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1 Introduction

Leptospirosis, an infectious disease that affects humans and animals, is considered the most wide-spread zoonosis in the world (WHO, 1999). The infection is however more common in the tropical and sub-tropical countries, since favourable conditions for its transmission exists there and most of the countries in this region are developing ones (Bharti et al., 2003, WHO, 2003). Leptospirosis is also considered as an emerging infectious disease in the developed world (Levett, 2001). For example, in countries like the U.S., the cases of canine leptospirosis are significantly increasing (MacAllister, 2003). Recreational activities like water-sports are also believed to be an important factor for the emergence of this infection (Morgan et al., 2002).

More than 200 serovars of *Leptospira* bacteria are found to infect the mammals, and the organism has been isolated from reptiles, amphibians, fish, birds and invertebrates throughout the world (Binder and Mermel, 1998; MacAllister, 2003). The worldwide annual incidence of human leptospirosis is estimated to range from 0.1-1 per 100 000 in the temperate countries to 10-100 per 100 000 in the humid tropical areas (WHO, 2003). About 30-50% of the human cases are believed to result from occupational exposure (Christova et al., 2003). In these high-risk groups or during outbreaks, the incidence rate may increase to as much as 100 per 100 000 (WHO, 2003). In the U.S., 100-200 cases of human leptospiral infection are identified per year (CDC, 2003).

The organism enters the host-body when they come in contact with abraded skin or mucus membranes. The infection results in a systemic illness leading frequently to renal, hepatic and pulmonary dysfunction (Trevejo, 1998; McKenzie, 2004). Leptospirosis has a protean manifestation and the only most common symptom is Jaundice. Laboratory tests are needed for the confirmatory diagnosis which is not readily available, mainly in the developing countries. Consequently, leptospirosis is unnoticed and underreported in developing countries of the world (WHO, 2003).

Leptospirosis is believed to be endemic as ideal conditions exist for transmission of this infection in Nepal (Brown et al., 1981), however, as with other developing countries, the infection is largely underreported. For the first time, a significant number of human cases have recently been reported (Murdoch et al., 2004). This essay discusses and describes

the epidemiology of Leptospirosis in humans and animals in Nepal, mainly by
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extrapolation of information from the tropical countries, particularly from the Indian Sub-continent, as only a few studies has been done in Nepal. Besides, control measures are reviewed and the most appropriate option and it implementation in the Nepalese context is discussed.

2 The etiological agent

The etiological agents of leptospirosis are the bacteria belonging to the genus *Leptospira*, the family *Leptospiraceae*, and the order *Spirochaetales*.

2.1 Morphology

The genus *Leptospira* comprises flexuous, helical, tightly coiled spirochaetes which are characterized by very active motility. The nature of the growth medium determines the appearance and motility of leptospire (Bharti et al., 2003). Three types of movements have been observed; rotation around the central axis, progressive movement in the direction of straight end, and circular motion (Bharti et al., 2003). The organisms are very thin (0.1-0.2 μm by 6-20 μm), so pass through the filter that retain most other bacteria (Torten, 1979). Usually they are hooked at one or both ends, but straight forms also occur under *in-vitro* conditions in culture media (Torten, 1979). These straight forms rotate and travel less quickly than the hooked forms. Leptospire gain their high motility by means of an axistyle, a flagellar analogue composed of two axial filaments inserted sub-terminally at both ends (Torten, 1979).

The leptospiral cell is surrounded by a 3-5 layered membrane, referred to as outer membrane or outer envelope. This outer membrane encloses the cellular components described as the protoplasmic cylinder. Two flagella, one at each end of the cell, are found between the outer envelope and the protoplasmic cylinder. The free ends of these flagella extend towards the central part of the cell but do not overlap.

Since these organisms do not stain readily with Gram's stain, they are neither classified as Gram positive nor Gram negative (Bharti et al., 2003). Their visibilities is impaired by the narrow diameter and failure to stain with aniline dyes, but are best visualized by dark-field illumination or phase-contrast microscopy (WHO, 1982). The common staining

procedure is silver impregnation (Torten, 1979) but may also be stained using carbol fuchsin counterstain (Levett, 2001).

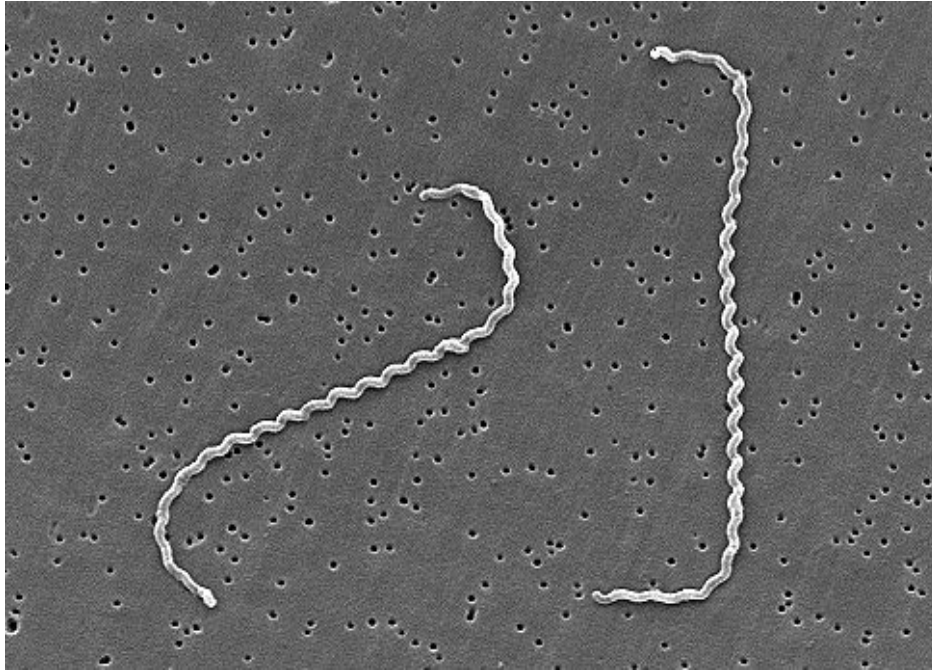


Figure 1. Scanning electron micrograph of *L. interrogans* serovar *icterohaemorrhagiae* strain RGA bound to a 0.2 µm membrane filter (from Levett, 2001).

2.2 Cultural characteristics

Leptospire are slow growing obligate aerobes with a preference for microaerophilic conditions (Torten, 1979). Optimum growth temperature for culture is 28°C to 30°C however, at temperatures up to 37°C, rapid multiplication may occur for up to 2 days (Torten, 1979; Levett, 2001). Since the generation time ranges from 7-12 hr, cultures may be contaminated by other bacteria that have shorter generation times resulting in death of leptospire (WHO, 1982). These organisms are very sensitive to dryness and will die within minutes of exposure to dry conditions (Torten, 1979).

Leptospire have simple but unique nutritional requirements. They require vitamin B1, B12, long chain fatty acids, ammonium salts, and pyruvate (WHO, 1982). As leptospire are resistant to the antibacterial activity of 5-fluorouracil, this compound is used in selective media for the isolation of leptospire from contaminated sources (WHO, 1982). The artificial media used for cultivation of leptospire are enriched with 10% rabbit

serum or 1% bovine serum albumin (BSA) and long-chain fatty acids (Bharti et al., 2003). Protein-free synthetic media are also used for isolation of leptospire (Levett, 2001).

2.3 Taxonomy and classification

Since the morphological and cultural characteristics of different leptospire are fairly similar, serological features are used for their classification and identification. Originally, the genus *Leptospira* was differentiated on the basis of serological reactions into two species; *L. interrogans* containing the pathogens, and *L. biflexa* containing the saprophytes (McKenzie, 2004). The present classification of leptospiral species is by DNA relatedness; 17 species have been identified on the basis of “being at least 70% DNA-related and whose related DNA sequence contain at most 5% unpaired bases (divergence)” (Bharti et al., 2003). Various serovars are identified within each species (genomospecies) on the basis of serological reactions, and serovars with common antigens are assembled into a serogroup (Quinn et al., 2002). Currently more than 200 serovars have been arbitrarily assembled into 24 serogroups within *L. interrogans* (Bharti et al., 2003).

Under present definition, “two strains are considered to belong to different serovars, if after cross-absorption with adequate amounts of heterologus antigen, more than 10% of the homologous titre regularly remains in at least one of the two antisera in repeated tests” (WHO, 2003). Leptospiral strains are still commonly referred to by serovar as the serovar concept and the classification of serovars in serogroups is widely accepted and used in epidemiology. The recent genotypic classification coexists with the older serological classification as shown in table 1.

Table 1. Classification (genotypic and serological) of *Leptospira* species (from Bharti et al., 2003).

Species	Serovar	Serogroup
Pathogenic species		
<i>L. interrogans</i>	<i>australis</i> <i>bratislava</i> <i>bataviae</i> <i>canicola</i> <i>hebdomadis</i>	Australis Australis Bataviae Canicola Habdomadis

	<i>icterohaemorrhagiae</i> <i>copenhageni</i> <i>lai</i> <i>pomona</i> <i>pyrogenes</i> <i>hardjo</i>	Icterohaemorrhagiae Icterohaemorrhagiae Icterohaemorrhagiae Pomona Pyrogenes Sejroe
<i>L. alexanderi</i>	<i>manhao3</i>	Manaho
<i>L. fainei</i>	<i>hurstbridge</i>	Hurstbridge
<i>L. inadai</i>	<i>lyme</i>	Lyme
<i>L. kirschneri</i>	<i>bim</i> <i>cynopteri</i> <i>grippotyphosa</i> <i>mozdok</i> <i>panama</i>	Autumnalis Cynopteri Grippotyphosa Pomona Panama
<i>L. meyeri</i>	<i>semaranga</i>	Semarang
<i>L. borgpetersenii</i>	<i>ballum</i> <i>castellonis</i> <i>javanica</i>	Ballum Ballum Javanica
<i>L. weillii</i>	<i>celledoni</i>	Celledoni
<i>L. noguchii</i>	<i>fortbragg</i>	Autumnalis
<i>L. santarosai</i>	<i>brasiliensis</i> <i>Georgia</i>	Bataviae Mini
Genomospecies 1	<i>pingchang</i>	Ranarum
Genomospecies 4	<i>hualin</i>	Icterohaemorrhagiae
Genomospecies 5	<i>saopaulo</i>	Semarang
Saprophytic species		
Genomospecies 3	<i>holland</i>	Holland
<i>L. biflexa</i>	<i>patoc</i>	Semarang
<i>L. wolbachii</i>	<i>codice</i>	

2.4 Infection sources

The most important source of infection is the infected urine and tissues from infected animals (WHO, 1982). Leptospirosis infection in humans and animals results primarily from direct or indirect exposure to the urine of infected animals (Bharti et al., 2003). Examples of indirect exposure are contact with animal beddings, soil or mud. Infection may also occur during handling of infected animal tissues or ingestion of contaminated food and water (McKenzie, 2004). Besides urine, other body fluids that contain viable leptospire are also a potential source of infection. Some uncommon modes of transmission, mainly in farm animals, are the congenital and venereal transmission (WHO, 2003). Rarely, transmission may occur via laboratory accidents, blood

transfusions or animal bites (McKenzie, 2004), and direct human-to-human transmission has also been reported (WHO, 1982; Levett, 2001).

Usually, the organism enters the host body via the abraded skin or the mucous membrane of the nose, eye, mouth and esophagus. Inhalation of aerosols or droplets of fluids containing leptospire may also result in infection via the mucous membrane of the respiratory tract (Levett, 2001). Rarely, the organism may also gain entry into the host body via intact skin (McKenzie, 2004), which occurs more readily when the skin is submerged in water for prolonged periods (Levett, 2001; Hakke et al., 2002).

3 Epizootiology and Epidemiology

Leptospirosis has a worldwide distribution. The tropical countries, most of which are developing countries, has the higher annual incidence rate which is estimated to be of up to 100 per 100 000 during outbreaks (WHO, 2003). Nepal is one among these developing countries, and there are greater opportunities for exposure of the humans to the animals. Besides, favourable environment for survival of the organism, like warm temperature, moist soil and plenty of surface water, exists in Nepal.

3.1 Country background

Nepal is a small country in South-Asia, situated between China and India, at 80° 04' E to 88° 12'E longitude and 26° 22' N to 30° 27' latitudes. It has a total area of about 14.7 million hectares and a rectangular shape, extending from east to west, and covering a length of roughly 800 km and a width of from 130-140 km. Three different climatic conditions exist in Nepal; temperate, subtropical, and tropical, respectively, in the northern Himalayan region, Mid-hills and in the southern Terai belt. The Terai is the lowland region bordering India, having similar climatic conditions. The climate in the Mid-hills and the Terai varies from cool, dry winters (December to February) to hot monsoon season (June to September), during which plenty of rainfall occurs which averages 1600 mm per year.



Figure 2. Map of Nepal

The human population is about 23 million, most densely populated areas being the Terai and the Mid-hills. More than 80% people are engaged in agriculture which is of substance type, and involves mixed crop-livestock farming. Rice, maize, and wheat are the three most important crops, occupying about 55%, 29%, and 23% of the total cultivated area. Sugarcane is also an important cash-crop in the Terai region. Mostly indigenous breed of animals are reared in Nepal. There are approximately 7.0 million cattle, 3.6 million buffaloes, 6.5 million goats, 0.9 million pigs, and 0.8 million sheep in Nepal (FAO, 2002).

Nepal seems to have ideal conditions for the transmission of leptospirosis. Families keep their livestock in very close proximity to their own house, and rice-fields are abundant where exposure to water contaminated with urine from infected animals occurs. Heavy rainfall during monsoon and floods make the conditions even more vulnerable for the transmission of infection.

3.2 Epizootiology

3.2.1 Prevalence

The organism affects at least 160 mammalian species and has been recovered from rodents and rats, swine, dogs, cats, raccoons, cattle and other animals. The infection is more common in cattle, pigs, rats, mice, and hedgehogs whereas less common in sheep, goats, dogs, and horses (Wilks and Humble, 1997).

Limited information is available on prevalence of Leptospirosis infection in animals in Nepal. Joshi and Joshi (2000, 2001) conducted the first serological survey in 200 animals (114 cows and 86 buffaloes) with infertility problems. Screening of serum samples for *L. hardjo* antibodies by microscopic agglutination test (MAT) showed positive reaction in 8.5% of the serum samples (n=200). The prevalence rate in cattle and buffaloes was found to be 5.5% and 11% respectively. Similar serological survey was done throughout Nepal by Dyson et al., (2000). They found the prevalence rate of 17% in different species of livestock, and suggested that chronic infection is very common.

No information is available regarding the prevalent serovars, and leptospirosis in other species of animals in Nepal. In India, serovars *icterohaemorrhagie* and *canicola* has been isolated from dogs and rats in an outbreak (Venkataraman and Nedunchelliyan, 1992).

3.2.2 Transmission pattern/Mode of spread

Almost all the animal and rodent hosts of *Leptospira* occur in Nepal. As transmission of leptospiral infection in animals occurs in a cyclical pattern worldwide, this is believed to occur in Nepal as well. A carrier animal usually infects its young directly or via contamination of soil, surface water and nesting or foraging area in the periphery of the animal's habitat (WHO, 1982). Transmission within cattle or sheep population occurs by two means; first is by congenital or neonatal infection followed by recovery and a carrier state, and second is by contamination of farm premise from the infected urine. The latter mode of transmission also poses a threat of human infection. Besides their own species, rodents are also an important source of infection to farm animals and humans. In the rice-growing parts of the world, where the rodent carriers contaminate the water, the latter serves as a potential source of infection to human and animals. Transmission of infection

in dogs usually results from contact with urine of other dogs or rat urine polluted areas (WHO, 1982). Indirect exposure to contaminated soil and water is an important risk factor for infection in horses (Barwick et al., 1998).

It has been mentioned that there is an association between certain leptospiral serovar and particular species of animal, and each serovar has a maintenance host (Wilks and Humble, 1997). Animals that are maintenance hosts to a particular serovar usually show no or comparatively few ill effects following infection with that serovar. The disease is subclinical in maintenance hosts and is characterized by prolonged leptospiruria resulting generation to generation transmission (Wilks and Humble, 1997; Quinn et al., 2002). Maintenance hosts thus serve as the source of environmental contamination and facilitate the natural transmission of infection to other animal species referred to as incidental or accidental hosts. Although the susceptibility is low, once infected, the incidental hosts develop severe illness but do not transmit the infection to other animals efficiently (Quinn et al., 2002). The maintenance hosts and the commonly infected incidental hosts of some serovars of *L. interrogans* are presented in Table 4. Genetic factors may be responsible for variations in the degree of severity of infection in different host species (Quinn et al., 2002).

Table 4. Maintenance and incidental hosts for important serovars of *Leptospira interrogans* (from Quinn et al., 2002)

Serovar	Maintenance hosts	Incidental hosts
<i>bratislava</i>	Pigs, hedgehogs	Horses, dogs
<i>canicola</i>	Dogs	Pigs, cattle
<i>grippotyphosa</i>	Rodents	Cattle, pigs, horses, dogs
<i>hardjo</i>	Cattle, (sheep occasionally)	Humans
<i>icterohaemorrhagiae</i>	Brown rat	Domestic animals, humans
<i>pomona</i>	Pigs, cattle	Sheep, horse, dogs

3.2.3 Clinical manifestation

The clinical manifestations of leptospirosis in animals can be categorized into two distinct phases: an acute (initial and late) phase whose beginning corresponds with the bacteraemic phase of infection, and a chronic phase which occurs much later and whose effects are more evident on the reproductive tract (Ellis, 1984). During the initial acute stage, the clinical symptoms in different species are basically similar rather than typical (Table 2). An eye problem called periodic ophthalmia may occur in horses. The

symptoms include increased tearing, conjunctivitis, photophobia and keratitis with eventual blindness (MacAllister, 2003). Later in the acute stage, as the disease advances, more characteristic signs and symptoms are evident. These symptoms include haemorrhages, jaundice, nervous symptoms, liver and kidney failure, abortion, stillbirths, and mastitis in lactating animals (WHO, 1982; Ellis, 1984).

Table 2. Main clinical features of leptospirosis in Animals (from WHO, 1982).

Symptoms	Bovines	Equines	Ovines and Caprines	Swine	Canines	Rodents
Acute infection (initial)						
Fever (temperature up)	1-2.5°C	+	0.5-2°C	0.5-1.5°C	+	+
Malaise	+					
Weakness		+			+	
Depression		+		+	+	+
Listlessness				+		+
Anorexia	+	+			+	+
Vomiting					+	
Diarrhoea	+			+		
Convulsions				+	+	
Conjunctivitis	+				+	+
Haemorrhages	+				±	±
Anaemia	+		+			+
Jaundice	+	+		+	±	±
Anuria	+					
Haemoglobinuria	+	+	+			
Mastitis, agalactia	+					
Acute infection (late)						
Pneumonia	+					
Abortion/Stillbirth	1-3*	+	+	2-4*		
Chronic infection						
Nephritis	+		+	+	+	
Periodic ophthalmia		+				
Encephalitis	+			+		
Grey-white spots on kidneys postmortem	+			+	+	

* Weeks after onset of initial infection

+ Regularly present

± Occasionally present

As the disease progresses towards chronicity, the organisms may remain symbiotically localized in renal tubules of the kidneys without producing any pathological changes (McKenzie, 2004). The progression of the disease and severity depends on the type of *Leptospira* causing infection (MacAllister, 2003). Chronic *Leptospira* infection generally remains subclinical in animals. Animals in this carrier state thus serve as reservoirs of host-adapted serovars as high concentration of the organisms are shed in the urine (McKenzie, 2004). Detection of chronic infection with no clinical signs is possible only by the laboratory tests. If present, the symptoms resemble to that of nephritis- excretion of large volumes of urine of low specific gravity (WHO, 1982).

3.3 Epidemiology of human Leptospirosis

3.3.1 Prevalence

Human cases of leptospirosis in Nepal have been reported since 1980 (Brown et al., 1981). These workers carried out a serological survey in 188 individuals from Mid-hills of Eastern Nepal. Sensitised-erythrocyte-lysis (SEL) test showed that 12% of the subjects were positive for *Leptospira* infection. Microagglutination of serum sample of a patient suspected of leptospiral infection revealed the organism as *L. interrogans* serovar *icterohaemorrhagiae*.

In a recent study (Murdoch et al., 2004), leptospiral infection was found to be one among the four most important cause of febrile illness in Nepal. Identification of *Leptospira* was done by PCR which gave the prevalence rate of 2% (9 subjects) during winter months and 5% (27 subjects) during the summer. These authors also mentioned that this prevalence rate is an underestimation as diagnosis by PCR only detects acute infection. However, in this study investigation, serological identification of the leptospire were not done, so the involving serovars are not known.

As the prevalence of different leptospiral serovars within a human population depends on the reservoir animals present and the serovars that they carry, as well as local environmental conditions, occupational, agronomical, and agricultural practices (Bharti et al., 2003), and as Nepal have these features in common with India, some information has been extrapolated from the Indian context.

A recent outbreak of leptospirosis in Orissa, India has been reported by Jena et al. (2004). These workers found the involvement of three serovars of *L. interrogans*: *canicola*, *pomona* and *hebdomadis*, in this outbreak. The attack rate was found to be 5.95% and the case fatality rate (CFR) to be 7.69%. Similar outbreak was reported from Madras, India in 1988 (Venkataraman and Nedunchellian, 1992). In this outbreak, 50.5% of the 95 human sera tested were positive for leptospirosis. Two serovars; *icterohaemorrhagie* and *canicola* were found to be involved. Serovar *javanica* was isolated from a case of human leptospirosis with renal involvement in India (Saravanan et al., 1998). Serovar *autumnalis* is also prevalent in India (cited in Saravanan et al., 1998). A new serovar has been isolated from Kerala, India, belonging to the serogroup *Australis* and named as *bharathy* (Kuriakose et al., 1997).

3.3.2 Patterns of occurrence

No information is available regarding the sex and age group susceptible to leptospiral infection in Nepal. In general, all ages and sexes are susceptible. As adult men work outdoors in high-risk jobs, they are believed to be more vulnerable to infection (WHO, 1982). In India, higher incidence of leptospiral infection was found in 20-40 years age group (Kuriakose et al., 1997). Frequency of infection varies with season, and it was observed that leptospiral infection was higher in Nepal and India during the summer months/ monsoon as shown in figure 3 (Kuriakose et al., 1997; Murdoch et al., 2004).

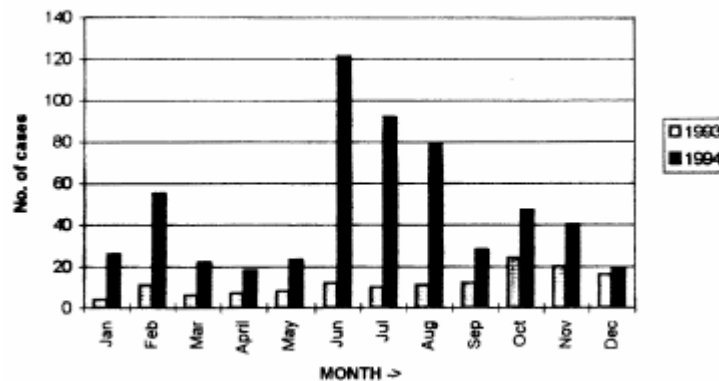


Figure 3. Seasonal incidence of cases of Leptospirosis in Kerala, India (from Kuriakose et al., 1997).

This may be due to the high rainfall during this time period, and also most crop-raising is done during this time. The amount of rainfall and the intensity of flooding may be responsible for variation in incidence of leptospirosis in a country like Nepal. Group activities like rice-plantation and harvesting may lead to outbreaks of leptospirosis, but has not been documented yet in Nepal.

Animal sources of human infection

In Nepal, people keep their livestock in close proximity to their housing, and almost every household raise some animals. Pet animals like dogs are also a part of lifestyle. Rodents like rats are very common as most village people live in mud-houses, and store the grains inside their house which attracts these animals. Usually people work bare foot in the fields without any protective clothing. These circumstances keep these village people at high-risk of leptospiral infection. Cattle, buffalo, pigs, and dogs seem to be most important animal sources of human infection. Serovar *hardjo* has been found to be infecting buffaloes (Joshi and Joshi, 2000; 2001), of which humans are potential incidental host (Quinn et al., 2002). Wallowing habit of buffaloes may contaminate the water, eventually posing higher risk for human infection. Besides, cow-urine is used in religious ceremonies, and people also drink it as a medicine. Cow-dung is used to coat the mud houses. All these practices seem to keep the people in Nepal, especially those residing in the villages, at high-risk of leptospiral infection. People living in the urban areas are equally vulnerable to leptospiral infection as stray dogs are roaming everywhere and rodents are common because of the inappropriate garbage disposal system.

Water borne infection

Water is the most common vehicle for transmission of leptospiral infection (Thiruventhiran and Tan, 2000). Outbreaks of Leptospirosis due to water-borne infection have been reported from India (Jena et al., 2004). The raising of wet-land crops like rice is mentioned as one of the most important risk-factor for leptospiral infection (WHO, 1982). In Nepal, 55% of the total cultivable land is used to grow rice. Rice field-workers in Nepal work with their bare feet and hands submerged in water for prolonged durations. As the skin becomes penetrable to leptospores due to excessive immersion in water, and as small cuts and abrasion are common in rice field-workers, infection may occur.

During summer months, children frequently swim in the rivers, ponds, and streams which

might have been recently flooded. As water sports (Hakke et al., 2002; Morgan et al., 2002), and activities like pond-cleaning (Phraisuwan et al., 2002), has been identified as an important-risk factor, swimming, especially in flooded rivers and ponds may be hazardous.

Occupational hazards

The main occupational groups at risk include dairy farm workers, veterinarians, pet shop owners, field agricultural workers, slaughter-house workers, plumbers, coal miners, workers in the fishing industry, military troops, and sewer workers. In India, farmers and agricultural workers were found to have the highest incidence of infection followed by housewives and students (Kuriakose et al 1997). No such information is available on Nepalese context, but it can be said the agricultural workers, specifically rice field workers and dairy farmers are the occupational groups that are at most risk.

3.3.3 Clinical manifestation

Leptospirosis infection is frequently misdiagnosed in humans because of its wide variety of clinical manifestations (McKenzie, 2004). These manifestations may range from a mild “flu”-like sickness to a serious and life-threatening disease. It may resemble other illness like dengue fever and other viral haemorrhagic diseases (WHO, 2003).

The typical symptoms of leptospirosis in man are: sudden onset of fever, headache, prostration, severe myalgia or cramps- especially in the calves and thighs, and conjunctival suffusion (WHO, 1982). Table 3 shows the frequency of clinical signs in leptospirosis patients with available clinical data.

Table 3. Frequency of clinical signs in 154 leptospirosis patients in Bulgaria, 1989-2001 (from Christova et al., 2003).

Sign or symptom	Frequency	Percentage (%)
Fever	144	93.5
Myalgia	103	66.9
Jaundice	92	59.7
Arthralgia	65	42.2
Hepatomegaly	63	40.9
Acute renal failure	52	33.8
Nausea	35	22.7
Vomiting	28	18.2
Headache	19	12.3
Meningitis	18	11.7

Weakness	17	11.0
Photophobia	12	7.8
Conjunctival suffusion	11	7.1
Abdominal pain	11	7.1
Pneumonia	7	4.5
Endocarditis	5	3.2
Paresis of facialis nerve	1	0.6

The clinical presentation can be divided into four categories (WHO, 2003):

- i) a mild influenza like illness;
- ii) Weil's syndrome characterized by jaundice, renal failure, haemorrhage and myocarditis with arrhythmias;
- iii) meningitis/meningoencephalitis;
- iv) pulmonary haemorrhage with respiratory failure.

Clinical presentations may also vary with the types of human dwellings. In urban settings, leptospirosis predominately results in pulmonary haemorrhage, renal failure and jaundice because of the variation in baseline clinical immunity in humans. On the contrary, in rural areas throughout the developing countries, leptospiral infection remains largely subclinical (Johnson et al., 2004).

The usual incubation period is 7-12 days, ranging from 2-20 days (McKenzie, 2004). The disease, if not treated within the first 2-3 days, may progress towards severity like: meningitis, anuria, iritis, liver failure and toxic delirium which may eventually lead to death (WHO, 1982). The case-fatality rate have been recorded to vary from <5% to 30% (WHO, 2003), and usually the patient recovers after a prolonged convalescence (WHO, 1982).

4 Diagnostic methods

Since the clinical signs of Leptospirosis are pathognomonic neither in humans nor in animals, they can not be used alone for the purpose of diagnosis. Laboratory findings coupled with individual and herd histories, clinical signs, and lesions provide a reliable diagnosis (WHO, 1982). For this, appropriate samples should be collected which is largely determined by the stage (acute or chronic) of the disease.

4.1 General laboratory tests

General laboratory tests are aimed either at demonstrating the organism or leptospiral antibodies in the sample. Some of the non-specific findings taken into consideration for general laboratory tests are mentioned here (Bharti et al., 2003). ESR may increase, mild increase may be seen in transaminases, alkaline phosphate, and bilirubin; abnormal urinalysis shows proteinuria, pyruia, microscopic haematuria, increased CSF protein, decreased CSF glucose, and xanthochromia in patients with severe jaundice. In severe cases, renal failure is indicated by increased plasma creatinine concentrations (Bharti et al., 2003). These findings are only suggestive of leptospirosis, and should be confirmed by specific microbiological tests (Levett, 2001). The organism may be demonstrated in CSF, blood or urine by dark-field microscopy, but the technique is rather insensitive (Quinn et al., 2002).

4.2 Culture

Recovery of leptospire from clinical samples by culture is one of the definitive diagnostic tests of Leptospirosis (Bharti et al., 2001). A variety of clinical specimen may be used for isolation of leptospire. Blood or CSF sample may be used during the first 7-10 days of infection (Quinn et al., 2002; Bharti et al., 2003) whereas urine may be used from second week of symptomatic illness (Levett, 2001). In animals like dogs and pigs, urine samples may be used for culture for up to one year or even more (WHO, 1982). Ground tissues of animals are sometimes used as specimen for culture of leptospire. Examples are the fetal tissues from an aborted animal, and tissue specimen of kidneys, liver or brain from an animal that had died following a fatal course of the disease (WHO, 1982). Isolated bacteria are then identified either by serological or by molecular techniques. There are several drawbacks of this method. Slow-growing serovars such as *hardjo* may require incubation for six months in liquid medium at 30°C (Quinn et al., 2002). Since it takes several weeks or months to culture leptospire from clinical specimens, only retrospective diagnosis can be made (WHO, 1982). Besides, culture has low sensitivity, and culture media are not readily available in the clinical laboratories (Bharti et al., 2003).

4.3 Molecular methods

Polymerase chain reaction (PCR) has been described as a substitute for direct demonstration of leptospires in clinical specimens (Bharti et al., 2003). The PCR methods are sensitive than the culture methods (Levett, 2001). A real-time PCR has been developed to identify leptospires in clinical and environmental samples which can distinguish pathogenic and non-pathogenic species (cited by Bharti et al., 2003). PCR can also be used for the detection of leptospiral DNA in bovine urine samples (Levett, 2001). However there are some drawbacks of PCR-based diagnosis, the most important being inability to identify the infecting serovar (Levett, 2001; Murdoch et al., 2004). DNA hybridization and dot-blotting are the examples of other molecular methods of diagnosis of leptospiral infection.

4.4 Serology

Leptospiral infection is most commonly diagnosed by the use of serological techniques. As the antibodies are detectable in the blood 4-6 days after exposure to *Leptospira* (Phraisuwan et al., 2002), these techniques involve the detection of antibodies in the infected animals by agglutination of live or formalized leptospires with titrated amounts of serum from humans or animals (WHO, 1982). The reaction is then read microscopically under dark-field illumination.

4.4.1 Tests available

The available serological tests can be categorized as screening tests and as specific tests. Screening tests include agglutination, indirect haemagglutination (HA), complement fixation (CF), latex agglutination, immunofluorescence (IF), immunoelectrophoresis, Lepto-Dipstick, and ELISA. Specific tests are the definitive diagnostic tests and include microscopic agglutination test (MAT), immunofluorescence, and ELISA. Among these tests, MAT is regarded as the standard serological reference test (Quinn et al., 2002).

“The standard criterion for a positive MAT are a fourfold increase in antibody titer, or a conversion from seronegativity to a titre of 1/100 or above” (Bharti et al., 2003). This serological test is used for the identification of serogroup or serovar of leptospires. The

main drawbacks of this test are requirement of expensive equipments and reagents, need of expertise, and a well-equipped laboratory (Sehgal et al., 1999; Eapen et al., 2002).

4.4.2 Choice of serological tests

IgM antibodies are produced in acute infection and IgG are produced later as the disease progresses to chronicity. Serological tests used for the detection of IgM are MAT, HA, Lepto-Dipstick, CF or ELISA, where as MAT or ELISA can be used for the detection of IgG (WHO, 1982). Thus detection IgM indicates acute infection and detection of IgG gives an indication of chronic infection. Table 5 shows the usefulness of various serological tests for the diagnosis of leptospirosis.

Table 5. Use of various diagnostic serological tests for leptospirosis (from WHO, 1982)

Sera of	Agglutination			CF	HA, HL	IF	ELISA
	Microscopic		Macroscopic				
	Nonspecific	Specific					
Man	+	++	+	+	+	+	+
Dog	?	++	+	?	?	+	+
Cattle	-	++	-	+	-	+	+
Swine	-	++	+	?	?	+	?
Horses	-	++	+	?		+	?
Sheep	-	++	-	+	?	?	+

++ = widely used + = useful - = not useful ? = little or no information

CF= Complement fixation HA= immune (indirect) Haemagglutination

HL= Immune haemolysis IF= Immunofluorescence

5 Control measures

Control measures are aimed at avoiding the direct contact of humans or animals with the reservoirs, breaking the transmission route, and avoiding the exposure to the infected environment.

5.1 Control of infection in man

5.1.1 Rodent control

The association of rodents along with humans is almost universal. Food storage areas and domestic environments are usually preferred by these species. One way of

controlling them is to avoid their access to these areas. This may be achieved by design and maintenance rodent-proof food storage premises, construction of new buildings in an area of low rodent infestation, maintenance of high level of hygiene, clearance of dense vegetation of the surroundings, removal of potential nesting materials, and use of rodent-proof storage containers. Use of rodenticide is another option but certain types of rodenticides like those available in dust forms are undesirable in food processing premises. Water-soluble anticoagulants and poisons like sodium fluoroacetate that can be administered in solution form seems to be comparatively better (WHO, 1982). Fumigation by the use of methyl bromide or carbon dioxide is recommended in extreme levels of infestations whereas traps may be useful in low level of infestation.

5.1.2 Control of exposure to an infected environment

As urine of infected animal is the most important source of infection, whenever possible, direct contact with urine should always be avoided. Personal protective equipment (PPE) should be used while working with animals and farm premises to avoid splashes or urine or contaminated water. Floors of the animal barns should be made of an impervious material with proper drainage system and drains should be kept covered. Manure and effluents from animal houses should be disposed in a predestined disposal area.

If an animal suspected of leptospirosis has aborted or died, disposal of the fetal contents or the dead animal should be done hygienically, by cremation or deep burial. The person carrying out the post-mortem examination should wear proper protective clothing. Contamination of water and soil may be avoided by making availability of good drainage of pastures and other agricultural land vulnerable to access of infected rodents and feral animals. Control attempts should also be aimed to reduce the population of these animals.

5.1.3 Occupational hygiene

As abraded skins and mucus membranes are the points of entry of leptospires, occupational hygiene should be aimed at reducing the number of leptospires in the environment, and making the wounds, lacerations and cuts inaccessible to these organisms. Any wound or lacerations should be well treated and covered with a water-

proof dressing material. Whenever there is risk of urine or contaminated water splash, protective clothing including waterproof footwear and gloves should be worn. Meat workers should wear waterproof aprons and other protective clothing. Wearing of waterproof gloves is must for the meat worker who handles kidneys and urinary bladder. Veterinarians, while attending a suspected leptospirosis case or during post-mortem examination, should wear protective clothing. Sewer workers should wear protective boots and gloves in addition to protective clothing. Rice field workers should treat their skin wounds immediately and should stop working if any wound is sustained while working.

5.1.4 Immunization

Vaccines offering total protection in humans against leptospirosis is yet to be developed (Massarain, 2004). At present vaccine is available only in some countries of the world, e.g. China, which offers only a limited degree of protection and the protection is largely serovar-specific, and is of relatively short duration (WHO, 2003). Besides, vaccines may also produce the side-effects like pain at the injection site and fever.

5.1.5 Awareness and Education

Leptospirosis is largely an underreported disease in most developing countries of the world. Even the physicians are not aware of infection among human populations. Awareness and education on leptospirosis will assist the people to understand the disease avoid the risks, and seek medical attention. Awareness among medical practitioners and veterinarians will remind them to consider leptospirosis as part of differential diagnosis in any febrile illness and treat the patient accordingly, while it will compel public health authorities to formulate appropriate control measures (WHO, 2003).

5.2 Control of infection in animals

5.2.1 Rodent control

Livestock may have contact with rodents whether they are stall-fed or are grazed. Control of rodents in animal houses is essentially similar to that mentioned in section 5.1.1. In

addition to rodents, grazing animals may come in contact with several wild reservoirs of leptospires, and their control is almost impossible. However, some methods may prove helpful in reducing the number of wild reservoirs and rodents in pasturelands. Poisoning of the wild animals by using zinc phosphide, sodium monofluoroacetate or thallium sulfate has been done successfully. Use of trap and predator animals may also be helpful when the intensity of the problem is low. Screening and fencing targeting a particular species of wild animal may stop its accessibility to the pasturelands. Alternatively, animals should not be allowed to graze in the area suspected to be contamination by wild reservoirs.

5.2.2 Isolation and slaughter of domestic animals

Suspected or positive testing domestic animals may be separated from uninfected animals and kept in isolation. Chemotherapeutic treatments of these isolated animals should be done to facilitate the eradication of leptospires from their kidneys. Disinfection of the premises should be done with a suitable disinfectant. Pigs and dogs should be isolated from other species of animals as they are potential shedders of large number of leptospires for longer periods (WHO, 1982).

Alternatively, if the animal is not recovering fast, it may be slaughtered to keep the rest of the herd free from leptospirosis. The disposal of dead animals should be done properly, e.g. by burning or deep burial.

5.2.3 Chemotherapy

Dihydrostreptomycin, tetracyclines or penicillin may be used to reduce or eliminate the infection in animals. Dihydrostreptomycin (25 mg/kg) is the drug of choice for the complete elimination of infection from animals (WHO, 1982; Ellis, 1984). The drug must be administered parenterally, usually over a period of days.

5.2.4 Vaccination

The most practical approach for control of leptospirosis in animals is the appropriate and timely vaccination of maintenance hosts to stop the spread of their infection resulting from leptospiruria. It has been suggested that leptospiral bacterins stimulate the optimum

production of antibodies for a period of at least 6 months post vaccination (WHO, 1982). Vaccines have been successfully used for the immunization of pets and farm animals. However, the protection is serovar specific and bacterins should contain all the antigens against which protection is sought. For example, vaccines used for dogs should contain the antigens of the serovar *icterohaemorrhagiae* and *canicola*; for cattle: *hardjo* and *pomona*; for pigs: *pomona*, *tarassovi* and *bratislava*.

However, vaccination will not stop leptospirosis. Vaccination along with antibiotic therapy (Dihydrostreptomycin @25mg/kg) is reported to offer effective control and prevention of clinical leptospirosis in bovines (Ellis, 1984). Implementation of successful vaccination programs in the developing countries is hindered by the lack of identification of the prevalent serovars against which protection is to be sought.

6 Discussion and Conclusion

The most important risk-factors for leptospiral infection in humans in Nepal have been mentioned here as working in the dairy farms and in the rice fields. Although very common (Murdoch et al., 2004), leptospirosis is an unnoticed disease in Nepal. Doctors, veterinarians, public health and general authorities, and the general public – all are unaware of the magnitude of this disease burden. In such a situation, it is difficult to suggest a control program, especially for a disease condition that has not even been noticed as a problem. For the present moment, raising of awareness about the disease and educational campaigns are the best options to reduce the incidence of leptospirosis among animals and humans in Nepal. Implementation of education campaign can be done as following:

Education and updates for Physicians: Medical practitioners and health care providers should be informed on the symptoms of leptospirosis, risk-factors, diagnostic methods and therapeutic strategies. This may be done by direct mailings, articles in the local medical journals or health department newsletters, presentations at hospitals or by combination of all of these activities.

Education for the public: The general people should be educated about the nature of the disease so that they can identify and avoid risks, and seek timely medicinal help in

leptospirosis suspected conditions. The educational materials can be produced in a variety of forms like brochures written in the community language, a descriptive videotape, display boards, tee-shirts printed with a suitable message. Besides, display of warning signs in the high-risk areas may also be helpful.

After a certain level of awareness has been achieved following the implementation of this educational program, the prevalent serovars in animal populations should be identified to suggest an appropriate vaccination program. This control strategy seems to be the most appropriate for Nepal.

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