RISK FACTORS OF LEPTOSPIROSIS IN ANDAMAN ISLANDS - A MATCHED CASE-CONTROL STUDY

by

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CERTIFICATION

This is to certify that this dissertation entitled **'Risk factors of leptospirosis in Andaman Islands - a matched case-control study'** submitted by Dr. A.P. Sugunan in partial fulfilment of the requirements for the degree of Master of Applied Epidemiology is the original work done by him and has not been submitted earlier in part or whole for any other (Publication or degree) purpose.

DIRECTOR

Date

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CHAPTER 1. INTRODUCTION

1.1. Leptospirosis – the disease, its distribution and public health importance

Leptospirosis is an acute bacterial infection caused by spirochetes belonging to the genus *Leptospira* (Waitkins SA, 1997) that can lead to multiple organ involvement and fatal complications. It has a wide geographical distribution and occurs in tropical, subtropical and temperate climatic zones. In the developed world, the incidence has come down substantially and most cases that occur now are associated with recreational exposure to contaminated water. In contrast, the incidence seems to be increasing in developing countries. Some countries such as Thailand, where leptospirosis is under surveillance, have recorded this increase in incidence (Tangkanakul W *et al*, 2000). Most countries in the South East Asia region are endemic to leptospirosis. The International Leptospirosis Society (ILS) made an attempt to compile data on occurrence of leptospirosis in various countries (World Health Organization, 1999). The data shows that on an average 10,000 severe cases requiring hospitalization occur annually all over the world.

A number of leptospirosis outbreaks have occurred during the past few years in various places such as Nicaragua (Zaki SR & Sheih WJ, 1996), Salvador (Ko AI *et al*, 1999) and Rio de Janeiro (Barcellos C & Sabroza PC, 2001) in Brazil and Orissa (World Health Organization, 1999; Sehgal SC *et al*, 2001), Mumbai (Karende *et al,* 2002) and Andaman Islands (Sehgal SC *et al*, 1995, Singh SS *et al*, 1999) in India. Some of these were as a result of natural calamities such as cyclone and flood.

1.2. Risk factors of leptospirosis

Leptospires are maintained in nature by a large variety of animal hosts. These include free-living animals such as rats, bandicoots and mongoose, domestic animals, wild animals and laboratory animals. Leptospires shed in the urine of carrier animals can survive in the environment for long period of time. The source of human leptospiral infection is either the carrier animals themselves or environment contaminated with leptospires. Hence, the commonly considered risk factors and behaviours are those that expose people to animal reservoirs or contaminated environment. Contact with various species of animals, animal tissue, animal urine and wet environment and occupational and recreational exposure to contaminated water bodies have been implicated as risk factors in various studies. A detailed review of several studies on risk factors of leptospirosis is presented in the next chapter.

1.3. Leptospirosis in Andaman Islands

Leptospirosis is known to be endemic in Andaman Islands since early years of 20th century. Case series with clinical features resembling those of Weil's disease were reported by Chowdry AK (1903), Woolley JM (1911 & 1913), de Castro AB (1922) and Barker FA (1926). The first report of bacteriologically confirmed leptospirosis in India originated from Andaman Islands in 1931. Taylor J and Goyle AN (1931) investigated several cases of Weil's disease among the free-living convicts at the penal settlement of Port Blair during a four-month period in 1929 and recovered 24 isolates of leptospires. They also reported the first common-source outbreak of leptospirosis in India among bund construction workers at *Mithakadi* village near Port Blair. During the period 26 June – 19 October 1929 they identified 64 cases confirmed either bacteriologically or serologically at Port Blair. The population of Port Blair in 1929 was 7,901. Assuming that no more cases were detected in 1929, the incidence was 810/100,000 population, which is probably the highest ever recorded incidence of leptospirosis.

No information about the status of leptospirosis in Andamans after 1931 is available. In the post-monsoon season of 1998, an outbreak of febrile illness with haemorrhagic manifestations appeared, first in Port Blair and other areas of South Andaman and then in Diglipur of North Andaman. Since the aetiology of the disease was unknown, it was named as Andaman Haemorrhagic Fever (AHF). AHF outbreaks recurred every year since then, but the aetiology remained unknown till 1993. Sehgal SC *et al* (1995) carried out studies during an outbreak at *Diglipur* in North Andaman in 1993 and on the basis of serological evidence they reported that the aetiology AHF was leptospires. Since there is no data on the status of leptospirosis in Andaman Islands between 1931 and 1988, it is generally believed that leptospirosis re-emerged in the islands after a gap of more than 50 years in a different clinical form. However, Roy S *et al* (2003) who studied the genetic characteristics of the strains isolated in the islands in 1929 and during the outbreaks of AHF since 1993 report that these strains were similar. This suggests that AHF outbreaks were not the result of reintroduction of a new strain of leptospires.

AHF continues to occur in Andaman Islands commonly as post-monsoon outbreaks and occasionally as sporadic cases (Singh SS *et al*, 1999). Reported incidence ranges between 40 – 80 per 100,000 population (Smythe LD, 1999). A surveillance system based at a rural primary health centre in South Andaman serving a population of about 9,500 detects 40 - 70 cases of leptospirosis every year (Vijayachari P *et al*, unpublished data). A serosurvey conducted in North Andaman (Murhekar MV *et al*, 1998) showed a seroprevalence of 54%. The prevalence rate showed a linear trend with age and was more than 72% in those aged above 30 years.

Outbreaks usually occur during the harvesting season and a large proportion of the affected persons are either farmers or members of farming families. No published report on risk of infection among different occupational groups in Andaman Islands exists. A study on risk factors was conducted in North Andaman in 1996 (Murhekar MV *et al*, 1998). The study used seropositivity as the outcome variable. Seropositivity does not necessarily indicate recent leptospiral infection. This would have resulted in under-estimation of the strength of association.

1.4. Need for a study on risk factors of leptospirosis in Andaman

For the past one decade, leptospirosis has been recognized as a disease of public health importance in Andaman Islands. Other than a few general awareness campaigns focusing on rodent control and hygienic practices that used to be organized during the times of epidemics, no specific control measure has been implemented yet. The outbreaks continue to occur and the disease incidence remains almost the same as when it reappeared in 1988. No efforts have been made to identify risk factors of acute leptospirosis in the islands. A study on risk factors conducted in North Andaman used seropositivity as the outcome of importance rather than acute symptomatic leptospirosis. Although this study gave some hints about the possible risk factors, this was not followed up further by studying the risk factors associated with incident acute cases. Seroprevalence among animal population has been estimated in some areas. But, no precise data on the relative importance of different animal species as the source of infection to human beings exist. Identifying the risk factors associated with acute leptospirosis is the essential first step in understanding the epidemiology of the disease in the islands and would generate useful information for planning control strategies. Hence the present study on risk factors of leptospirosis was conducted in Andaman Islands.

1.5. Objectives

The objective of the present study was to identify potentially modifiable independent risk factors associated with acute leptospirosis in Andaman Islands.

CHAPTER 2. LITERATURE REVIEW

TRANSMISSION & CONTROL OF LEPTOSPIROSIS

2.1. Introduction

Leptospirosis is the most widespread zoonosis in the world (Faine S, 1982). It is caused by spirocheates belonging to various pathogenic species of the genus *Leptospira* (Faine S 1998). Leptospirosis affects human beings and many other species of vertebrates. It can present in a wide spectrum of clinical manifestations in human beings (Levett PN, 2001). The syndrome of icteric leptospirosis with renal involvement is referred to as Weil's disease. Another recognized clinical form is that presenting with severe pulmonary haemorrhage (Sehgal SC *et al*, 1995; Zaki SR & Sheih WJ, 1996; Singh SS *et al*, 1999). In some countries such as China, pulmonary haemorrhage is considered as the complication of infection with a specific serovar of leptospires (Vinetz JM, 2001). Other complications include Acute Respiratory Failure (Silvia RRV & Brauner JS, 2002), myocarditis (Ramachandran S & Perera MVF, 1977), meningitis and renal failure (Muthusethupathy *et al*, 1994). Uveitis has recently been recognized as a late complication of leptospirosis (Rathinamsivakumar *et al*, 1996; Rathinam SR *et al* 1997).

2.2. Historical aspects

Diseases clinically resembling leptospirosis were recognized as occupational hazards of rice farmers in ancient China (Faine S, 1994). In 1886, Adolf Weil published his account of a clinical syndrome characterized by splenomegaly, jaundice and nephritis (Weil A, 1886 as quoted in Levett PN, 2001). This syndrome is usually referred to as Weil's disease and this has become synonymous with leptospirosis.

Leptospires were first identified as the cause of Weil's disease in Japan, where it was common among coal miners (Faine S, 1994). In 1915, Inada and Ido successfully transmitted the infection to guinea pigs and from the blood of the infected animals they grew the responsible organism. Unaware of this development, Huebener and Reiter reported the successful transmission of Weil's disease to guinea pigs in October 1915. They demonstrated flagella like bodies in Giemsa stained blood smears. Ten days later Uhlenhuth and Fromme also reported similar findings. Several years before this, Stimson had reported the presence of spiral organisms in kidney specimens stained with Levadeti technique (used to demonstrate spirocheats) from a patient, who was thought to have died of yellow fever (Stimson AM, 1907 as reproduced in Faine S, 1994). A World Health Organization Scientific Group on Research in Leptospirosis (1962-65) recognized Stimson's description as the first demonstration of leptospira (Abdussalam M *et al*, 1965).

Soon after the discovery that Weil's disease was caused by leptospires, several other disease entities were recognized to have a leptospiral etiology. These include '*nanukayami*' or the Japanese seven-day fever and '*akiyami*' the harvest fever. The same Japanese group that identified leprospires described the role of rats as their carriers (Faine S, 1994). Much of the basic current knowledge about leptospires and leptospirosis was understood within a decade of the discovery leptospires. Several types of leptospires such as *L. icterohaemorrhagiae, L. canicola, L. grippotyphosa, L. andamana, L. australis, L. bataviae, L. tarassovi* and *L. Pomona* were recognized during this period (Kmety E & Dicken H, 1988 & 1993).

2.3. Public health importance

Vast majority of leptospiral infections are either subclinical or result in very mild illness and the patients may not seek medical assistance (Levett PN, 2001). In a small proportion of patients, severe complications may set in. In such cases, there would be clinical manifestations of multiple organ involvement and the case fatality ratio could be more than 40% (Singh SS *et al*, 1999). Because of the protean manifestations of leptospirosis it is often misdiagnosed (Flannery B *et al*, 2001).

Leptospirosis is currently identified as a worldwide public health problem (World Health Organization, 2003). An increase in the incidence of the disease has been recorded in some countries where leptospirosis surveillance exists. In endemic areas, leptospirosis is a major cause of various clinical syndromes such as jaundice, renal failure (Muthusethupathy MA *et al*, 1995), myocarditis and atypical pneumonia. The annual incidence of leptospirosis increased from 0.3/100,000 persons during the period 1982 – 1995 to 3.3/100,000 persons in 1997 – 98 in Thailand (Thangkankul W *et al* 1998). A multi-centric study in India showed that leptospirosis accounts for about 12.7% of cases of acute febrile illness attending hospitals (Sehgal SC *et al*, 2003). Leptospirosis is the cause of a significant proportion of cases of non-hepatitis A – E jaundice, non-malarial febrile illnesses and non-dengue haemorrhagic fever in South East Asian countries (Laras K *et al*, 2002).

During the past several years, large outbreaks of leptospirosis have occurred in many countries, particularly in Southeast Asian countries and central and south America. Sehgal SC *et al* (1995) investigated an outbreak of Andaman Haemorrhagic Fever (AHF) in North Andaman in 1993 and identified leptospires as the aetiological agents. AHF had been occurring as outbreaks during post-monsoon periods in Andaman Islands since 1988. Zaki SR & Sheih WJ (1996) reported that the aetiology of the acute febrile illness with pulmonary haemorrhage that occurred as a large outbreak in Nicaragua in 1995 following floods was leptospires. Ko AI *et al* (1999) reported the detection of a large urban outbreak of leptospirosis in Salvador in Brazil through a hospital based surveillance system. During an eight-month period in 1996, the surveillance system detected 326 cases of leptospirosis among the two million population of Salvador. The case fatality rate in this case series was 15%. About 42% of the cases detected by the surveillance system were initially misdiagnosed as dengue fever at the outpatient clinic. Another outbreak was reported in the same year in Rio de Janeiro following heavy rainfall (Barcellos C & Sabroza PC, 2001). Analysis using Geographic Information System (GIS) techniques showed that the cases were clustered around waste accumulation inside the flooded area. Sehgal SC *et al* (2001) conducted a study among the population of villages in Orissa that were submerged following the super-cyclone in 1999 (World Health Organization, 2001). About 14% of the studied subjects had febrile illness during the post-cyclone period and had serological evidence of leptospiral infection in the recent past. Karende S *et al* (2002) conducted a study among paediatric patients in Mumbai during heavy rainfall and floods in July – August 2001. Among the 93 children who had clinical presentation matching the Indian Leptospirosis Society's working definition for clinically suspecting leptospirosis, 30 (32%) were confirmed to have leptospirosis based on the results of rapid diagnostic tests.

In many tropical endemic areas, a significant proportion of the population is exposed to leptospires. A survey conducted among a random sample of 1067 persons in Seychelles showed a seroprevalence rate of 37% (Bovet P *et al*, 1999). A seroprevalence study in North Andaman showed a prevalence of 54% among apparently healthy population (Murhekar MV *et al*, 1998). The study also showed an increasing trend in seroprevalence with age. Seroprevalence was more than 72% in older adults. High seroprevalence was observed among some of the primitive tribes of Andaman and Nicobar Islands (Sehgal SC *et al*, 1999a). Ratnam S *et al* (1983) studied the seroprevalence among the people of a village near Chennai City following an outbreak of leptospirosis in cattle. Among the 75 persons studied, 47% showed antibodies against leptospires. High seroprevalence has also been seen even in some sub-tropical and temporate regions. A sero-epidemiological survey conducted in North-eastern Alpine regions of Italy detected 10% – 12% seroprevalence of leptospirosis among farmers and forestry workers (Nuti M *et al*, 1993). In a seroprevalence study conducted in Yucatan State of Mexico situated in the inter-tropical belt, 57 (14.25%) of the 400 randomly selected subjects were found to be sero-positive to leptospires (Vado-solis I *et al*, 2002).

In endemic areas, the prevalence and incidence of assymptomatic infection could be very high. A survey conducted in Seychelles (Bovet P *et al*, 1999) showed 9% point prevalence of assymptomatic leptospiral infection as proved by positive Polymerase Chain Reaction Test (PCR). In a study conducted in the North Andaman, 27% of the 396 persons followed up serologically had evidence of leptospiral infection during the follow up period of 12 weeks in the post-monsoon season (Sehgal SC *et al*, 2000a).

2.4. Leptospira – the pathogen

Leptospira is a genus of gram-negative bacteria that comes under the family *Leptospiraceae* of order *Spirochaetales*. Like other spirochetes, these are helically coiled thin bacteria and are motile propelled by a flagellar mechanism (Faine S, 1994). Although they are stained by simple stains or by Gram stain, they are very thin and pale and are difficult to visualize microscopically under direct illumination.

The natural habitat of leptospires is the renal tubules of their animal host. Although they are susceptible to environmental factors, particularly drying, they can survive for long periods in water and wet soil. Some serovars of leptospires have been found to survive retaining their infective potential in soil for up to 74 days (Smith DJW & Self HRM, 1955). There is also indirect evidence that leptospires can grow and multiply in environment under favourable conditions (Baker MF & Baker HJ, 1970).

2.5. Animal reservoir

The transmission cycle of leptospirosis involves the maintenance hosts, the carrier hosts, the environment and human beings (Waitkins SA, 1987). Almost every known species of rodent, marsupial and mammal can be carrier and excretor of leptospires (Faine S, 1994). The role of rodents in the transmission of leptospirosis was understood soon after the discovery of leptospires as the cause of Weil's disease. Although, leptospiral infection in domestic animals particularly dogs, was identified, leptospirosis in animals as a veterinary problem and a possible source of infection to human beings was identified only in late 1930s and 1940s (Faine S, 1994).

2.5.1. Rodents

Rodents are the first recognized carriers of leptospires. They are often incriminated as the source of infection to human beings. A large number of studies on seroprevalence and leptospiral carrier state in rodents have been conducted in various countries. Although serovar Icterohaemorrhagiae has been often associated with rodents, other serovars have also been isolated. Matthias MA & Levett PN (2002) studied mouse and mongoose in Barbados and found that the seroprevalence rates were 28.2% and 40.7% respectively. Leptospires belonging to serovars Arborea and Bim were isolated from mouse. As part of the strengthened leptospirosis surveillance in Thailand following the sudden increase in the incidence since 1996, a simple test to detect anti-leptospiral antibodies in mammals was developed by modifying the lepto-dipstick test (Gussenhoven GC *et al*, 1997) used as a rapid diagnostic test to detect human leptospirosis. Eighteen of the 60 (30%) wild rodents screened were positive in the test (Kollars TM Jr *et al*, 2002). Leptospires were isolated from nine rodents. Alder H *et al* (2002) carried out a study among rodents and shrews in Zurich, Switzerland to establish the leptospiral carrier rate. Kidney specimens from 190 small animals were screened using polymerase chain reaction (PCR) and 12.6% were positive. In a similar study conducted among rodents of the species *Rattus norvegicus* in Turkey, 27.1% of the kidney samples and 16.9% of the brain samples were positive (Sunbul M *et al*, 2001). Coypu (*Myocastor coupus*), a rodent living in stagnant fresh water, is a widespread pest in France. Michel V *et al* (2001) screened 738 coypus from six areas during 1996 – 99 using serological and bacteriological techniques. Seroprevalence ranged from 16.5% - 66%. Three isolates of *L. interrogans* serovars Icterohaemorhagiae and Sejroe were obtained from the rodents. Very high seroprevalence rate and carrier rates (90.9% and 82.9% respectively) were observed among house mice in Terceira Island in Azores (Collares-Pereira M *et al*, 2000). A multiple logistic regression analysis with environmental and biological factors as independent variables and leptospiral infection as dependant variable identified male *Mus domesticus*, sexually active and living in humid biotopes 500 m above the sea level as the most likely reservoir.

Saravanan R *et al* (2000) studied 28 rats and 58 bandicoots at Avadi, a suburb near Chennai, India following isolation of leptospires from a suspected human patient. Anti-leptospiral antibodies were seen in 14.3% of the rats and 16.1% of the bandicoots. Leptospires were isolated from one rat and four bandicoots. The human and rodent isolates were typed as *L. interrogans* serovar Autumnalis. Natarajaseenivasan K *et al* (2003) screened field rodents near the rice mills of Salem, a town in Tamil Nadu, India following isolation of leptospires from the urine of a rice mill worker suspected to have leptospirosis. Seroprevalence rate among rates was 52.1%. A serosurvey conducted among the rat population of Andaman and Nicobar Islands, where leptospirosis is highly endemic (Sharma S *et al*, 2003) showed 7.1% seroprevalence. Two isolates were obtained from rats.

2.5.2. Cattle

Leptospiral infection among cattle was first recorded in Russia (Faine S, 1994). Cattle all over the world may be infected with serovars Hardjobovis, Pomona, and Grippotyphosa. Infection with Icterohaemorrhagiae, Bratislava, Hebomedis, Autumnalis, Australis, Sejroe, Canicola and Bataviae also occurs (Faine S, 1982). Leptospirosis in cattle could be totally unapparent or may result in acute febrile illness or severe complications. Infection in buffalos and deer is also similar to that in cattle (Bahaman AR *et al*, 1988; Flint SH *et al*, 1986).

W.A. Ellis and colleagues carried out a series of studies on leptospirosis in cattle in Northern Ireland. In a serological study conducted on aborted bovine foetuses in 1978 (Ellis WA *et al,* 1978), 6.9% of the aborted foetuses showed presence of anti-leptospiral antibodies, whereas none of the 196 non-aborted foetuses had antibodies. The study shows the potential of trans-placental transmission of leptospirosis in cattle. In another study in Northern Ireland (Ellis WA *et al*, 1981) a randomly selected sample of 200 cattle at a Belfast abattoir, 143 mice trapped from the fields of seven farms that reported abortions in cattle due to leptospirosis and 197 badgers trapped from various parts in Northern Ireland were screened for leptospiral carriage in kidneys and presence of anti-leptospiral antibodies. Leptospires were isolated from kidneys of 28.5% cattle, none of the mice and 9.6% of the badgers. Seroprevalence in cattle was 34.7%. However, 19.6% of the renal carrier cattle were seronegative to the infecting serogroup and 16.1% were seronegative even to the infecting strain. Leptospires isolated from cattle were of serovar Hardjo, whereas those isolated from badgers were mostly Icterohaemorrhagiae. This study brings out a few points that are important from public health point of view. Certain serotypes seem to be strongly associated with some animal species. Whether this is because of biological or ecological reasons is an issue still being debated. Seropositivity is not a reliable indicator of leptospiral carrier state in cattle as 16% of the carrier cattle were seronegative to even the strain that was infecting them. In a study conducted among 60 cows and heifers from an abattoir, leptospires belonging to serovar Hardjo were isolated from the genital tract of 57% of the animals and from the urinary tract of 62% (Ellis WA *et al*, 1986). The findings of the study show the possibility of sexual transmission of leptospirosis in cattle.

Several studies on cattle leptospirosis were conducted in other countries such as Spain and Australia also. Alonso-Andicoberry C *et al* (2001) studied 762 diary cattle belonging to 81 herds in Spain using microagglutination test (MAT) with 11 leptospiral serovars as antigens. Leptospiral antibodies were detected in 8% of the cattle. Infected cattle were present in 43% of the herds screened. Bratislava was the most prevalent serovar followed by Grippotyphosa and Copenhegani. No significant association was found between herd size and seroprevalence rate. In another study in Spain (Guitian FJ *et al*, 2001) 18.3% of 442 cows screened were seropositive. Monthly follow up of 219 cows for a period of 12 months showed that about 25% of the cows seroconverted during this period. Espi A *et al* (2000) reported 10.4% seroprevalence among 3,578 cattle tested in Northern Spain. The common serovars were Pomona and

Grippotyphosa. Black PF *et al* (2001) screened 2857 female cattle from 68 herds in central Queensland using MAT. Antibodies against serovar Hardjo was detected in 15.8%, against Tarassovi in 13.9%, against Pomona in 4% and against Szwajizak in 2.2% of the cattle screened.

A serosurvey among animal populations of Andaman and Nicobar Islands (Sharma S *et al*, 2003) showed that about 40% of the cows and 26% of the bulls were seropositive. Ratnam S *et al* (1983) screened 40 cows in a village near Chennai following an outbreak of leptospirosis in cattle. Antibodies against leptospires were found in 68% of the cows. A limited survey conducted in Kottayam District of Kerala in India during an outbreak of leptospirosis in 2000 (Vijayachari P *et al*, unpublished data) showed 8% seropositivity among cattle brought to a slaughterhouse. A survey in villages affected by an outbreak of leptospirosis in Thane in India in 2001 showed a similar seroprevalence among cattle (Sehgal SC *et al*, unpublished data).

2.5.3. Other domestic animals

Pigs are commonly infected with serovars Pomona, Tarassovi, Grippotyphosa, Bratislava, Sejroe, Icterohaemorrhagiae and Canicola (Faine S, 1994). Adult non-pregnant infected pigs are usually symptom-free and become chronic carriers. Young pigs may show various signs and may die of renal failure. Ellis WA *et al* (1985) studied piglets aborted by 10 sows in a herd of 100 for various bacteria and viruses. Leptospires were isolated from pooled tissues of two litters while no other pathogens were detected. Leptospires were also isolated from the kidneys and genital tracts of both the sows and the boar of the herd. A study conducted in Mekong delta in Vietnam, 73 (29%) of the 424 sows screened were seropositive (Boqvist S *et al*, 2002). The commonest serovar was Bratislava. Contact with sows in neighbouring pens, age, being born in herds with infected animals, lack of rodent control measures and artificial insemination were identified as the risk factors of acquiring leptospiral infection. In the seroprevalence study conducted in Andaman and Nicobar islands (Sharma S *et al*, 2003), pigs had the lowest seroprevalence. Culture of urine sample from 17 pigs in a village in Middle Andaman where an outbreak of leptospirosis occurred, did not yield any isolates (Sharma S *et al*, unpublished data).

Cousins DV *et al* (1989) followed up 15 sheep over one year period by urine culture. Initial cultures yielded leptospires in 10 out of 15 sheep. At the end of one year follow up, three of these were still excreting leptospires. Isolated leptospires were of serovar Hardjo, commonly seen in cattle. All the leptospira excreting sheep became negative to IgM antibodies within three months. However, they were IgG seropositive. The study shows that sheep can also act as long-term carriers of leptospires. Although IgM antibodies may not be a reliable indicator of leptospira carrier state, presence of IgG antibodies can be used as an indicator of past exposure in sheep.

Historically, leptospirosis was recognized as a disease of dogs before it was known in any other animals. Canicola and Icterohaemorrhagiae are the common serovars that infect dogs (Faine S, 1994). Acute leptospirosis in dogs is known as Stuttgart disease, which is characterized by vomiting, dehydration, bloodstained faeces, mucosal sloughing and death. Some dogs survive with chronic nephritis and continue to excrete leptospires. Leptospirosis due to a variety of serovars is reported rarely in cats and it is not significantly different in course from the disease in dogs. In a serosurvey conducted among 245 kennelled dogs in Italy (Scanziani E *et al*, 2002), 72 were found to be seropositive. Bratislava and Grippotyphosa were the common infecting serovars. Venkataraman KS & Nedunchelliyan S (1992) reported an outbreak of leptospirosis in human beings and dogs in Madras City. Following the outbreak, a serosurvey was conducted among humans and dogs. Seroprevalence was 50.5% among humans and 21.3% in dogs. Leptospira belonging to serovar Icterohaemorrhagiae was isolated from a human patient and Canicola from a dog.

2.5.4. Wild animals and laboratory animals

Leptospires have been isolated from almost all warm-blooded animals. Ruiz-Pina HA *et al* (2002) reported a 4.9% seroprevalence among 91 captured opossums (*Didelphis virginiana*) in Mexico. Colagross-Schouten AM *et al* (2002) screened 225 free-ranging California sea lions presented to coastal marine mammal rehabilitation centres using MAT. They found an overall seroprevalence rate of 38.2%. Seropositivity was highest among adult and sub-adult males. The seropositives had elevated serum urea, creatinine and phosphorus. Smythe LD *et al* (2002) screened 271 Australian flying foxes (pteropid bats) in Queensland and found 28% seropositivity. *L. krishneri* serovar Australis was the commonest infecting serovar. Bunnell JE *et al* (2000) conducted a study among wild mammals in Peruvian Amazon basin. Wild mammals were trapped and their kidney samples were tested for leptospiral DNA using PCR. A total of 148 wild mammals of various species were trapped. This includes 72 rodents, 55 marsupials (opossums of various species), bats and carnivores. Seroprevalence was highest among the opossums (39%) followed by bats (35%) and rodents (20%). The study indicates that in certain ecological systems wild mammals other than rodents could be more important reservoirs of leptospires. Perolat P *et al* (1992) reported an outbreak of leptospirosis in a squirrel monkey (*Saimiri sciureus*) colony at the Pasteur Institute, French Guyana. Eleven animals among the 109 in the colony had acute febrile illness with jaundice and haemorrhages. Ten of them died. Another five pregnant monkeys had miscarriages. All the affected monkeys were MAT positive. Urine specimens from two monkeys yielded positive culture. A serosurvey conducted among the unaffected animals showed a prevalence of 26%. Newly trapped feral squirrel monkeys also showed seropositivity.

2.6. Modes of transmission

Leptospires are ubiquitous. They are found wherever experts in leptospirosis, astute and aware medical and veterinary practitioners and epidemiologists, and adequate specialist laboratory facilities exist (Fane S, 1994). The primary source of leptospires is the excretor animal, from whose renal tubules leptospires are excreted into the environment with the animal urine.

Transmission can be direct or indirect. Direct transmission occurs when leptospires from tissues, body fluids or urine of acutely infected or asymptomatic carrier animals enter the body of the new host and initiate infection. Direct transmission among animals can be transplacental, haematogenous, by sexual contact or by suckling milk from infected mother. Presence of leptospires in genital tracts as well as transplacental transmission has been demonstrated in animals (Ellis WA *et al* 1978; Ellis WA *et al*, 1985; Ellis WA *et al* 1986). Direct transmission from animals to human beings is common in occupational groups who handle animals and animal tissue such as butchers, veterinarians, cattle and pig farmers, rodent control workers etc. Accidental infection to veterinarians has been recorded (Bolin CA & Koellner P, 1988). Demers RY *et al* (1985) studied the risk of leptospiral exposure to rodent control workers in Detroit by a comparative cross-sectional study among the workers and two control groups. A statistically significant higher risk was found in rodent control workers (OR: 4.37; 95% CI: 3.0, 6.3 and OR: 11.08, 95% CI: 5.6, 22). Human-to-human transmission through breast-feeding has also been recorded (Bolin CA & Koellner P, 1988).

Indirect transmission occurs when an animal or human being acquires leptospirosis from environmental leptospires, originating in the urine of excretor animals (Faine S, 1994). Leptospires can survive for long periods of time in the environment (Smith DJW & Self HRM, 1955) and probably multiply when the conditions are favourable (Baker MF & Baker HJ, 1970). It is considered that the most common portal of entry of leptospires into the host body is through intact skin (Faine S, 1994). High incidence has been recorded among people who are exposed to wet environments because of occupational or other activities. Leptospirosis is a known health hazard of rice farmers in countries such as Indonesia and Thailand. High incidence of leptospirosis has been recorded in provinces with large populations of farmers (Tangkanakul W *et al*, 1998). Outbreaks have occurred in Korea on several occasions when the fields were flooded before harvest (Park S *et al*, 1987).

Outbreaks have occurred among general population when people are exposed to floodwaters that have high chance of leptospiral contamination (World Health Organization, 2000; Sehgal SC, 2001; Zaki SR & Sheih WJ, 1996).

Leptospirosis has been recognized as a potential hazard of water sports and other recreational activities that exposes people to possibly contaminated waters. Outbreaks associated with recreational exposure to water have been reported from several countries. Anderson DC *et al* (1978) investigated a cluster of seven cases of leptospirosis among the residents of Stewart County, Tennessee. Frequency of exposure to a creek was the only factor significantly associated with leptospirosis. Sejvar J *et al* (2003) investigated an outbreak of febrile illness among the athletes who participated in the eco-challenge sabah 2000 multi-sport expedition race in Borneo, Malaysia. Among the athletes studied, 54% were found to have serological evidence of leptospiral infection. Swimming in the river was significantly associated with leptospirosis.

Ingestion of contaminated water has been reported to cause leptospirosis by some investigators. Cacciapouti B *et al* (1987) investigated an outbreak of leptospirosis that occurred in a small town in Central Italy. In a case-control analysis, drinking water from a fountain was found to be significantly associated with leptospirosis. Corwin A *et al* (1990), who investigated an outbreak of leptospirosis at Okinawa, Japan, reported that swallowing water while swimming was significantly associated with leptospirosis.

2.7. Risk factors

A number of studies on risk factors of leptospirosis have been conducted in different settings and using different designs. Some of the studies were part of investigation of outbreaks, whereas others were planned case-control studies. In some studies symptomatic leptospiral infection was used as the outcome whereas in some others presence of anti-leptospiral antibodies was used as the outcome. Both matched and unmatched case-control designs have been used. Five case-control studies conducted in various countries and reported during the past one decade are reviewed in detail here. The studies are listed in Table 1.

Table 1. Details of five studies on risk factors of leptospirosis reviewed in detail

Authors	Year	Title	Place, Country
Sasaki DM *et al*	1993	Active surveillance and risk factors of leptospirosis in Hawaii	Hawaii, USA
Bovet P *et al*	1999	Risk factors associated with clinical leptospirosis: a population-based case-control study in the Seychelles (Indian Ocean)	Seychelles Island
Tangkanakul W *et al*	2000	Risk factors associated with leptospirosis in North-eastern Thailand 1998	North eastern Thailand
Ashford DA *et al*	2000	Assymptomatic infection and risk factors of leptospirosis in Nicaragua	El Sauce, Nicaragua
Sarkar U *et al*	2002	Population-based case-control investigation of risk factors of leptospirosis during an urban epidemic	Salvador, Brazil

2.7.1. Active surveillance and risk factors of leptospirosis in Hawaii

Sasaki DM *et al* (1993) present the results of a hospital-based surveillance and a matched case-control study conducted at Kauai Island and east coast of Hawaii (big island) during 1988 – 89. The objectives of the study were to determine the incidence of leptospirosis in big island and to identify risk factors specific to the area. Patients attending the hospitals and clinics in the two islands who fulfilled a pre-defined case definition were included in the study. The study was conducted for a period of one year in east coast of big island, but for only six months in Kauai. Annual incidence was estimated using the data from big island.

Patients identified by the surveillance system based on the case definition were screened using blood culture for leptospires and microaglutination test (MAT) on paired samples. Patients who could be diagnosed definitely by a positive culture, by a four-fold rise in titre or seroconversion in MAT or were positive in fluorescent antibody testing of tissue specimens in the case of fatal cases were considered as definite cases. Eligible controls were the remaining patients who met the clinical criteria but did not have leptospiral infection as proved by negative culture and negative results in MAT on paired samples. Age, sex, island and season matched controls were drawn from the list of eligible controls. On an average there were 2.3 controls per case (it is not mentioned whether one-to-one matching or frequency matching was employed). Case patients and control patients were interviewed over telephone using a standard questionnaire.

From the big island 172 patients fulfilling the case definition were identified. Among them 20 fulfilled the laboratory criteria for leptospirosis. Based on various other factors such as the number of cases detected outside the surveillance system, predictive value of the case definition, sensitivity of clinicians' initial diagnosis etc., three estimates of incidence were made. These were 58 cases/100,000, 128 cases/100,000 and 444 cases/100,000.

Case-control analysis was based on 33 confirmed cases and 77 matched controls. Crude (unmatched) analysis showed that use of water catchment systems, drinking surface water, skin wounds, contact with cattle and cattle urine and handling of animal tissues were associated with leptospirosis. A grouped analysis (matched analysis) confirmed the association of leptospirosis with use of water catchment systems, skin wounds and handling animal tissues. Prevalence of these factors among cases and controls and p-value were presented in a table. Mean number of times surface water was drunk by cases and controls were compared and was found to be not different among cases and controls. Interaction between skin wounds and various high-risk activities were studied by stratified analysis. For some of the factors studied, the odds ratio for skin wounds showed great variation between those with the presence of the factor and those without (e.g. 12.5 and 2.5 respectively for those who had garbage contact and those did not have; 5.2 and 1.4 respectively for those did gardening and those did not). However the statistics used to test interaction showed that no interaction was present.

The study had several drawbacks. Although it was stated that cases were identified through an active surveillance, it was a hospital and clinic based surveillance system depending on voluntary reporting by patients. Hence it was a passive surveillance system. The compliance by physicians/clinics was poor. Review of hospital records revealed that about 300 cases fulfilling the case definition had attended the clinics/hospitals in one month, but the total number of cases detected during the whole year was only 172, which was less than 5% of the expected number of cases. In an attempt to overcome this deficit, various indirect methods were used to make estimates of annual incidence of leptospirosis. Confidence intervals for the estimates were not presented. The possibility of variability in the factors used in making the estimates such as seasonal variation in the occurrence of cases fulfilling the case definition, changes in the prevalence of leptospirosis cases among the suspects due to seasonal variation in the incidence of leptospirosis etc. were not considered. Because of these possible errors, the estimates can be inaccurate.

Since a large number of cases were missed by the surveillance system, it is possible that the cases detected were actually the more severe cases requiring hospitalisation. This could have resulted in an over-estimation of the strength of association. The controls did not represent the healthy population of the area. They were patients with symptoms similar to those of leptospirosis, but without leptospiral infection. They might have had some other infection that had risk factors similar to those of leptospirosis resulting in an under-estimation of the strengths of association.

There was variable number of controls per case. In the first step of analysis unmatched analysis was done on the matched data, which is inappropriate. Factors that were significantly associated with leptospirosis in the unmatched analysis were then tested by matched analysis. Strengths of association and confidence intervals were not calculated. Only the prevalence of the factors among cases and control and the p-value were presented. No efforts were made to look for confounding effects. Neither stratified analysis, nor logistic regression was performed.

Two of the identified risk factors *i.e.* skin wounds and handling animal tissues are known risk factors of leptospirosis. The third one *i.e.* use of water catchment system in the household is specific to the area. The first two can be modified to some extent by advocating proper care of wounds and abrasions and use of protective clothing while handling animal tissues. The study methodology had certain defects. Appropriate statistical analysis was not performed. There is possibility of selection bias and confounding. Because of these, the estimates of strength of association between the studied factors and leptospirosis could be erroneous.

2.7.2. Risk factors associated with leptospirosis in North-eastern Thailand 1998

Tangkanukul W *et al* (2000) report the results of a case-control study conducted in Nakhornratchasrima Province in the Northeastern region of Thailand in 1998 to identify potentially modifiable risk factors for leptospirosis. Incidence of leptospirosis in Nakhornratchasrima Province was higher than the national incidence in 1997 (16/100,000 Vs 3.3/100,000). The study was conducted between August and December when the incidence of leptospirosis peaks in Thailand. Suspects were identified based on a case definition at community and provincial hospitals. They were tested for leptospiral IgM antibodies using commercially available ELISA kits. ELISA positive suspects were considered as cases. Persons from the neighbourhood of the cases who did not have any illness during the previous 30 days and were negative in IgM ELISA test were selected, after matching to the corresponding case for age (±5 years) and sex, as controls. Information regarding activities associated with water and animals and environmental conditions of house and workplace was collected from cases and controls using a standardized questionnaire. An environmental survey was also conducted at the house and workplace. Univariate matched analysis was performed to obtain odds ratios. Stepwise conditional logistic regression was done to identify independent risk factors.

In 1998, 262 suspected leptospirosis patients were reported to the public health division that includes Nakhornratchasrima Province. Case Fatality Ratio was 10%. During the study period 80 suspects were identified from Nakhornratchasrima Province and IgM ELISA confirmed 62 cases. Three patients died (CFR: 4.8%) and they were excluded from the study. Among the remaining 59 cases, 51 had two matched controls each and 8 had only one control each. Univariate matched analysis showed that ploughing, pulling out sprouts, replanting sprouts and fertilizing in wet fields for more than 6 h/day, walking through water, having cuts on feet and keeping pet dog were significantly associated with leptospirosis. Sowing, fishing and not wearing boots were not significantly associated. In the logistic model, walking through water, pulling out sprouts, ploughing, and fertilizing in wet fields for more than 6 h/day emerged as significant independent variables.

IgM ELISA was used to diagnose cases and to exclude assymptomatic infection in controls. The test has sensitivity and specificity of about 90%. False positivity and negativity of the test would result in some diferential misclassification leading to an under-estimation of the strength of association. IgM antibodies remain for about three months after infection and hence the test wouldn't be able to detect infection in controls occurred more than three months earlier. This again would result in misclassification. The study was conducted during a particular season, apparently during rice cultivation. Hence the study would have failed to identify risk factors resulting from other seasonal activities. Three fatal cases had to be excluded from the study for cultural reasons. So the cases selected were relatively milder ones. If the risk factors have dose-response relationship with leptospirosis in terms of severity, excluding severe cases would result in an under-estimation of the strength of association.

The study used a very liberal case definition i.e. presence of fever, headache and muscle pains. All these symptoms are seen in most acute bacterial and viral infection. In spite of this, 77.5% of the suspects identified based on this case definition were positive in IgM ELISA and three of them died.

This high predictive value of such a liberal case definition is difficult to explain.

The study has some minor drawbacks such as possible misclassification error due to false results of the laboratory test and inability to detect risk factors that may be important during seasons other than the study period. The limitations of the laboratory test are unavoidable, as an alternate test would have its own problems. Perhaps the objective of the study was primarily to detect risk factors that contribute to the bulk of the cases occurring in the peak season. The identified risk factors pertain to agricultural practices and could be modified possibly through introduction of alternate cultivation techniques or through programmes targeting behaviour modification of farmers.

2.7.3. Risk factors associated with clinical leptospirosis: a population-based case-control study in the Seychelles (Indian Ocean)

Bovet P *et al* (1999) conducted this study in Seychelles island during a one-year period starting from 1 April 1995 to determine the incidence of acute leptospirosis and the prevalence of subclinical infection and to identify risk factors of acute leptospirosis in an endemic tropical setting. Seychelles has high incidence of leptospirosis (40 – 60/100,000). There are two major hospitals where leptospirosis cases could be treated. The study was conducted at these hospitals. Suspects were identified by physicians based on a case definition and were referred to one of these hospitals. Suspects who showed seroconversion (to a minimum titre of 1:400) or four-fold rise in titres in microagglutination test (MAT) or had a positive result in PCR assay were considered as cases. Eligible controls were selected from a list of 1067 persons randomly selected from census data. For each case, age, sex and occupation matched persons were selected from the list and were screened by MAT and PCR. Those with a negative result in both were considered as a matched control for the case. Controls were selected and investigated within 15 days of identification of case, thus partially matching for period and consequently for rainfall. Information about social, educational, occupational, environmental and behavioural variables were obtained through interviews using a questionnaire. Difference in the frequency of the variables among cases and controls was tested using χ^2 statistic. Strength of association between the variables and acute leptospirosis was tested by three statistical analyses i.e. a univariate conditional logistic regression using the matched data for each variable, a univariate unconditional logistic regression adjusted for the matching variables and a multivariate stepwise logistic regression. It is not mentioned whether the multivariate logistic regression was conditional or unconditional.

During the study period, 125 suspects were identified and 75 of them matched the laboratory criteria for a definite diagnosis. Among 125 matched controls selected for these cases, 60 were positive in either MAT or PCR and hence were excluded. Among the 75 cases and 65 controls available, 38 original matched pairs remained. Ten variables in the univariate conditional regression and 15 variables in unconditional univariate regression (using unmatched data) had statistically significant association. Eight variables remained in the multivariate logistic model with statistical significance. These were Gardening, indoor occupation (protective), corrugated iron house, wet soil around house, refuse not being collected by public service, presence of cats at home, skin wounds and drinking home brews. The OR for these factors was in the range of 4.60 – 9.68.

The study used paired MAT, which is still the gold standard for serological diagnosis of leptospirosis, and PCR, which has a very high sensitivity and specificity during early stages of the disease, to identify cases. Hence there is little chance of misdiagnosis. The same tests were used to exclude leptospirosis in controls also and therefore it is fairly certain that the controls did not have leptospiral infection in the recent past. The incidence of leptospirosis calculated based on the number of cases detected (101/100,000) was higher than the previously reported annual incidence. This indicates that the case finding in the study had a better sensitivity than that in the routine surveillance system. The study was conducted during a period of one year and hence seasonal changes in risk behaviour might not have affected the results. If multiple controls were selected for each case and then screened using laboratory tests, it would have been possible to identify matched controls for all cases detected. The list of eligible controls was sufficiently large for this. Because of laboratory evidence of current or past leptospiral infection controls for half of the cases had to be excluded. Since the set of 75 cases and 65 controls were partially matched, it was not appropriate to perform an unmatched analysis on this data. It is not mentioned whether the final multivariate logistic regression was a conditional one or unconditional one.

Some of the identified risk factors could be targeted in control programmes. This includes more hygienic ways of preparing home brews, proper care of skin wounds, regular removal of house refuse, wearing of footwear and protective clothing while doing risky activities such as gardening.

2.7.4. Asymptomatic infection and risk factors of leptospirosis in Nicaragua

Ashford DA *et al* (2000) conducted a cross-sectional survey among a random sample of households in El Sauce in Nicaragua, approximately two months after an outbreak of leptospirosis to detect symptomatic/asymptomatic infection. The surveyed subjects were classified as cases and controls based on serological evidence of recent leptospiral infection and an unmatched cases-control analysis was done to identify risk factors. Serological evidence of leptospiral infection was defined as a positive result in commercially available IgM ELISA test. Households were selected at random using detailed map of El Sauce and information was collected about the household and about all the members present at the time of house visit using a standardized questionnaire. A univariate unmatched analysis was done to obtain the Odds Ratios. Variables that were significantly associated with seropositivity to leptospirosis were then included in a stepwise multiple logistic regression by backward elimination to determine the independent risk factors. Age and sex were retained in the model throughout to adjust for the confounding effect of these.

Households enrolled (218) had a total of 867 persons. However, blood samples could be collected from only 566 persons and only they were included in the analysis. IgM ELISA detected antibodies in 85 persons and among them 25 had febrile illness during the previous two months. Among household factors, rural household, availability of indoor water source and having electric lights and radio in the house were identified as as protective factors in the univariate analysis. Public facility, private well and river or lake as household water sources were risk factors. Among individual factors, bathing and washing clothes in river or brook, gathering wood, grinding grain and shelling/husking corn were risk factors. Multivariate analysis showed that rural household and gathering wood were independent risk factors and indoor water source is a protective factor. Rural household + shelling/husking corn was identified as a significant interaction term. Stratified analysis showed that shelling corn is a risk factor in urban households and a protective factor in rural households, but the associations were not statistically significant.

The study used presence of anti-leptospiral IgM antibodies detectable by ELISA test as the outcome. Presence of IgM antibodies indicates infection sometime during the previous three to six months. The test results in about 10% of false positive and negative results. Seventy percent of the identified cases had subclinical infection and the remaining 30% had mild illness. It is possible that the severity of the disease depends upon the dose of infection, which in turn, may depend upon frequency and dose of exposure to risk factors. Identified cases had very mild infections and hence might have had minimal exposure to risk factors, resulting in an under-estimation of strength of association. There were 85 cases and 481 controls, or the control to case ratio was 1:6. Except water source, the other identified risk factors are difficult to modify. The significance of finding a factor as a risk factor in urban setting and as a protective factor in rural setting is difficult to explain.

2.7.5. Population-based case-control investigation of risk factors of leptospirosis during an urban epidemic

The study (Sarkar U *et al*, 2002) was a matched case-control study in Salvador, a Northeastern coastal city in Brazil, during an urban epidemic in 2000. Suspects were defined as patients with conjunctival suffusion, jaundice and renal failure. Suspects were screened using an IgM ELISA test, prepared in-house using a circulating strain of leptospira as described by Terpstra *et al* (1985), during acute and convalescent phase. Diagnosis was confirmed by paired MAT. Age and sex matched controls were selected from the neighbourhood of cases. IgM ELISA positive controls were excluded. Information regarding potential risk factors was collected from cases and controls using a standardized questionnaire. Univariate odds ratios were calculated by matched analysis. Factors found significant in univariate analysis were included in a multivariate logistic model. A backward elimination method was used in conditional logistic regression.

During March – October 2000, 157 patients were hospitalised with suspected leptospirosis as per the case definition. Paired samples were available for 108 patients and 101 were found to have serological evidence of leptospirosis in IgM ELISA test. A random sample of 83 patients was selected from among these 101 patients as cases. However, 17 cases either did not agree to participate in the study or were not located at their residences. Therefore, the study was confined to the remaining 66 patients. The diagnosis of all these patients was confirmed by MAT. From the neighbourhood of the cases, 134 individuals, matched for age and sex to the cases, were identified and 132 among them agreed to participate. Serum samples were obtained from only 103 subjects and seven were positive in to case ratio was 1:6. Except water source, the other identified risk factors are difficult to modify. The significance of finding a factor as a risk factor in urban setting and as a protective factor in rural setting is difficult to explain.

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IgM ELISA. The case-control analysis was done on 66 cases and 125 controls (including 101 IgM ELISA negatives and 24 who did not give a blood sample). In the univariate analysis, 12 variables were found to be significantly associated with leptospirosis. Four variables were found to be significant in the multivariate analysis *i.e.* open sewer in proximity to residence, peri-domiciliary sighting of rats, sighting groups of five or more rats and workplace exposure to contaminants (floodwater, sewer or mud).

Cases were those admitted to hospitals with jaundice and renal failure *i.e.* they were cases of severe leptospirosis with signs of organ failure. Selecting only severe cases might have led to an over-estimation of strengths of association. The reason why only 83 patients were selected when 101 confirmed by ELISA were available is also not clear. Since the cases were positive in both IgM ELISA and MAT there is little chance for any misclassification of cases. Some of the controls were not tested by ELISA and hence the possibility of sub-clinical infection among them cannot be excluded. Risk factors identified were contact with rats, floodwaters, sewer and mud.

2.8. Prevention and control

Prevention of leptospirosis essentially is by identifying the source and interrupting the transmission (Faise S, 1994). In different epidemiological setting, different animal species could be the primary source of infection. In two of the risk factor studies reviewed here, significant association of leptospirosis with possible carrier animals was found. In the case-control study conducted in Seychelles (Bovet P *et al*, 1999) cats were found to be associated and in the study in Brazil (Sarkar U *et al*, 2002) rats were found to be associated. In a case-control study conducted in Andaman, cattle were found to be associated with leptospiral seropositivity (Murhekar MV *et al*, 1998). Even in the absence of statistical evidence of association between contact with an animal species and human leptospirosis, if significant carrier state is detected in animals, it could be taken as a potential source of infection to humans. However, since seropositivity is not a reliable indicator of carrier state (Ellis WA *et al*, 1981) a demonstration of excretion of leptospires in the urine by bacteriological or molecular tools are required.

Several strategies have been described to reduce the load of carrier domestic animals. Total herd immunization controlled leptospirosis in cattle in a trial (Little TW *et al*, 1992). A proposed clean herd policy in UK, which categorized cattle into different levels based on infection and immunization status and imposing restrictions on pasture, restocking herds etc. was later abandoned as it could not eradicate infection (Faine S, 1994). An alternate 'test and slaughter' strategy can be considered if no alternatives exist (Faine S, 1982).

Measures targeting human beings include vaccination and chemoprophylaxis. Vaccines have been developed for use in man (Torten M *et al*, 1973), however the existence of a large number of serovars of leptospires makes it difficult to develop a universally effective vaccine. Chemoprophylaxis with doxycycline has been tried in soldiers from non-endemic areas visiting endemic areas and was found to be almost 100% effective (Takafuji ET *et al*, 1984). However, a study conducted in endemic area during an outbreak showed only 54% protection (Sehgal SC *et al*, 2000). Chemoprophylaxis can only be used in outbreak situations or in travelers.

2.9. Summary

Leptospires are widespread pathogens and have a large number of animal hosts. Human infection results from accidental interaction of people with carrier animals or environment contaminated with leptospires. Although the basic principles of prevention such as reduction of source, environmental sanitation, more hygienic work-related and personal practices etc, are same everywhere, there is no universal control method applicable to all epidemiological settings. A good understanding of the ecological, epidemiological and cultural characteristics of a community that faces the problem of leptospirosis is the essential prerequisite for evolving an effective and acceptable control measure

CHAPTER 3. METHODOLOGY

3.1. Study area and study population

The study was conducted in Rangat (Fig. 1) and adjoining villages in Middle Andaman, Andaman & Nicobar Islands during October – November 2002. Andaman and Nicobar is an archipelago of 572 islands, islets, reefs and rocks in the Bay of Bengal situated about 1,200 km east of Indian subcontinent. The Union Territory of Andaman & Nicobar comprises of two districts *viz.* the Andaman district and the Nicobar district and the total population is 356,265 as per the 2001 census (Directorate of Census Operations, Andaman & Nicobar Islands, 2001).

About 86% of the land area of the islands is covered by evergreen forest. The climate is tropical throughout the year with temperature in the range of 25° C – 32° C and relative humidity in the range of 70% - 100%. The islands receive an annual rainfall in excess of 3300 mm. Most of the islands have an undulating topography with hills and small hillocks surrounding basins that fill with water during monsoons.

Andaman district has three main islands *i.e.* South Andaman, Middle Andaman and North Andaman and several small islands. About 75% of the population resides in rural areas and the remaining 25% at Port Blair, the only town in the Union Territory. In rural areas, 50% of the working persons are engaged in outdoor work (cultivators, agricultural labourers, marginal workers, livestock farmers, fishermen, forestry workers, hunters etc.) whereas in urban area 59% are white collar workers (trade, commerce and service sector) (Directorate of Census Operations, Andaman & Nicobar Islands, 1991).

3.2. Outbreak of leptospirosis at Rangat, Middle Andaman

Leptospirosis outbreaks occur usually in South Andaman and North Andaman. Although sporadic cases occasionally occur at various places in Middle Andaman and other islands of Andaman district, outbreaks had not been reported. During October – November 2002 a suspected outbreak of leptospirosis occurred at Rangat in Middle Andaman (Fig. 1). The outbreak was investigated to confirm the diagnosis and to identify the risk factors associated with the disease.

Fig. 1. Map showing the location of Rangat, Middle Andaman where the outbreak of leptospirosis occurred during October – November 2002

3.3. Suspected cases

A suspected case of leptospirosis in Rangat was defined as any patient who reported to CHC, Rangat on or after 1 October 2002 with complaints of fever associated with any of the following symptom/sign:-

(a) Severe muscle tenderness

(b) Any bleeding tendencies including sub-conjunctival haemorrhage

(c) Jaundice

(d) Cough, haemoptysis and breathlessness

(e) Oliguria

(f) Signs of mengeal irritation

3.4. Confirmed case of leptospirosis

A confirmed case was defined as a suspect who showed serological evidence of current leptospiral infection

3.5. Selection of controls

A set of eligible controls for each case was selected from the neighbourhood of the cases matching for age (±5 years) and sex. The selected persons were screened for serological evidence of asymptomatic or past leptospiral infection. Two seronegative persons were selected for each case as controls.

3.6. Clinical specimens

A blood sample was collected during the acute phase and another sample 10 – 14 days later from the suspected cases. One blood sample was collected from all the eligible controls. The blood samples were collected with aseptic precautions using pre-sterilized disposable syringes and needles into sterilized test-tubes. Samples were allowed clot for 6 h at 4°C (in a refrigerator). Test tubes were then centrifuged and the serum transferred to sterile serum vials. Serum samples were kept at -70°C until processed.

3.7. Laboratory tests

3.7.1. Lepto-dipstick

Lepto-dipstick test (Gussenhoven GC *et al*, 1997) was done following standard procedure on the acute serum samples of the suspected cases at the

CHC as an aid to rapid diagnosis. The results were recorded as negative, 1+, 2+, 3+ or 4+ depending on the intensity of the colour of the test band.

3.7.2. Microscopic Agglutination Test

Microscopic Agglutination Test was done on all acute and convalescent serum samples from suspected cases and the samples from eligible controls at the National Leptospirosis Reference Centre following standard procedure (Wolff JW, 1954). A panel of 12 leptospiral strains representing the common circulating serogroups of leptospires in India was used as antigens in MAT. Strains used as antigens in MAT are shown in Annexure I. MAT was done at doubling dilutions starting from 1:40. Positive samples were titrated up to a titre of 1:2560.

3.7.3. Isolation of leptospires

Isolation of leptospires was attempted from the acute blood samples of suspected patients. Two to three drops of blood was inoculated into tubes containing Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and incubated at 30°C. Cultures were examined under darkground illumination weekly till six weeks.

3.7.4. Polymerase chain reaction

Polymerase chain reaction (PCR) was done on some of the samples. DNA was purified from 1 ml serum following the method described by Broom R *et al* (1990) with the modifications suggested by Gravekamp C *et al* (1991). Two pairs of primers were used in multiplex PCR. These were G1 (5' CTG AAT CGC TGT ATA AAA GT) and G2 (5' GGA AAA CAA ATG GTC GGA AG) derived from recombinant plasmid pLIPs60 and B64-I (5' CTG AAT TCT CAT CTC AAC TC) and B64-II (5' GCA GAA ATC AGA TGG ACG AT) derived from the recombinant plasmid pBIM64.The former primer pair detects DNA from non-Krischneri group of leptospires and the later detects Krischneri group.

3.8. Criteria for laboratory diagnosis

A positive dipstick test was considered as an indication of possible current leptospiral infection for the purpose of initiating specific treatment.

Isolation of leptospires from the blood is considered as a confirmatory evidence of current leptospiral infection.

Seroconversion (negative to a titre of 1:160 or more) or four fold rise in titre was considered a definite evidence of current leptospiral infection in cases.

A titre of 1:160 or more was considered as an indication of possible current leptospiral infection among controls and such persons were eliminated.

A titre less than 1:160 among controls was considered as an indication of past leptospiral infection and such persons were eliminated.

A titre of 1:160 was taken as the cut-off for seroconversion as well for the diagnosis of possible current leptospiral infection in the controls as it is the closest titre to the ideal cut-off of 1:200 in endemic areas (Vijayachari P *et al*, 2001).

3.9. Information about potential risk factors

Information about potential risk factors was obtained by interviewing the cases and controls using a structured questionnaire (Annexure II). The questions were under three broad categories i.e. : -

Environmental: Type of house, house surroundings, proximity to water bodies, ownership of house and agricultural land.

Contact with animals: Cattle, swine, cat, dogs and rat infestation of houses.

Behavioural/occupational: Participation in agricultural activities, fishing, contact with garbage and sewage, cleaning animals, direct contact with animal urine, and recreational activities such as swimming.

Information was obtained by interviewing the subjects and by direct observation. All the questions except that regarding source of water for drinking and washing elicited a 'yes' or 'no' response and hence the corresponding variables were dichotomous. For water source, the response was one of the four categories *i.e.*

indoor tap, public tap, well or stream. Other sources such as tube well or river are not used in the study area. Questions regarding behavioural and occupational exposure to possible sources of leptospires sought information about such exposure during a four week period prior to onset of symptoms in the case of cases and during the corresponding period for their matched controls.

3.10. Population at risk

Information about population at risk was obtained from the list of residents of Rangat *tehsil* maintained by the Andaman & Nicobar Administration (available at http://www.andaman.nic.in; last accessed on 13 August 2003). This was used to calculate the attack rates in different villages in Rangat.

3.11. Information about acute febrile illness cases

Data on the reported number of cases of acute febrile illness cases was collected from three sub-centres serving the villages affected by the outbreak. The data was collected for the period 1 September – 15 December 2002. This data was used to see whether there was an increase in the number of cases corresponding to the outbreak.

3.12. Data analysis and statistical tests

3.12.1. Data entry

A database file was created using FoxBase 2.10 (Fox Software). All dichotomous variables were coded as 1 for 'Yes' and 0 for No. Categorical variables were coded as single digit numeric values and later were converted into dichotomous by creating dummy variables for each possible value except the reference value. Cases were coded as 1 and controls as 0. A numeric variable was created to uniquely identify each group of one case and two controls. Data was entered through a FoxBase data entry programme written specifically for the study. The FoxBase database file could be directly read by EpiInfo 6.04 (Dean AG *et al*, 1995) and EpiInfo 2002.

3.12.2. Descriptive statistics

A line graph was plotted using weekly moving average of reported cases of acute febrile illness at the three sub-centres by date of reporting to see the trend in the occurrence of fever cases in the affected villages. Frequency distribution of suspected cases and confirmed cases by date of onset of symptoms was plotted to study the characteristics of the epidemic curve. Attack rate of acute leptospirosis was calculated for each of the affected villages. Prevalence of the potential risk factors among cases and controls was calculated and the statistical significance of the difference in the prevalence was tested using χ^2 test (with Yate's correction) or Fisher's exact test as applicable.

3.12.3. Univariate Analysis

Univariate odds ratios for the potential risk factors were calculated by a matched analysis and 95% confidence intervals were calculated using maximum likelihood ratio method. McNamar χ^2 and p values were calculated to test the statistical significance. Analysis was done on EpiInfo Ver 6.04 (Dean AG *et al*, 1995).

3.12.4. Interaction terms

Biologically plausible first order interaction terms were listed. A stratified analysis was done for each pair of interaction terms and χ^2 for evaluation of interaction was calculated. If the test suggested (at 5% level) that the ORs differ by stratum (interaction), the interaction term was used for further analysis. The statistic was calculated using EpiInfo Ver 6.04 (Dean AG *et al*, 1995).

3.12.5. Multiple logistic regression

Conditional multiple logistic regression was done to identify independent risk factors and interaction terms using EpiInfo 2002. A backward elimination method following the procedure described in the online manual of the software was used. In the initial step all the variables and identified interaction terms that were associated with leptospirosis with statistical significance at 0.20 level were included. In each subsequent step, the variable/interaction term with lowest significance was removed. Individual variables of interaction terms present in the model were retained irrespective of statistical significance. The process was repeated till all the variables in the model were significant with p value < 0.05.

CHAPTER 4. RESULTS

4.1. Confirmation of outbreak

Fig. 2 shows the distribution of cases of leptospirosis admitted to CHC, Rangat during the period January 2000 to December 2002. The largest number of cases admitted in a month before November 2002 was ten in October 2000. In November 2002, 50 cases were admitted, which is in excess of the number of cases admitted during the corresponding period in the previous two years. Therefore, the existence of an outbreak is confirmed.

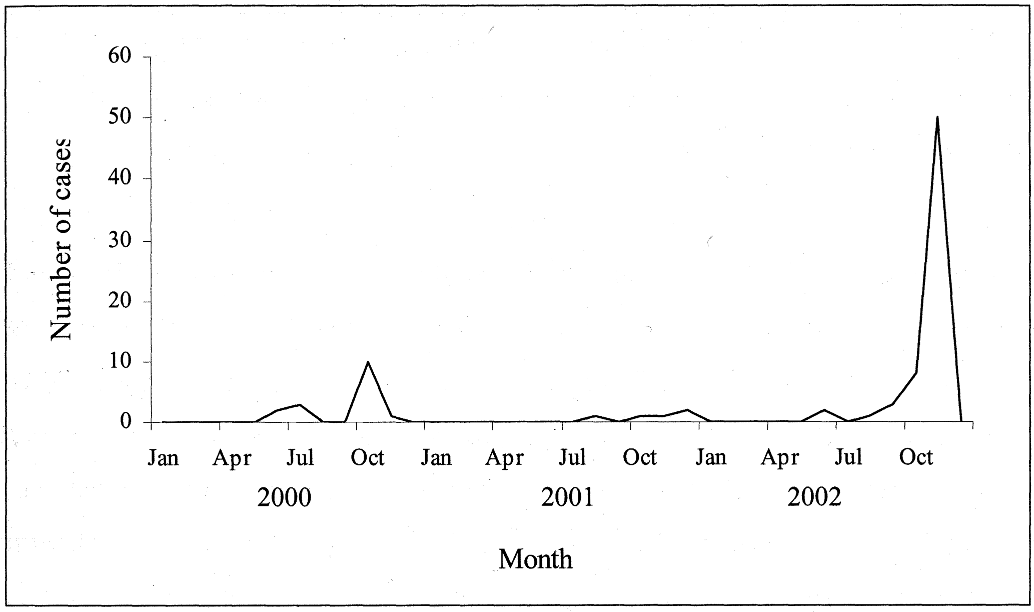

Fig. 2. Distribution of cases of leptospirosis admitted at CHC, Rangat during the period January 2000 to December 2002 by month of admission

4.2. Suspects, confirmed cases and deaths

During the period 24 October – 30 November 2002, 114 suspected cases of leptospirosis were admitted to CHC, Rangat. Lepto-dipstick test was done on blood samples from 100 patients and 45 were positive. The test was not done on the remaining 14 patients as they had recovered or died before the start of investigations. Among the six persons who died, dipstick test was done in the case of four patients and two of them were positive.

Table 2. Results of MAT on samples from suspected cases, outbreak of leptospirosis at Rangat, Middle Andaman, 2002

Result	No.	(%)
Patients with paired samples	**79**	
Seroconversion	38	(49.4)
Four-fold rise in titre	17	(22.1)
High titre in first sample negative in second	1	(1.3)
Low titre in first/both	2	(2.5)
Negative in both samples	21	(26.6)
Patients with single samples	**35**	
High titre in acute sample, no convalescent sample	2	(5.7)
No acute sample, negative in convalescent sample	1	(2.9)
Negative in acute sample, no convalescent sample	32	(91.4)

Paired blood samples were available for 79 patients and 38 of them showed a seroconversion and 17 had a four-fold rise in titre. Table 2 shows the details of MAT done on samples from suspected cases. Three patients (one with paired samples and two with only a single sample) showed a titre of 1:320 or more, which in a patient with symptoms can be considered as an indication of current leptospiral infection (Vijayachari P *et al*, 2001). Hence a diagnosis of leptospirosis was confirmed in 58 patients. Assuming that the six fatal cases were due to leptospirosis, the case fatality ratio was 9.4% (6/64). Leptospires could not be isolated from the blood of any of the patients. PCR was done on acute samples from 42 suspected cases and 17 were positive. All positive samples amplified the sequence specific for non-Krischneri group of leptospires (primers G1, G2).

4.3. MAT titre distribution

Distribution of acute and convalescent samples by MAT titre is shown in Fig. 3. Out of the 114 suspected cases, one did not have acute sample and hence the distribution shown is that among the remaining 113 samples. Most of the samples

(71.7%) did not show any titre. Among MAT positive samples the commonest titre was 1:80.

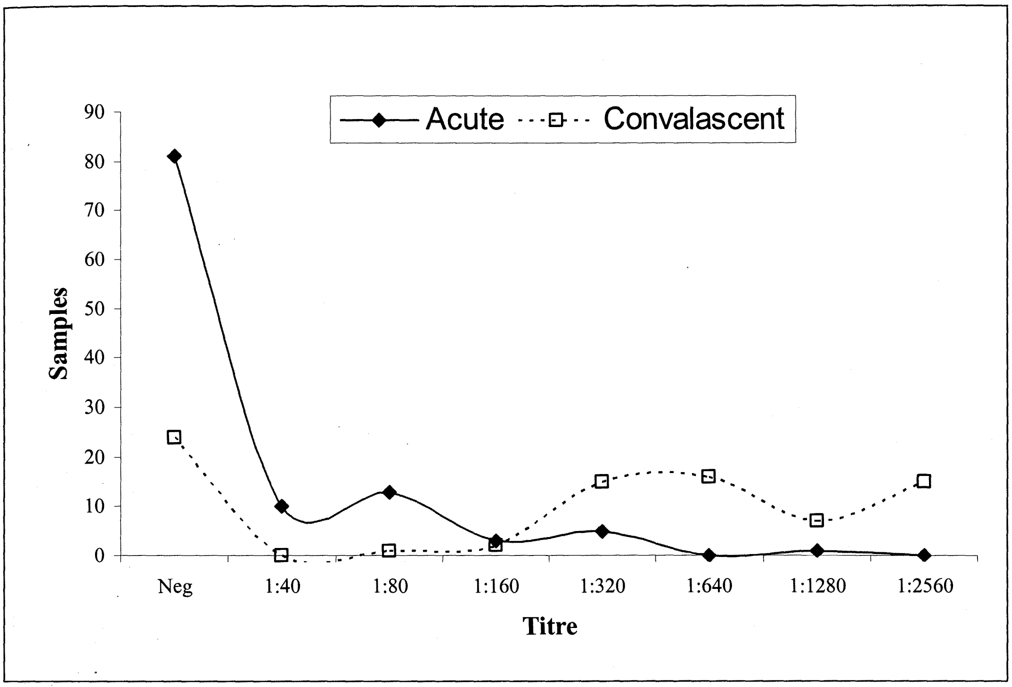

Fig. 3. Distribution of acute (n=113) and convalescent(n=80) serum samples from suspected cases of leptospirosis by MAT titre, outbreak of leptospirosis at Rangat, Middle Andaman, 2002 (n=113).

Convalescent samples were not available from 34 cases and hence the distribution is that among the remaining 80 samples. The most common titre among MAT positives was 1:640. The apparent second peak corresponding to the titre 1:2560 is because the samples were not titrated beyond that titre.

4.4. Infecting serogroups

Presumptive information about infecting serogroups was obtained from MAT titres against the reference strains of the serogroups represented in the panel of antigens used. Out of the 58 confirmed cases, 39 (67.2%) had the highest titre against serogroups Icterohaemorrhagiae and 16 (27.6%) had against serogroups Grippotyphosa. In one case the first sample was negative and the second sample reacted only against serogroups Sejroe (titre 1:320). In two cases, the first samples were negative and the second samples reacted to both Icterohaemorrhagiae and

Grippotyphosa at equal titres (1:160 and 1:320 respectively) and hence it was not possible to determine the infecting serogroups. Cross-reactions at low titres were observed against serogroups Australis, Autumnalis, Canicola, Hebdomedis, Pomona and Pyrogenes.

4.5. Distribution of cases

4.5.1. Distribution in time – epidemic curve

Weekly moving average of number of fever cases reported to three sub-centres under Rangat CHC that serve several of the villages affected by the outbreak (Fig. 4) shows an increase in the number during the period of outbreak. Cases started increasing by third week of October, peaked during first week of November and declined to baseline level by first week of December.

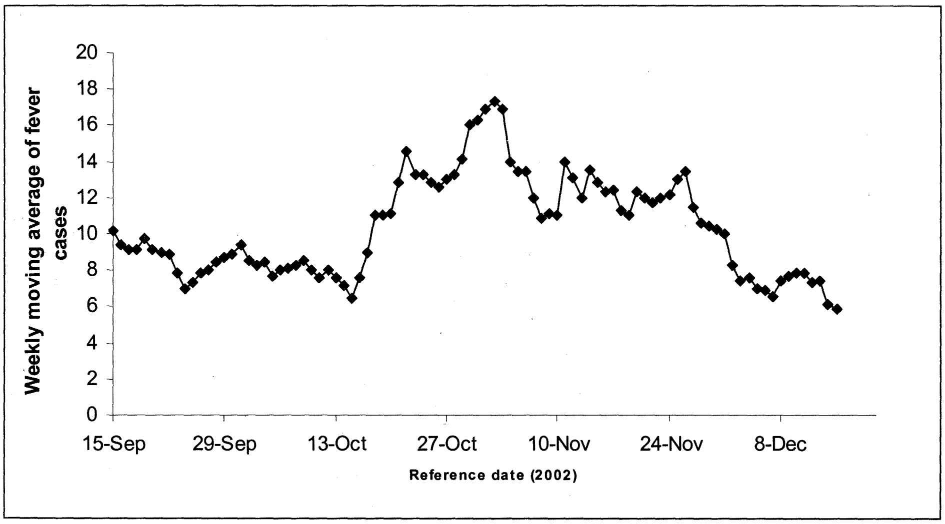

Fig. 4. Weekly moving average of number of fever cases reported to three sub-centres under Rangat Community Health Centre during the leptospirosis outbreak at Rangat, Middle Andaman, 2002

The first suspected case occurred on 24 October 2002 and the last on 25 November 2002. Distribution of confirmed cases (58) and deaths (6) by date of onset of symptoms (in four day interval) is shown in Fig. 5. The overall trend is a slow increase in the number of cases from third week of October to a peak in the third week of November and then a rapid decline.

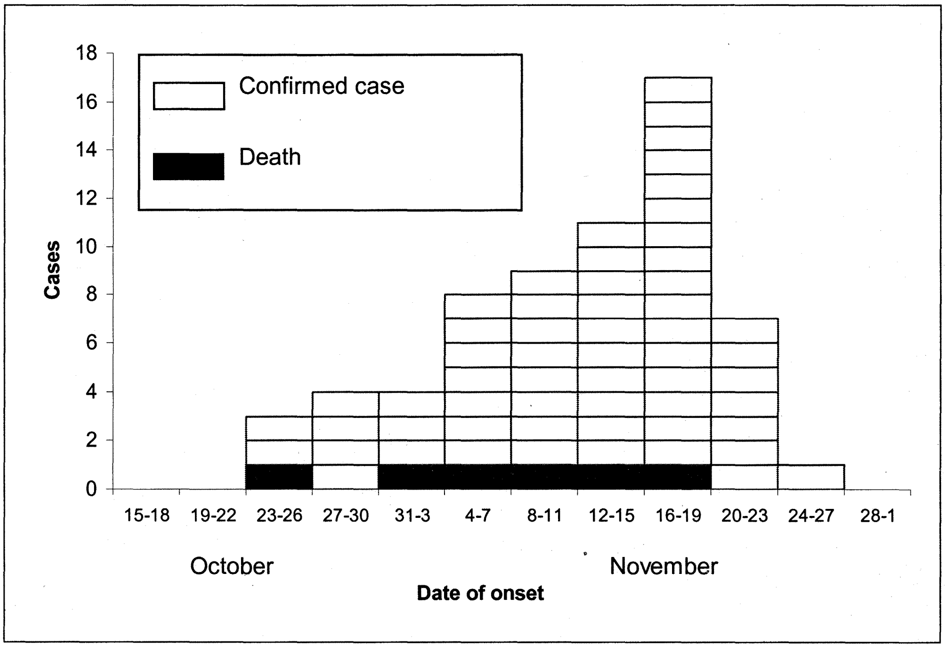

Fig. 5. Frequency distribution of confirmed cases (n=58) and deaths (n=6) by date of onset of symptoms, outbreak of leptospirosis at Rangat, Middle Andaman, 2002

4.5.2. Distribution in villages

There are 89 inhabited villages under Rangat tehsil. Out of this 17 villages were affected. These 17 villages together have a population of 12,996 and 58 confirmed cases occurred in these villages. Assuming that the six fatal cases were due to leptospirosis, though laboratory confirmation was lacking, the overall attack rate was 492.5/100,000 population. Attack rate in individual villages are summarized in Table 3. The largest number cases occurred at Rangat, which is the tehsil headquarters and the second largest populated area in the tehsil. Three villages *viz* Yeratta, Shaktighar and Amkunj had attack rates in excess of 1,000/100,000 population. Five villages *viz*. Parnasala, Rampur, Shyamkhund, Janakpur and Khalsi had attack rates between 500 and 1,000 per 100,000 population. The largest number of cases occurred in Rangat followed by Nimbutala and Bakultala. All these villages are more populated than the other villages. Two deaths occurred in Amkunj village and one each in Rangat, Nimbutala, Bakultala and Janakpur.

Table 3. Attack rates and case fatality ratios (CFR) in affected villages, outbreak of leptospirosis at Rangat, Middle Andaman, 2002

No	Village	Population	Cases	Deaths	Attack rate	CFR (%)
1	Yeratta	170	2	0	1176.5	0.0
2	Shaktighar	433	5	0	1154.7	0.0
3	Amkunj	378	4	2	1058.2	50.0
4	Parnasala	417	3	0	719.4	0.0
5	Rampur	736	5	0	679.3	0.0
6	Shyamkhund	295	2	0	678.0	0.0
7	Janakpur	752	5	1	664.9	20.0
8	Khalsi	194	1	0	515.5	0.0
9	Sabari	814	4	0	491.4	0.0
10	Rangat	2074	10	1	482.2	10.0
11	Nimbutala	1948	9	1	462.0	11.1
12	Laxmanpur	225	1	0	444.4	0.0
13	Sitapur	249	1	0	401.6	0.0
14	Bakultala	2199	7	1	318.3	14.3
15	Kousalyanagar	1120	3	0	267.9	0.0
16	Bharatpur	426	1	0	234.7	0.0
17	Dasaratpur	566	1	0	176.7	0.0
	Total	**12996**	**64**	**6**	**492.5**	**9.4**

4.5.3. Socio-demographic characteristics

Age of the patients ranged from 3 years to 62 years. The 64 patients included 41 (64.1%) males and 23 (35.9%) females. The mean age was 25.9 yrs (SD: 11.9 years). Females were slightly older (mean age: 29.1 years, SD: 14.1 years) than males (mean age 24.1 years, SD: 10.3 years). Age and sex distribution of the patients is shown in Table 4. Male to female ratio was 1.78:1. About 50% of the patients were in the age group 15 – 34 years and 15% were children below 15 years of age. Two confirmed cases were in preschool age group. Age-specific attack rates could not be calculated as age structure of the population of the affected villages was not readily available.

Table 4. Distribution of cases by age and sex, outbreak of leptospirosis at Rangat, Middle Andaman, 2002.

Age group (yrs)	Male	(%)	Female	(%)	Total	(%)
0-4	1	2.4	1	4.3	2	3.1
5-9	1	2.4	0	0.0	1	1.6
10-14	4	9.8	3	13.0	7	10.9
15-19	8	19.5	3	13.0	11	17.2
20-24	8	19.5	1	4.3	9	14.1
25-29	6	14.6	5	21.7	11	17.2
30-34	0	0.0	0	0.0	0	0.0
35-39	3	7.3	3	13.0	6	9.4
40-44	2	4.9	3	13.0	5	7.8
45-49	0	0.0	3	13.0	3	4.7
50-54	0	0.0	0	0.0	0	0.0
55-59	1	2.4	0	0.0	1	1.6
60-64	7	17.1	1	4.3	8	12.5
Total	**41**	**100**	**23**	**100**	**64**	**100.0**

Table 5. Distribution of cases by occupation, leptospirosis outbreak at Rangat, Middle Andaman, 2002

Sl No	Occupation	Cases	(%)
1	Cultivators	18	28.1
2	Housewives	10	15.6
3	Students	15	23.4
4	Agricultural labourers	11	17.2
5	Other manual labourers	3	4.7
6	White collar workers	2	3.1
7	Vendors/traders	3	4.7
8	Preschool children	2	3.1
	Total	**64**	**100.0**

The households of most of the cases owned agricultural land and even if the patients were formally employed, they were working in their agricultural land. Except for married ladies who were not doing any work other than household duties, most adults reported that they used to work in the agricultural fields. Hence none of them were categorized as unemployed. Distribution of the patients by occupation is shown in Table 5. Cultivators and agricultural labourers constituted 45% of the patients and students 23.4%.

4.6. Seroprevalence among controls

Eligible controls were selected after the cases were diagnosed from the neighbourhood of the cases. For most of the cases, three age and sex matched eligible controls each were selected. Thus there were a total of 152 eligible controls. MAT showed positive result in 23 giving a seroprevalence rate of 15.03% (95% CI: 9.96, 21.91). Six among the MAT positive controls had titres ≥1:160 and the remaining had titres of 1:40 or 1:80. Presumptive infecting serogroups identified among these seropositive controls were Icterohaemorrhagiae in 12 (52.2%), Grippotyphosa in 6 (26.1%) and Hebdomedis in 4 (17.4%). One had equal titres to Icterohaemorrhagiae and Grippotyphosa.

4.7. Cases and matched controls

Among the 64 cases, the six fatal cases were excluded as their diagnosis was not confirmed as per the laboratory criteria. Among the remaining 58 cases, the diagnosis in three was based on MAT results on a single sample and hence they were also excluded. After removing the 23 seropositives, All the eligible controls of another three cases were seropositive they were also removed. Among the original matched groups 52 cases with two controls each remained in the analysis.

4.8. Prevalence of risk factors

Prevalence of various potential risk factors among cases and controls is summarised in Table 6 and Table 7. Among the factors relating to house, environment and animals in house, the difference in the prevalence among cases and controls were statistically significant in the case of house with thatched roof, stream water as the source of drinking water and keeping cattle and pig in the house. Among these factors, keeping cattle had the highest prevalence among the cases (84.6%) followed by house with thatched roof (50.0%).

Table 6. Prevalence of potential risk factors among cases and controls, leptospirosis outbreak at Rangat, Middle Andaman, 2002. I. Factors relating to house and environment

Sl No	Factor	Cases (n=58)		Controls (n=116)		χ^2	P
		No	(%)	No	(%)		
1	Own house	43	82.7	85	81.7	0.01	0.941
2	Own land	40	76.9	82	78.8	0.00	0.945
3	House in low-lying land	32	61.5	62	59.6	0.00	0.954
4	House compound wet	31	59.6	60	57.7	0.00	0.954
5	Ponds in compound	18	34.6	23	22.1	2.19	0.139
6	Streams near house	30	57.7	66	63.5	0.27	0.601
Type of house							
7	Thatched roof	26	50.0	28	26.9	7.17	0.007
8	Mud floor	41	78.8	71	68.3	1.43	0.232
9	Thatch walls	30	57.7	73	70.2	1.89	0.169
Drinking water source							
10	Stream	13	25.0	9	8.7	6.36	0.012
11	Well	5	9.6	2	1.9	3.16	0.075
Water source for washing							
12	Stream	15	28.8	23	22.1	0.53	0.468
13	Well	9	17.3	8	7.7	2.38	0.123
Animals in house							
14	Cattle	44	84.6	51	49.0	16.96	0.000
15	Pigs	18	34.6	17	16.3	5.64	0.018
16	Goats	17	32.7	28	26.9	0.32	0.533
17	Cats	22	42.3	51	49.0	0.39	0.533
18	Dogs	31	59.6	47	45.2	2.34	0.126
19	Rat Infestation of house	41	78.8	71	68.3	1.43	0.232

Among occupational behaviour factors, prevalence of walking barefoot, skin wounds, harvesting, cleaning sewage, clearing garbage and working in water were significantly different among cases and controls. Harvesting and staying in water while working had the highest prevalence among cases (71.2%).

Table 7. Prevalence of potential risk factors among cases and controls, leptospirosis outbreak at Rangat, Middle Andaman, 2002. II. Occupational and behavioural factors

Sl No	Factor	Cases (n=58)		Controls (n=116)		χ^2	P
		No	(%)	No	(%)		
1	Agricultural worker	21	40.4	43	41.3	0.00	0.954
2	Walks barefoot	26	50.0	26	25.0	8.66	0.003
3	Open defecation	44	84.6	97	93.3		
4	Skin wounds	26	50.0	29	27.9	6.49	0.011
5	Crosses water on way	23	44.2	29	27.9	3.47	0.063
Activities during the previous one month							
5	Harvesting	37	71.2	43	41.3	11.96	0.001
6	Cleaning sewage	40	76.9	58	55.8	5.77	0.016
7	Clearing garbage	33	63.5	40	38.5	7.73	0.005
8	Fresh water fishing	25	48.1	36	34.6	2.10	0.147
9	Cleaning animals	21	40.4	31	29.8	1.30	0.254
10	Slaughter animals	8	15.4	9	8.7	1.00	0.318
11	Swimming in streams	27	51.9	46	44.2	0.54	0.461
12	Stayed in water while working	37	71.2	45	43.3	9.72	0.002
13	Contact with animal urine	33	63.5	50	48.1	2.71	0.100

4.9. Strength of association – univariate analysis

Univariate matched analysis was performed to determine the strength of association of the potential risk factors with acute leptospirosis. Table 8 and Table 9 show the univariate odds ratios with 95% confidence intervals of the potential risk factors.

Among the factors relating to house, environment and animals in the house, thatched roof, use of stream water for drinking and keeping cattle and pigs in house were significantly associated with leptospirosis.

Table 8. Univariate matched odds ratios, confidence intervals and p-values of potential risk factors, outbreak of leptospirosis at Rangat, Middle Andaman, 2002. I. Factors related to house, environment and animals

Sl No	Factor	OR	95% CI	χ^2	P
1	Own house	1.07	0.42, 2.86	0.01	0.942
2	Own land	0.89	0.38, 2.24	0.00$	0.945
3	House in low-lying land	1.08	0.55, 2.14	0.00$	0.957
4	Streams near house	0.79	0.38, 1.64	0.27	0.606
5	Ponds in compound	2.08	0.85, 5.27	2.45	0.118
6	House compound wet	1.07	0.53, 2.24	0.00$	0.955
Type of house					
7	Thatched roof	2.85	1.28, 6.53	7.03	0.008*
8	Mud wall	1.65	0.75, 4.02	1.37	0.242
9	Thatched walls	0.59	0.28, 1.24	1.79	0.181
Source of drinking water					
10	Stream	3.83	1.27, 11.98	6.32	0.012*
11	Well	5.00	0.82, 52.51	3.02	0.082
Source of water for washing					
12	Stream	1.44	0.60, 3.31	0.54	0.461
13	Well	2.25	0.78, 7.64	2.26	0.133
Animals kept in the house					
14	Cattle	5.63	2.20, 16.41	15.37	0.000*
15	Pigs	2.90	1.18, 7.45	5.67	0.017*
16	Goats	1.29	0.60, 2.81	0.30	0.585
17	Cats	0.75	0.35, 1.58	0.41	0.523
18	Dogs	1.75	0.85, 3.57	2.17	0.191
19	Rat infestation	1.69	0.74, 3.79	1.25	0.263

* *Factors with statistically significant association at 5% level and included in logistic regression*
$ *Less than 0.01*

Among occupational and behavioural factors, walking barefoot, having wounds, harvesting, cleaning sewage, clearing garbage were significantly associated working standing in water. The strength of association was highest for keeping cattle in house (OR: 5.63) followed by harvesting (OR: 5.43) and using well water for drinking (OR: 5.0). Having goats, cats and dogs in house, rat infestation of houses, fresh water fishing, swimming in streams and having direct contact with animal urine were not found to be significantly associated with acute leptospirosis.

Table 9. Univariate matched odds ratios, confidence intervals and p-values of potential risk factors, outbreak of leptospirosis at Rangat, Middle Andaman, 2002. I. Occupational and behavioural factors

Sl No	Factor	OR	95% CI	χ^2	P
1	Agricultural worker	0.94	0.36, 2.48	0.01	0.941
2	Crosses water on way	2.13	0.98, 4.83	3.64	0.056
3	Open defecation	2.29	0.72, 7.40	1.88	0.171
4	Walks barefoot	2.73	1.31, 5.86	7.90	0.005*
5	Skin wounds	2.64	1.19, 5.48	6.08	0.014*
Activities during the previous one month					
6	Harvesting	5.43	1.89, 12.93	12.80	0.000*$
7	Cleaning sewage	3.20	1.31, 10.00	7.25	0.007*
8	Clearing garbage	2.73	1.26, 5.58	6.98	0.008*
9	Fresh water fishing	1.78	0.86, 4.45	2.37	0.124
10	Cleaning animals	1.69	0.76, 4.07	1.56	0.212
11	Slaughtering	2.40	0.61, 9.48	1.26	0.262
12	Swimming in streams	1.32	0.67, 2.69	0.50	0.478
13	Staying in water	3.23	1.47, 7.21	9.45	0.002*
14	Contact with animal urine	2.00	0.91, 4.49	2.84	0.092

* *Factors with statistically significant association at 5% level and included in logistic regression*
$ *Less than 0.01*

4.10. Multivariate analysis

4.10.1. Interaction terms and other independent variables

Nineteen factors that had a p-value of < 0.20 were selected for inclusion in the multivariate model. Out of the possible 171 first order interaction terms, 55 were selected based on biological plausibility. Stratified analysis of these 55 pairs of variables identified three possible interaction terms based on χ^2 for evaluation of interaction terms. All these terms involved presence of cattle in house. These terms, χ^2 for evaluation of interaction and p-values are listed in Table 10.

Table 10. Factors interacting with presence of cattle, statistic for evaluation of interaction and p-value, outbreak of leptospirosis at Rangat, Middle Andaman, 2002.

Interacting term	χ^2	**P**
Barefoot walking	4.82	0.028
Harvesting	6.35	0.012
Contact with animal urine	7.09	0.008

Table 11. Results of univariate analysis of presence of cattle in house stratified separately for three factors, outbreak of leptospirosis at Rangat, Middle Andaman, 2002

Factor	Odds Ratio		OR_{MH}*	χ^2	P
	Presence of cattle in house	No cattle in house			
Barefoot walking	9.92	2.22	5.11	14.04	0.000
Harvesting	6.08	2.84	4.08	9.44	0.001
Contact with animal urine	7.28	3.89	5.39	15.59	0.000

**Mantel-Haenszel weighted odds ratio*

These factors that had interaction with presence of cattle in the house were barefoot walking, harvesting and contact with animal urine. Odds ratios for presence of cattle when these factors are present and absent and the Mantel-Haenszel weighted odds ratios are shown in Table 11. Odds ratio for presence of cattle was 9.92 for those walking barefoot whereas it was only 2.22 for those wearing footwear. Odds ratios for presence of cattle were significantly higher, though to a smaller extent than in the case of barefoot walking, for the other to factors also.

4.10.2. Multiple logistic regression

Table 12 shows the results of conditional logistic regression with potential risk factors as independent variables and acute leptospirosis as dependent variable. All the three interaction terms were removed during the backward elimination process. Five variables remained in the model. Presence of cattle in the house had the strongest association with leptospirosis (OR: 5.1) followed by use of streams as a source of drinking water.

Table 12. Results of conditional logistic regression of potential risk factors and acute leptospirosis, outbreak of leptospirosis at Rangat, Middle Andaman, 2002

Term	β	OR	95% C.I.	P
Cattle in house	1.6	5.1	1.8, 14.6	0.002
Stream as source of drinking water	1.5	4.4	1.1, 18.0	0.041
Cleared garbage	1.3	3.7	1.5, 9.3	0.006
Walks barefoot	1.3	2.7	1.1, 6.9	0.035
Worked standing in water	0.9	2.5	1.0, 5.9	0.043

CHAPTER 5. DISCUSSION

5.1. Outbreaks of leptospirosis in Andaman Islands

Although leptospirosis used to occur as sporadic cases in Middle Andaman, outbreaks have not been reported. In North and South Andaman, outbreaks occur during October – November months almost every year (Sehgal SC & Sugunan AP, 2000). These outbreaks have been so regular that its appearance is usually anticipated by the people. What changes, or combination of changes, in the determinants of disease transmission trigger the outbreak at a specific short period is a question still unanswered. Although climatic changes, social events or specific farming activities often precede leptospirosis outbreaks, it is the change in the specific determinants of disease transmission brought about by these events that causes the outbreak. It is important to understand these determinants to plan control strategies. Recognizing the risk factors and behaviours is the essential first step in identifying epidemiologically important and modifiable disease determinants. The present study was conducted with this objective.

5.2. Outbreak at Rangat, case definition and its predictive value

The outbreak at Rangat during October – November 2002 was due to leptospirosis as 58 of the 108 patients studied showed serological evidence of current leptospiral infection. Cases were detected at the Community Health Centre, Rangat, which is the only hospital with facilities for inpatient treatment in the area. Among the 114 suspected cases, 32 including the six fatal cases could not be adequately followed up either to make a diagnosis of leptospirosis or to rule out it. A diagnosis of leptospirosis was confirmed in 58 and in the remaining 22 leptospirosis was ruled out. The case definition used to detect cases showed 69.1 % predictive value among the 84 patients who were adequately followed up.

Some leptospirosis surveillance systems use a more stringent case definition, *e.g.* the case definition used by the hospital-based surveillance system in Brazil requires evidence of hepatic or renal dysfunction to make a clinical diagnosis of leptospirosis (Ko AI *et al*, 1999) and the predictive value was more than 90%. The case definition used in Thailand is slightly more liberal. Presence of fever, headache and myalgia is sufficient to make a clinical diagnosis of leptospirosis during outbreaks. The reported predictive value was 77.5% (Tangkanakul W *et al*, 1998), which is slightly higher than the predictive value in the current study. An interesting case definition was the one used by Sejvar J *et al* (2003) while investigating a suspected outbreak of leptospirosis among the participants of Eco-challenge Sabah 2000, a multi sport expedition race in Malaysia. Their case definition included diarrhoea as one of the symptoms. During a water-borne outbreak of leptospirosis in Stewart County, Tennessee, three of the seven cases were initially diagnosed as acute gastroenteritis and one as shigellosis (Anderson DC *et al*, 1978). In the current series also, one of the fatal cases presented with high grade fever and diarrhoea and later developed jaundice and renal failure.

5.3. Timing of outbreak and the affected population

As in the case of the outbreaks that occur in North and South Andaman, this outbreak in Middle Andaman also coincided with the rice harvest season. Suspected cases were admitted to CHC, Rangat during a period of one month. Although the epidemic curve looks like a common source epidemic, cases were widely distributed among the villages of Rangat tehsil. Attack rate in the affected villages ranged between 176/100,000 to 1176/100,000. This is very high compared to the annual incidence reported earlier in Andaman Islands (Smythe LD, 1999). However, that was based on the number of cases reported at various hospitals and the population of the whole of Andaman Islands was taken as the population at risk. The incidence is very low in the urban area which constitute a significant proportion of the population of Andaman and in many villages. Even during the present outbreak only 17 of the 89 inhabited villages of Rangat tehsil were affected. If the whole population of the tehsil was considered to be at risk, the attack rate would have been much smaller.

Although, the outbreak coincided with rice harvest season it was not confined to farmers. Among the confirmed patients, 23% were students and 15% housewives who did not participate in agricultural activities. There was a male predominance among cases, which though is usual for leptospirosis elsewhere, has not been predominant in Andaman Islands. People of all ages were affected and about 16% were children below 15 years of age. Age specific attack rates could not be calculated as information about age structure of the population at risk was not available. As per 1991 census children in the age group 0 14 constitute about 12.5% of the population. Since the proportion of cases in this age group was higher that this, it can be assumed that the attack rate among children was slightly higher than that among adults. There were even preschool children among the confirmed cases. Leptospirosis is not a common disease of children. Recently paediatric cases have been observed during an outbreak in Mumbai (Karande S *et al*, 2002).

5.4. Infecting serogroups

In the absence of isolation of leptospires, it is not possible to determine the infecting serogroups conclusively. However, MAT titres give presumptive information about the infecting serogroups. Cross reactions are common during acute stage. During convalescent stage, antibodies against the infecting serogroups persist at high titres while the cross-reacting antibodies decrease and disappear. Hence the serogroups against which high titre persists in the convalescent samples can be considered as the infecting serogroups. In the present study, Icterohaemorrhagiae was the commonest infecting serogroups (67%). In 27% the infecting serogroups was Grippotyphosa. This pattern is different from that usually seen in South and North Andaman. In North Andaman the commonest infecting serogroups is Grippotyphosa followed by Australis. Although leptospires belonging to serogroups Icterohaemorrhagie have been isolated from occasional patients at North Andaman (Sehgal SC *et al*, 2000b), Icterohaemorrhagiae is responsible for only a small proportion of infections.

Multiple transmission cycles involving different serovars of leptospires existing in the same ecological space with different maintenance animal hosts has been recorded (Hathaway SC, 1981). Since MAT titres indicate that infection with leptospires belonging to two serogroups was predominant, it is possible that infection was occurring through two separate transmission cycles during the present outbreak. An earlier study in North Andaman had identified different sets of risk factors for seropositivity to different serogroups (Murhekar MV *et al*, 1998).

5.5. Results of laboratory tests

Dipstick test (Gussenhoven GC *et al*, 1997) was used as a rapid diagnostic test for initiating treatment. In the present study dipstick test showed 82.8% sensitivity (95% CI = 70.1, 91.0) and 83.3% specificity (95% CI= 61.8, 94.5) These indices were similar to those reported earlier (Sehgal SC *et al*, 1999b).

It would be interesting to examine retrospectively, how many patients with a confirmed diagnosis of leptospirosis based on the results of paired MAT, would have been identified as cases if only acute samples were available. The distribution of MAT titres in acute samples from confirmed cases shows that among the 55 confirmed cases, 24 (43.6%) had a titre of 1:40 or more and hence the sensitivity of a single MAT on acute samples would have been equal to this. But if a cut off titre of 1:40 was used five confirmed negatives would have been falsely diagnosed as cases and hence the specificity would have been 81.5%.

Although isolation of leptospires was attempted from acute samples from suspected cases, none of the cultures yielded leptospiral isolates. It is hard to isolate leptospires from blood after 5-7 days of illness (Faine S, 1994). The patients in the present study were severe cases admitted to CHC, Rangat. Most of them had reported to sub-centres near their homes and had taken medication. Since an outbreak of leptospirosis was suspected, use of antibiotic to treat febrile illness was widespread. The delay in reporting to CHC and prior use of antibiotics might be the reasons for the cultures being unsuccessful. Out of the 42 acute samples tested using PCR, 17 showed a positive result, thus confirming the serological diagnosis.

5.6. Seroprevalence among controls

The seroprevalence observed among the controls was 15%. This is much less than the prevalence observed among the population of North Andaman (Sehgal SC *et al*, 1994; Murhekar MV *et al*, 1998) and among some of the tribes of the islands (Sehgal SC *et al*, 1999a). Controls were screened immediately after the outbreak was over and hence at least some the seropositives would represent those who had an asymptomatic infection during the outbreak. This lower seroprevalence in Rangat even after an outbreak indicates that leptospirosis is not highly endemic here. MAT titres among seropositives indicate that the infecting serogroups among them was similar to that in the cases. It needs further studies to understand the reason for Middle Andaman remaining relatively free of leptospirosis while the other two major islands in Andaman district have been affected severely since 1988.

5.7. Risk factors

The risk factors studied included factors relating to environment and housing, animals in the house and occupational and behavioural factors particularly during a period of one month prior to onset of symptoms. A few of the factors were such as use of stream water and well water were rare. Most other factors were fairly common with prevalence in the range of 25% - 80%. The factors that had a significantly different prevalence among cases and controls were house with thatched roof and use of stream water for drinking among the factors relating to house and environment, cattle and pig among the animals in house and barefoot walking, wounds, harvesting, cleaning sewage, clearing garbage and working standing in water among behavioural and occupational factors studied. Plausibility of these factors predisposing leptospiral infection can be explained. Houses with thatched roof might permit free access to rodents. Cattle and pigs could be carriers of leptospires. Walking barefoot and presence of wounds are known risk factors. Other occupational and behavioural factors that were significantly different among cases and controls were those leading to contact with possibly contaminated water and environment and hence can cause leptospiral infection.

In the second step of the risk factor analysis, matched univariate analysis was performed to determine the strength of association of these factors with leptospirosis. The factors that were significantly associated with leptospiral infection were the same ones that had significantly different prevalence among cases and controls. Age, sex and neighbourhood were considered as potential confounders and matching was done on these factors. Hence association of these factors with leptospirosis could not be examined. In addition to the 10 factors that were associated with leptospirosis with a statistical significance at 5% level, another nine factors had association with a p value < 0.20. These 19 factors were considered for further analysis.

Before performing multivariate analysis, interaction terms were evaluated. Among the interaction terms with explainable biological plausibility three were found to be significant in statistical testing (stratified analysis and statistical test using χ^2 for evaluation of interaction). Conditional logistic regression with backward elimination identified five factors as independent risk factors. These, in decreasing strength of association, were cattle in house, use of stream water for drinking, clearing garbage, walking barefoot and working standing in water.

5.8. Dose-effect relationship

In the logistic model, dose-response relationship was not demonstrated for any of the factors identified as significantly associated with leptospirosis. Quantitative measurement of exposure was not performed except for number of cattle present in the house. A univariate matched analysis was performed with number of cattle in the house (grouped as nil, 1, 2-4, 5 and above) as independent variable and leptospirosis as dependant variable. The odds ratios observed were 1.88 for one cattle (95% CI: 0.60, 5.73), 1.68 for 2 – 4 cattle (95% CI: 0.81, 3.68) and 2.55 for 5 or more (95% CI: 1.08, 6.62). The association was not statistically significant for the first two categories and the strength of association of the second category was slightly lower than that for the first category. Having five or more cows in the house had the highest strength of association and it was statistically significant. Although these results do not show a statistically significant dose response relationship between the number of cattle in the house and leptospiral infection, there is some indication that the risk of leptospirosis is highest in persons with five or more cattle in their house. Because it was difficult to measure quantitatively the other factors identified as having statistically significant association with leptospirosis, dose-effect relationship could not be tested for these.

5.9. Biological plausibility

Barefoot walking is a known risk factor of leptospirosis. In a study in Thailand, not wearing boots was studied as a risk factor, but was found to be not associated with leptospirosis (Tangkanakul W *et al*, 2000). The risk factors identified in that study were those related to agricultural activities. In rice farmers, who stay in water-logged fields while working, wearing footwear might not offer significant protection as exposed parts of the leg would be in constant contact with water whereas in peri-domiciliary transmission, as reported by Sarkar U *et al* (2002), exposure is mainly to wet mud or soil and wearing footwear may give some protection. In the present study the only factor associated with leptospirosis that is related to occupation is working standing in water. Although wearing footwear might not offer much protection against such exposures it may offer some protection when the transmission is due to exposure to contaminated environment in and around the house.

In the present study the factor with the strongest association with leptospirosis was presence of cattle in the house. Cattle are known maintenance hosts of leptospires. Although it is often implicated as a carrier of leptospires belonging to the serovar Hadjo, infection with other serogroups particularly Grippotyphosa is also common among cattle (Faine S, 1994). Infection with leptospires of serogroups Icterohaemorrhagiae has also been recorded in cattle. During the present outbreak, two predominant serogroups were involved *i.e.* Icterohaemorhagiae and Grippotyphosa. Among the five risk factors identified, one was work related and the remaining four were related to house, environment and personal behaviour. It is possible that infections with the two different serogroups of leptospires were spread through two separate transmission cycles during the outbreak and cattle were the source of infection in one of these. Because of the small number of cases it was not possible to study the risk factors associated with infection by individual serogroups. Further studies on leptospiral excretion rate among cattle in the region are required to understand the role of cattle in the epidemiology of leptospirosis in Middle Andaman. As it has been shown that seroprevalence is not a reliable indicator of leptospiral carrier state (Ellis WA *et al*, 1981), such studies should use bacteriological or the more sensitive molecular tools. A limited survey for leptospiral excretion was conducted among cattle and pigs in the affected area immediately after the outbreak. While no isolate of leptospires were recovered from pigs, two isolates were recovered from cattle. Serotyping of these isolates is being carried out.

Use of stream water for drinking was the factor with the second strongest association with leptospirosis. Although, drinking water is occasionally implicated as the source of leptospiral infection (Cacciapouti B *et al,* 1987), the role of this portal of entry in leptospiral transmission is unclear (Faine S, 1994). One argument against this portal of entry is the high susceptibility of leptospires to low pH. The leptospires are unlikely to survive the gastric acid barrier. During swallowing contaminated water, leptospires may penetrate oral, oro-pharyngeal or oesophageal mucosa. Abrasions in the mucosa may also facilitate entry of leptospires into body. There has been strong evidence of leptospirosis being transmitted through ingestion as in the case of transmission from a nursing mother with leptospirosis to her baby (Bolin CA & Koellner P, 1988) and the observed association between swallowing water while swimming and leptospirosis during an outbreak in Japan (Corwin A *et al,* 1990). In the present study, the possibility of some unstudied confounding factors being responsible for the observed association between use of stream water for drinking and leptospirosis cannot be rule out completely. People who use stream water are more likely to come into contact with these water-sources more frequently and this frequent contact may be the actual exposure. However, no association was found between use of stream water for washing and bating and leptospirosis. The role of drinking water as a source of leptospiral infection needs to be studied in detail. Leptospiral contamination of drinking water sources could be studied using bacteriological or molecular tools. It is also necessary to study whether filtering or disinfection reduces the risk.

Standing in water for prolonged period of time has been identified as a risk factor in other studies also (Tangkanakul W *et al*, 2000). Intact skin is considered to be the most common portal of entry of leptospires (Faine S, 1994). Prolonged contact with water would make the skin sodden and soft thus aiding penetration by leptospires. It needs to be studied whether there is a difference in the risk between standing in water continuously and intermittently for short period of time as if there is a difference, it could be the basis of control strategy through modification of occupational habits.

5.10. Opportunities for risk modification

The identified risk factors pertain to one possible maintenance host that act as a source of infection (cattle), two factors relating to environmental and water sanitation (use of stream water for drinking and contact with garbage) and two pertaining to occupation and personal habits (working standing in water and walking barefoot). Control measures targeting all these factors can be planned. One of the basic principles in leptospirosis control is reduction of source of leptospires. Present study indicates that cattle could probably be an epidemiologically important carrier host in the study area. This needs further proof. The next step should be to estimate leptospiral excretion rate among cattle population of the area. A limited survey conducted immediately after the outbreak showed that carrier state does exist among cattle. However, a systematic survey involving a larger sample size is required. Bacteriological technique is ideal as it gives an opportunity to serotype the isolates. Molecular tools such as PCR have a higher sensitivity and might be alternative techniques, though these cannot differentiate between serotypes. Once the role of cattle as a maintenance host is proved, various approaches such as testing and treating and immunization can be used to control carrier state. The role of other animals also needs to be assessed.

Leptospiral contamination of water sources can be tested by culture and serotyping or using PCR specific to pathogenic leptospires. Stream water is being used by the people because of lack adequate safe water supply. Improved water availability would reduce the risk due to use of unsafe water. Better environmental sanitation and prompt removal of garbage should also be components of control programmes as contact with garbage has been shown to be a risk factor. The habit of barefoot walking should be discouraged through health awareness programmes. Working standing in water is difficult to modify as it is unavoidable for rice farmers.

However, further studies can be conducted on the efficacy and acceptability of protective footwear

5.11. Limitations

Six fatal cases had to be excluded from the study as their diagnosis could not be confirmed. Since the fatal cases represent severe forms, the cases included in the study had relatively milder illness. This would have resulted in under-estimation of strength of association. Controls were screened using MAT to detect asymptomatic infection/past infection. MAT has very low sensitivity during early phase of diseases and it is possible that persons in early phase of asymptomatic infection among the controls might have been missed. This could have resulted in misclassification of cases as controls, though the number would be extremely small. Information about exposure was collected using a structured questionnaire. Most of the confirmed patients had information about their clinical diagnosis at the time of interview. This might have resulted in bias described as rumination bias, which would lead to an overestimation of strength of association. Exposure could not be assessed quantitatively and hence a dose-effect relationship could not be demonstrated for any of the identified risk factors except for presence of cattle in the house.

The study was conducted in a confined area. Epidemiology of leptospirosis differs from place to place. The risk factors identified in one area might not have any role in leptospiral transmission in other areas. Hence the findings cannot be generalized to larger populations. Because the cultural habits of people and the nature of environment and animal population are not much different in different parts of Andaman Islands, these findings can be generalized to the population of the Islands.

5.12. Conclusions

The outbreak at Rangat in Middle Andaman during October – November 2002 was due to leptospirosis. This was the first time an outbreak of leptospirosis is occurring in Rangat. Attack rates in all the affected villages were much higher than attack rates in Andaman reported earlier. The causative serogroups were

Icterohaemorrhagiae and Grippotyphosa. A large proportion of the patients were farmers. However other groups such as students and housewives were also affected. All age groups including preschool children were affected. There was a male predominance among the cases with a male: female ratio of 1.7: 1. A seroprevalence of 15% was observed among the healthy population indicating the area is less endemic to leptospirosis than North and South Andaman. Univariate matched analysis showed that ten factors were significantly associated with leptospirosis. A multivariate logistic model was developed by conditional logistic regression by backward elimination. The model had five independent risk factors *i.e* cattle in the house, use of stream water for drinking, barefoot walking, contact with garbage and working standing in water. Dose effect relationship could not be demonstrated for any of the factors except for the presence of cattle in the house. A univariate matched analysis after categorizing the study subjects into different groups based on the number of cattle in the house showed that those with five or more cattle in the house had the highest risk. Biological plausibility of all the risk factors can be explained. All the factors except working standing in water can be modified to reduce risk. The study had a few draw backs such as being confined to relatively milder cases, possibility of some minor degree of misclassification due to low sensitivity of the test used to exclude leptospirosis in controls and exposure suspicion bias as the cases were not blinded about their clinical diagnosis.

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Annexure I

Leptospiral serogroups, serovars and strains used as antigens in Microscopic Agglutination Test, outbreak of leptospirosis, Rangat, Middle Andaman, 2002.

Sl. No.	Serogroup	Serovar	Strain
1.	Australis	Australis	Ballico
2.	Autumnalis	Rachmati	Rachmat
3.	Ballum	Ballum	Mus 127
4.	Bataviae	Bataviae	Swart
5.	Canicola	Canicola	Hond Utrecht V
6.	Grippotyphosa	Grippotyphosa	Moskva V
7.	Hebdomedis	Hebdomedis	Hebdomedis
8.	Icterohaemorrhagiae	Icterohaemorrhagiae	Ictero No. 1
9.	Javanica	Javanica	Poi
10.	Pomona	Pomona	Pomona
11.	Sejroe	Sejroe	Mus24
12.	Tarassovi	Tarassovi	Perepelistin

Annexure II

Questionnaire used for interviewing cases and controls for the case control study on risk factors of leptospirosis, Rangat, 2002

NATIONAL LEPTOSPIROSIS REFERENCE CENTRE
(Regional Medical Research Centre, ICMR)
Port Blair

Identification

Name:
Address:

House and surroundings

Native Place	Rural	Urban	**House Location**	Low	High
Water bodies on the way	Yes	No	**Ponds in the compound**	Yes	No
Own house	Yes	No	**Owns land**	Yes	No
House surroundings	Wet	Dry	**Rat Infestation**	Yes	No
River/streams nearby	Yes	No	**Attached latrine**	Yes	No
Type of roof	Pucca	Thatch	**Type of floor**	Pucca	Mud
Type of walls	Brick	Wood	**No. rooms in house**		

Water sources

Drinking water source	Own tap	Pub tap	Well	Tube well	Stream	Pond
Other water source	Own tap	Pub tap	Well	Tube well	Stream	Pond

Animals in house

Cattle	Pigs	Goats	Cats
Dogs	Chicken	Ducks	Others

Activities during the previous three months

Sawing	Yes	No	**Harvesting**	Yes	No
Cleaning sewage	Yes	No	**Removing garbage**	Yes	No
Fishing in field	Yes	No	**Cleaning animals**	Yes	No
Slaughtering animals	Yes	No	**Swimming**	Yes	No
Stayed in water	Yes	No	**Contact with animal urine**	Yes	No