1984). Scanziani *et al.* (1994) has reported high titres to *leptospira* serovars among German shepherd dogs. Venkataraman *et al.* (1994b) have described experimental infection in non-descript dogs. Most of the non descript dogs are maintained by people of low income group, thereby this particular group is not properly kenneled and get more chance for roaming and picking up the infection, whereas the high population of German Shepherd dogs in Thrissur might be one important factor in the present finding.

Season

In the present study, more number of seropositive cases were during June, May and December. Though leptospirosis can occur at any time of the year, a strong association between periods of high rainfall and leptospirosis has been documented (Sullivan, 1974; Faine, 1982; Venkataraman and Nedunchelliyan, 1990a).

Summary

CHAPTER-VI

SUMMARY

Leptospirosis is an important zoonotic disease. Dogs being an important companion animal of human being, and with more contact with man than other domestic animals the prevalence of leptospirosis in dogs was studied.

The seroprevalence of leptospirosis in one hundred and fifty dogs including 138 dogs presented to the Hospital and twelve stray dogs was studied by microscopic agglutination test using a battery of live *Leptospira* serovars *canicola*, *icterohaemorrhagiae*, *autumnalis*, *australis*, *ballum*, *bataviae*, *hebdomadis*, *pomona*, *pyrogenes* and *grippotyphosa*.

Blood and urine sample of dogs with symptoms suggestive of leptospirosis, and from stray dogs were subjected to examination by dark field microscopy and isolation of leptospires. Kidney tissue from sacrificed stray dogs was also cultured for the isolation of the organism. Haematology and biochemical analysis were done in one hundred and twenty dogs.

Of the 150 cases 49 (32.67 per cent) were positive and reaction to *L. pomona* (53.06 per cent) was highest followed by *L. canicola* (32.65 per cent) and *L. icterohaemorrhagiae* (14.28 per cent). Among the stray dogs, 90 per cent reacted

to *L. pomona* and ten per cent to *L. canicola*. Higher seroprevalence of 83.33 per cent was recorded among the stray dogs.

Leptospiraemia was detected in two clinical cases (C 261 and C 11556) and in two stray dogs (S-6 and S-10) by DFM examination, among the 35 dogs.

On culture of blood from these cases, blood sample from stray dog S-6 showed evidence of growth in the medium but it was contaminated. Attempts to purify the culture was also not successful.

Leptospiruria could not be detected in the eight suspected cases by DFM examination or on cultural isolation.

Kidney tissue from six sacrificed stray dogs were cultured and the culture medium of S-6 revealed the presence of leptospires on DFM examination. But contaminants could not be reduced on further subculture, millipore filtration or guinea pig inoculation. Young dogs, below three years of age were more seropositive in this study. Leptospiral agglutinins were more prevalent among the male dogs; and among the breeds in the non-descript and German shepherd dogs. Seasonal prevalence was higher during June and , May correlated with rainfall and during December.

Among the haematological parameters monocytosis was highly significant ($P \leq 0.01$) in the seropositive dogs. Increase in the mean values of ESR, total leucocyte count and absolute neutrophil count were recorded, but the differences were not significant. A slight increase in the mean PCV, absolute lymphocyte count and absolute eosinophil count were recorded in the seropositive group. Mean haemoglobin level was slightly lower in the seropositive dogs, compared to the seronegative dogs.

The biochemical changes recorded were significant increase in creatinine level ($P \leq 0.05$) in the seropositive group, non-significant increase in total bilirubin level, albumin and Albumin globulin ratio in the seropositive group. Mean serum total protein, globulin, serum ALT values were lower in the seropositive group compared to the seronegative group.

Leptospirosis was hither to not surveyed in dogs in Kerala. Hence the finding of prevalence of 32.66 per cent in dogs in Thrissur is significant. Leptospirosis should be considered in the diagnosis of suspected cases. A change in the epidemiology of leptospirosis in dogs, with prevalence of agglutinins to *L. pomona* in addition to *L. canicola* and *L. icterohaemorrhagiae* has been highlighted based on this study. Hence the need to include *L. pomona* bacterin in vaccines.

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PREVALENCE OF LEPTOSPIROSIS AMONG DOGS IN THRISSUR

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

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ABSTRACT

The seroprevalence of leptospirosis in one hundred and fifty dogs from different parts of Thrissur including 138 dogs presented to the Hospital and twelve stray dogs were studied by microscopic agglutination test.

Among the 150 dogs, 49 (32.67 per cent) were seropositive to *Leptospira* serovars *pomona* (53.06 per cent), *canicola* (32.65 per cent) and *icterohaemorrhagiae* (14.28 per cent). 83.33 per cent of the stray dogs were seroreacative with agglutinins to *L.* serovars *pomona* (90 per cent) and *L. canicola* (10 per cent).

Seroprevalence recorded was higher age wise among young dogs, upto three years of age, among the male dogs and among non-descript and German Shepherd dogs. Monthwise prevalence was higher during June, May and December.

DFM examination were carried out on blood samples from 23 suspicious cases of leptospirosis and 12 stray dogs, and on urine sample from eight suspected cases. Leptospiraemia was detected in two clinical cases (C 261 and C 11556) and from two stray dogs (S-6 and S-10). Urine samples were negative for leptospires or DFM.

Culture and isolation were tried with blood sample from 23 suspected cases of leptospirosis and from 12 stray dogs, urine sample from eight suspected cases of leptospirosis and kidney tissue from six sacrificed stray dogs. Of these, blood sample from stray dog S-6 showed evidence of growth in the medium, but leptospires could not be isolated. The culture medium with kidney tissue from stray dog S-6 revealed the presence/growth of leptospires on DFM examination. But isolation of leptospires from amongst the contaminants was not possible on further subculture, millipore filtration or guinea pig inoculation.

Haematological findings in the seropositive dogs were monocytosis which was highly significant, non-significant increase in the mean values of ESR, total leucocyte count and absolute neutrophil count. A slight increase in the mean PCV, absolute lymphocyte and eosinophil count were recorded. Mean haemoglobin level was slightly lower in the seropositive dogs, compared to the seronegative dogs.

On biochemical analysis, significant increase ($P \leq 0.05$) in creatinine level was recorded in the seropositive dogs. There was increase in the mean total bilirubin, albumin and albumin-globulin ratio in the seropositive dogs, but it was non-significant. Mean serum total protein, globulin, serum ALT values were lower in the seropositive group compared to those of the seronegative group.

Appendix

APPENDIX

IN/OUT - PATIENT RECORD
VETERINARY HOSPITAL - MANNUTHY/KOKKALAI

O.P. No. ———————— Date of admission: ————————

Name & Add
of Owner ———————— Date of discharge: ————————
————————
————————

Animal: ————————

Breed : ————————

Sex : ————————

Age : ————————

Colour: ————————

Vaccination Status: ————————————————

Patient Profile In house Outside Chained Loose

History Present: ————————————————————————
————————————————————————

Past: ————————————————————————

Clinical Data ————————————————————————

R/minute) ———— P/minute) ———— T (°F) ————
MM ————

Clinical Observation

Digestive system

Appetite P/A

Defecation P/A Normal/Gastroenteritis

Vomiting P/A

——— Frequent

——— Immediately after feeding

——— 1-2 hrs. after feeding

Nature of vomitus:

Feed/Watery/Mucoid/Blood

Colour of vomitus:

Yellowish/Plain

Gastroenteritis P/A

——— Frequent

Passing blood/mucus

Smell - Foul/No. abnormality

Palpation of abdomen - pain/ No. pain

Oral Cavity

——— Normal

——— Haemorrhage

——— Erosion of mucosa

——— Foul smell

——— Discolouration, if any

Respiratory system

Rate

Character of respiration

Nasal discharge

P/A

Epistaxis P/A

Cough P/A

Trachea

Circulatory system

Pulse Rate Rhythm

Auscultation of heart:

Superficial lymph glands: Normal/Enlarged

Nervous/locomotor system

Behaviour

Posture Gait

Reflexes

Abnormal acts

Muscles

Eye

Visible m.m.

Ears

Urinary system

Micturition P/A colour

Kidneys

Reproductive system

Mammary gland Scrotum

External genitalia Penis & prepuce

Skin & external surface

Skin and coat

Muzzle

Ectoparasite P/A

Dehydration P/A

Lesions

Date	Major findings	Diagnostic plan samples collected	Therapy given

VETERINARY HOSPITAL - KOKKALAI/MANNUTHY

TEST REPORTS

O.P. No. ———————— Date: ————————

Species: ———————— Animal: ———————— Breed: ————————

Sex: ———————— Age: ———————— Colour: ————————

HAEMATOLOGY REPORT

Haemoglobin	:	Total W.B.C. Count:	
P.C.V.	:	Neutrophils	:
E.S.R.	:	Lymphocytes	:
		Eosinophils	:
		Monocytes	:
		Basophils	:

Interpretation/
inference

BIOCHEMICAL REPORT

Blood glucose	:	Albumin	:
Serum creatinine	:	Globulin	:
Serum bilirubin	:		
Serum ALT	:		
Serum total proetin:			

Inference

URINE EXAMINATION REPORT

Colour	:	Specific gravity	:
Transparency	:	Bile salt	:
Albumin	:	Bile pigment	:
		Urobilinogen	:

Deposits

RBC	:
Epithelial cells	:
Pus cells	:
Casts	:
Organisms	:

Inference

FAECAL SAMPLE EXAMINATION REPORT

Macroscopic	:	Colour	:
		Consistency	:
		Blood	:
Microscopic	:	Ova	:

Inference

171442

SAMPLE EXAMINATION REPORT

O.P. No. : _____

Date of admission : _____

Animal: Breed: Sex: Age:

I Blood

Date of collection
Date of examination

Subject to _____

Haematology

Dark field

Culture

II Serum

Date of collection
Date of examination

Subject to _____

Biochemical test

MAT

III Urine

Date of collection
Date of examination

Biochemical tests

Microscopic

Dark field

Culture