ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

SEROLOGICAL STUDIES ON BOVINE AND HUMAN LEPTOSPIROSIS IN WONJI, EAST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

MSc Thesis

BY

ROMAN YILMA GEBRE-SELASSIE

JUNE, 2009
DEBRE ZEIT, ETHIOPIA

SEROLOGICAL STUDY ON BOVINE AND HUMAN LEPTOSPIROSIS IN WONJI, EAST SHOA ZONE, OROMIA REGIONAL STATE, ETHIOPIA

BY

ROMAN YILMA GEBRE-SELASSIE

Board of External Examiners **Signature**

1. Dr Berhe Gebre-Egziabher (Ministry of Agriculture and Rural Development, Animal and Plant Health Regulatory Department) _____
2. Dr Desalegn Lidetu (National Animal Disease Investigation and Diagnostic Center) _____
3. Dr Gobena Ameni (Aklilu Lemma Institute of Pathobiology, Addis Ababa University _____
4. Dr Mohammed Abdela (Jijiga University) _____
5. Prof. Sayed Mohammed Zeidan (PANVAC) _____

Academic advisors: Moses N. Kule (BVM, MSc, MPVm, PhD, Associate Prof.)
Mahendra Pal (BVSc, MVSc, PhD, DSc, Prof.)

AKNOWLEDGEMENTS

It gives me an immense pleasure to pass my deep felt gratitudes and many thanks to my advisors Dr Moses N. Kyule and Prof. Mahendra Pal, for intellectual guidance, material provision and unreserved effort to correct this paper. My special thanks go to Dr Mosses N. Kyule for his assistance and patience during data analysis.

I am delighted to acknowledge the Food and Agriculture Organization of the United Nations for sponsorship of ELISA kit purchase. Emmanuelle Guerne Bleich has great part in fulfillment of the research, without whom it would have been hard to achieve. Prof. Getachew Abebe has also made incalculable effort towards the accomplishment of the research, and deserves my sincere thanks.

Though no words can express the effort my husband, Dr Gedlu Mekonnen, has made for my achievement, I use this as an opportunity to express my heartfelt appreciation, for he covered my expenditures, provided reference materials, and encouraged me during my difficulties, throughout the study period. My Daughter, Saron Gedlu is also appreciated for her patience and tolerance during my departures. Misrak Yilma, who has been with me from the very beginning, is the secret behind my success, as she has been a key person to fill the gaps I came across during the research period.

The cooperative assistance of the staffs (Ato Worku Masho, Tewodros, Tsegaye, Solomon and others) of Wonji Hospital and 7th Camp Health Center, who collected blood samples from human patients has considerable part in achievement of this research, they are really appreciated. Dr Yohannis Tesfaye deserves my honest gratitude for considerable contribution towards the success of sample collection from human cases. I also thank the animal health workers (Ato Megersa and Ato Lemi) of Wonji area for their assistance during blood sample collection from cattle.

I would like to pass my paramount regards to the National Veterinary Institute where I got permission for transport access using their service bus, which I take for a critical time service,

during my course work. The institute also cooperated to use the materials I needed to conduct the serological tests, which made my works easy and successful.

I would also like to forward my sincere regards to my parents, Ato Yilma G/Selassie and W/o Kabtamua Bekele and my brothers Tedy, Dawit, Yohanis, Tilahun, Elias and Sisay, for they have been my strength throughout my whole life. My deepest gratitude goes to Ato Shimelis Bekele and W/o Abaynesh Desta, W/o Wolansa G/Selassie and Ato Samuel Jima, Mulu Mekonnen and Askale Mekonnen whose concern plays an important role in my life.

My friends, Dr Redeat, Dr Akafete, Hirut, Dr Etsehiwot, Dr Yenehiwot and also others are deeply acknowledged for they have contributed in one or another way to accomplish my study successfully.

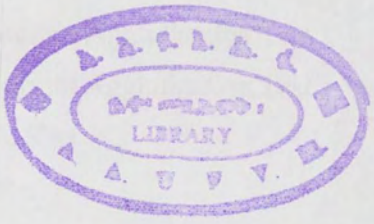
LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF APPENDICES



LIST OF ABBREVIATIONS

CI	Confidence Interval
CSF	Cerebro-spinal Fluid
DNA	Deoxyribonucleic Acid
DNR	Department of Natural Resources
ELISA	Enzyme Linked Immunosorbent Assay
ESS	Erythrocyte Sensitizing Substance
FUO	Fever of Unknown Origin
HA	Heamagglutination
HIV	Human Immunodeficiency Virus
HL	Haemolysis
IgM	Immmunoglobulin M
LAT	Latex Agglutination Test
MAT	Microscopic Agglutination Test
MCAT	Microcapsul Agglutination Test
n	Sample Size
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PFGE	Pulsed Field Gel Electrophoresis
REA	Restriction Enzyme analysis
RNA	Ribonucleic Acid
SEA	Sensitized Erythrocyte Agglutination
SEL	Sensitized Erythrocyte Lysis
sv	Serovar
UTI	Urinary Tract Infection
WHO	World Health Organization
TMB	Tetramethlybenzidine

ABSTRACT

A cross sectional study design, consisting serological and questionnaire surveys, was applied to assess the occurrence of leptospirosis in cattle and humans in Wonji from October 2008 to April 2009. Consequently, the seropositivity percentage for leptospirosis in human patients visiting hospital and health center in Wonji and the seroprevalence of *Leptospira interrogans* sv Hardjo in apparently healthy cattle was determined by using *Leptospira* IgM ELISA and *Leptospira* Hardjo antibody ELISA respectively. The overall seropositivity percentage of human leptospirosis was found to be 72.5% (n=298) and that of cattle was 30.4% (n=204). In this study assessment of risk factors associated with human leptospirosis was carried out based on semi-structured questionnaire survey. Regardless of wide distribution of the risk factors included in this study, statistical analysis of relationship between the variables showed that only occupation, animal contact and sampling sites were significantly associated ($p<0.05$) with seropositivity of leptospirosis.

Leptospirosis seropositivity occurred in all human age groups observed in this study, with more frequency of occurrence in individuals aged between 20 to 50 years of age although no significant statistical difference among age groups. There was no significant difference between sexes on ELISA test result. Signs and symptoms manifested by the sampled patients were highly distributed, but no statistical significance was observed.

Analysis of the occupational risk showed that housewives were found to be most frequently reactive to *Leptospira* IgM ELISA test as compared to sugarcane factory and government employees. Moreover, Leptospirosis seropositivity was significantly higher ($p<0.05$) in human cases having animal contact as compared to those who had no animal contact.

Leptospira interrogans sv Hardjo anitibody test was underway to measure the prevalence of leptospirosis in cattle. Out of 204 samples tested, 62 were positive, with no significant difference ($p<0.05$) between sex and sampling locations. The animals sampled were apparently healthy and the high prevalence (30.4%) of *Leptospira interrogans* sv Hardjo, which is pathogenic to humans, suggests the existence of considerable risk posed by animal contact. Generally, this study emphasizes the potential public health importance of leptospirosis in the study area, where the cause of fever usually remains obscure because of limited laboratory diagnostic facilities.

To contain the disease, public awareness on the transmission routes, and health and economic importance of leptospirosis should be implemented particularly in Wonji and generally in other similar areas that may support the maintenance of leptospirosis spread. Virtually no work has been done in Ethiopia regarding the veterinary and public health significance of leptospirosis that renders it as a forgotten disease with paramount health and economic importance. Veterinarians and physicians should make collaborative further investigation on leptospirosis epidemiology, control and prevention.

Keywords: Bovine, ELISA, Human Cases, Hardjo, IgM, Leptospirosis, Prevalence

1. INTRODUCTION

There are several diseases of public health importance that are associated with exposure to animals. Many of these diseases have been recognized as emerging or under diagnosed health challenges common to animals and humans or transmissible from animals to humans. Among such diseases, leptospirosis has been known to be a worldwide zoonosis of public health and economic importance, the incidence of which is higher in tropical climates (Meites *et* al., 2004; Senthilkumar *et al.*, 2007).

Though the number of human cases worldwide has not been well documented, it has been supposed to range from 0.1 to 1 per 100 000 per year in temperate climates to 10 or more per 100 000 per year in the humid tropics (WHO, 2009). During outbreaks and in high-risk groups, 100 or more per 100 000 may be infected. It has been indicated that leptospirosis is caused by different serovars of a spirochetal organism known as *Leptospira interrogans*. In addition to *Leptospira interrogans,* the genus *Leptospira* includes a free living (nonpathogenic) species named as *Leptospira biflexa* (Esen *et al.*, 2004). The pathogenic species, *Leptospira interrogans* affects many mammals mainly cattle, pigs, horses, dogs and humans (Feresu *et al.*, 1998; Hirsh and Zee, 1999; Pal, 2007). At least 300 serovars classified into 23 serogroups and 17 genomospecies have been identified within the pathogenic species (Quinn *et al.*, 1994; Maria *et al.*, 2008).

Leptospirosis is acquired by direct or indirect contact with the urine of infected animals such as rodents and domestic animals (Esen *et al.*, 2004). The clinical presentation of leptospirosis varies greatly due to diversity within this species coupled with its complex epidemiology. Several mammals develop chronic leptospirosis and thus act as reservoirs for the pathogenic leptospires. Human leptospirosis or Weil's disease has a high mortality rate and is caused by *Leptospira interrogans* serogroup Icterohaemorrhagiae (Shah *et al.*, 1999; Esen *et al.*, 2004). However, it is the relatively mild (flu-like) form which occurs most often as compared to its sever form (Levett and Whittington, 1998; Esen *et al.*, 2004).

Many reports have documented that leptospirosis infects humans through contact of cut or abraded skin or mucous membranes with contaminated urine of infected domestic and wild animals, of which rats are the most common source of infection with a 90% carriage rate.

According to ProMed-mail 2005, an outbreak of leptospirosis occurred and resulted in the death of 100 people within two days, following torrential rains and subsequent floods in Maharashtra in India. Between 1985 and 1993, 20,341 cases of human leptospirosis were recorded in Brazil, with a case fatality rate of 11% (Nassi *et al.*, 2003). Generally, those people who are at risk of acquiring leptospirosis are abattoir workers, livestock handlers, veterinary surgeons, poultry and fish handlers, sewer and canal workers (Pal, 1996; Gracey *et al.*, 1999). Occupational exposure probably accounts for 30 - 50% of human cases (Mckenzie and Shoff, 2008).

Leptospirosis occurs endemically as well as in serious epidemics in tropical and developing countries where highest morbidity rates have been reported (Oliveira *et al.*, 2001). Optimal conditions for survival of the organism are a warm and wet environment, with neutral or slightly alkaline water; however, leptospires are able to survive acidity of pH 5 - 6.2 for limited periods. They can also survive in cold water provided that it does not freeze (Baron *et al.*, 1994, Quinn *et al.*, 1994).

Reports have indicated that the first isolation of *L. interrogans* serovar Hardjo from cattle was made in America. Later on, different serovars of *Leptospira* have been isolated from domestic animals, wild animals and settled human sewage. In Africa, the serovar known as Tarrasovi serogroup has been detected in humans, wild life and cattle (Feresu *et al.*, 1998). Since then, further serovars were discovered throughout the world and now all pathogenic leptospires are classified into one species, *Leptospira interrogans* that includes different serovars (Gumussoy, *et al.*, 2009; Hussein and Nabi, 2009).

According to the serological study in cattle conducted by Abdollahpour (2007) in Iran, it was shown that 46.8% of the samples from 39 herds had a positive reaction against one or more serogroups. However, cattle susceptibility varies according to stress factors such as age, physiological state and population density (Oliveira *et al.*, 2001). In cattle and other domestic animals, septic illness is largely confined to the young while abortion is the principal manifestation in adults. Abortion and recurrent uveitis are the most common manifestations in other domestic animals (Radostits *et al.*, 1994). While poor reproductive performance in domestic animals has been investigated in some countries of the world, less attention has been given to *Leptospira* serovars than other causes of reproductive wastage that may be attributed to nonspecific clinical manifestations of the disease (Esen *et al.*, 2004).

Serological surveys have demonstrated that leptospirosis occurs in all African countries where they have been undertaken (Feresu *et al.*, 1998). In Ethiopia, there is a limited information on the prevalence and health significance of leptospirosis both in the public health and veterinary institutions. However, a study conducted several years ago demonstrated wide distribution of the organism in different species of domestic animals where 758 serum samples from domestic and wild animals were tested for leptospiral antibodies using the MAT (Moch *et al.*, 1975). The following proportions of seropositivity were obtained: 91.3 % in horses, 70.7% in bovine, 57.1% in pigs, 47.3% in goats, 43.4% in sheep, 15.4% in camels and 8.3% in dogs. In addition, these researchers tested 54 samples from birds and wild animals, but all were reported to be seronegative.

Yimer *et al.* (2004) also conducted a pilot study on human leptospirosis around Wonji, central Ethiopia, where out of 59 patients sampled, 47.46% were positive for leptospirosis. The natural environment of Wonji makes it favorable for the survival and spread of *Leptospira* species and the presence of such environmental conditions as sugar cane factory and plantation contributes to the high occurrence of leptospirosis in humans and animals. Moreover, the inevitable backyard livestock production system particularly in the nearby rural areas exacerbates the situation, since the epidemiology of the disease is maintained in such management conditions. The lack of leptospirosis investigation generally in Ethiopia and particularly in Wonji might have brought about a wide information gap on the economic, public and animal health importance of the disease. Yimer *et al.* (2004) hypothesized that Leptospirosis might have a wide distribution in Ethiopia where climatologic, socioeconomic and cultural factors are highly favorable for the occurrence and spread of the disease.

There is a limitation in epidemiologic investigation of leptospirosis as it is often hampered by difficulty of definitive microbiological diagnosis. Though isolation of the organism from clinical specimens provides a definitive diagnosis, the value of culture is limited due to the fact that the samples need to be collected prior to the antibiotic treatment and prolonged incubation is required (Levett, 2003). On the other hand, demonstration of bacterial motility under dark ground illumination in clinical samples seems to be helpful in the early diagnosis, but this technique has low sensitivity and depends on the technician's opinion. The Enzyme-linked immunosorbent assay (ELISA) is a useful alternative with a high sensitivity to detect specific IgM antibodies as a sign of current or recent leptospirosis which also gives a more

objective interpretation of results than other methods (Chaudhry *et al.*, 2002; Senthilkumar *et al.*, 2007; McKenzie and Shoff, 2008).

The probability of achieving a positive serologic test increases with the duration of disease and good correlation between results of MAT and ELISA has been reported (McKenzie and Shoff, 2008). MAT has been referred to as a dependable diagnostic tool for leptospirosis by providing serovar specific diagnosis. The weak side of this method is that there exists a large number of *L. interrogans* serovars and maintaining large number of the organisms for MAT is difficult in routine diagnostic laboratories. Moreover, MAT may fail to detect antibodies when specific serovars are not used (Chaudhry *et al.*, 2002; Maria *et al.*, 2007). MAT is also an inadequate assay for rapid diagnosis since it requires the testing of paired sera and the sensitivity has to be determined by cell culture and the specificity is related to the serovars included in the panel.

The current study was conducted with the following objectives:

- To assess the prevalence of *Leptospira interrogans* serovar Hardjo in apparently healthy cattle in Wonji
- To measure the positivity percentage of leptospirosis in human patients visiting Wonji Hospital and a health center
- To identify risk factors associated with the occurrence of leptospirosis in humans

2. LITERATURE REVIEW

2.1 Leptospirosis

Leptospirosis has been known to be a zoonosis, which is transmissible from animal to animal and from animal to human. Rodents have been mentioned as the original natural carriers. The disease has been reported for a long time in the world and is known under different names such as Weil's disease, canicola fever, hemorrhagic jaundice, sugarcane fever, mud fever, swamp fever, swine-herder's disease and Bragg fever (Acha and Szyfres, 2001; Pal, 2007). Leptospirosis is predominantly an occupational disease (sewer workers, plumbers, veterinarians, abattoir workers, butchers, sugarcane-field workers, dairy workers and military personnel) (Pal, 1996; Slack *et al.*, 2007).

It has also been increasingly recognized as a recreational disease contracted by those traveling to tropical and equatorial regions. Leptospirosis occurs worldwide, in both rural and urban areas and in temperate and tropical climates (Vinetz *et al.*, 1999). It is also a recreational hazard to those who swim or wade in contaminated waters (Meites *et al.*, 2004; Alberta Health Services, 2005). In endemic areas the number of leptospirosis cases may peak during the rainy season and even may reach epidemic proportions in case of flooding (WHO, 2009). Flooding after a heavy rainfall facilitates the spread of the organism, as water saturates the environment, leptospires present in the soil pass directly into surface water (WHO, 2009).

2.1.1 Epidemiology

Leptospirosis is a zoonotic spirochetal disease of global importance that is more prevalent in tropical countries as compared to the temperate due to higher humidity rainfall and temperature that promote survival of the organism in such environment (Ignacio,*et al.*, 2002; Slack *et al.*, 2007). It has major negative impacts on people living in urban and rural areas in developing countries with inestimable morbidity and mortality (Cachay and Vinetz, 2005). It occurs wherever there are risks of direct or indirect contacts with the urine of infected animals (Romero *et al.*, 2003). Leptospirosis can occur in all mammals, including domestic pets, livestock, wild animals and humans, although in some species it is rare (Eymann *et al.*, 2007). Fish and crustaceans seem rarely, if ever, to be infected, and birds appear unable to contract the infection in the wild except when very young. Reptiles and amphibians can also be infected but not as commonly as mammals (Pal, 2007). Insects are not considered to be involved except where they transfer blood between other animals.

All domestic animals, game, rodents and humans are susceptible to *L. interrogans* and its pathogenic serovars. Leptospires have also been isolated from poikilothermic organisms/animals (Radostits *et al.*, 1994; Braunwald *et al.*, 2003; Krawczyk, 2006). Because of the importance of water as a means of spreading infection, new cases are most likely to occur in wet seasons and low lying areas, especially when contamination and susceptibility are high. A different distribution has been observed in the prevalence of seropositives in cattle in Australia. *Leptospira interrogans* serovar Hardjo antibodies have a high prevalence through all rainfall areas, but *L. interrogans* serovar Pomona is much more common in low rainfall areas although, heavy rainfall followed by flooding is frequently associated with large outbreaks of leptospirosis (Matsunaga *et al.*, 2003).

However, an epidemiological study of bovine leptospirosis in Queensland, Australia, indicated different geographical distributions and prevalence of antibodies to the serovars Hardjo and Pomona (Seifert, 1996; Abdollahpour, 2007).

Rodents are considered to be the most important carriers for most serovars of *Leptospira*, but serovars Pomona and Hardjo are adapted to agricultural animals as carriers (Table 1). Infection with *Leptospira* organisms is maintained in the kidneys of carrier hosts. Organisms shed in urine contaminate the environment and infect other species, including human (Beers *et al.*, 2003; Braunwald *et al.*, 2003). Inevitably, conditions of housing determine the numbers of mice and rats present and thus the number of animals infected (Senthilkumar *et al.*, 2007). In some countries, leptospirosis is endemic and infection is much more common than clinical disease. Domestic animals may acquire the infection by accidentally ingesting reservoir rodents or getting into contact with contaminated urine of infected animals. These domestic animals then maintain the infection within the herds or flocks. The rate of transmission between mammals by indirect contact largely depends on environmental factors that favor the survival of leptospires. In general terms, the disease is most common in areas or seasons when the climate is warm and humid, soils are alkaline and there is an abundance of surface water (Radostits *et al.*, 1994).

In the case of leptospiral abortion, infection can be spread by the aborted foetus and uterine discharge. An infected foetus can carry the infection for up to 7 weeks after birth. The semen of an infected bull may carry leptospires and transmission from such a bull to heifers by coitus and artificial insemination has been documented. Field observations of herd outbreaks

of *L. interrogans* serovar Hardjo infection have frequently implicated a bull as the source of introduced infection. In cattle, excretion of leptospires in the urine may take up to 542 days. *Leptospira interrogans* serovar Hardjo is excreted from the genital tract of aborting cows for as long as 8 days after abortion or calving and is detectable in the oviducts and uterus for up to 90 days after experimental infection and in naturally infected cows. Infection may also persist in the mammary gland as organisms have been isolated from milk of an experimentally infected cow for as long as 91 days after infection (Radostits *et al.*, 1994; Seifert, 1996).

Leptospires are ubiquitous and abundant due to their abilities to infect a variety of animals and to persist outside the host. Transmission of leptospires may follow direct contact with urine, blood or tissue from an infected animal, transplacental transmission or exposure to a contaminated environment (Senthilkumar *et al.*, 2007). The urine of infected animals or healthy carriers that may contaminate soil, pasture, drinking water and feed is the main source of infection (Gracey *et al.*, 1999; Braunwald *et al.*, 2003).

The organisms have a predilection for the kidneys and the urinary tract where they may produce a persistent infection (Eymann *et al.*, 2007). Leptospires may be excreted with the urine by which transmission of the bacteria takes place. Infective urine constitutes the major sources of infection for humans, domestic animals and wild animals. Transmission usually occurs when there is direct contact between urine droplets or urine contaminated water and the mucous membranes of the eye, nose and mouth or through abraded skin (Ignacio *et al.*, 2002; Sharma and Kalawat, 2008). Transmission of the leptospire organisms may also occur through the food chain by the ingestion of leptospiral-infected carcasses by carnivorous species. This is the most significant mode of spread of leptospires among carnivores (NSW Public Health Bulletin, 2003; Wiwanitkit, 2006). Transmission of leptospirosis from domestic animals to human beings has been widely reported, often with a minimal contact exposure than animal owners. Human-to-human transmission of leptospirosis is rare; thus human beings usually are dead-end hosts for the disease.

Milk seems to have been the most likely route. Serovar Hardjo causes agalactia and mastitis in cattle. Leptospires have also been isolated from the milk of a woman with clinical disease. In the latter case, breast-feeding was discontinued during the mother's illness and the infant did not become infected. Transplacental infection with fetal death and abortion has also been reported in human beings.

Leptospira organisms enter into the body most often through cutanous or mucosal abrasions and rapidly disseminate to target organs. These organisms may be cleared by the humoral immune response, but in carriers they have the ability to colonize and persist in the kidney tubules. Oral transmission may occur when animals are feeding on contaminated pasture or feedstuffs or drinking or standing in contaminated water. The finding of *L. interrogans* serovar Hardjo in the genital tract of cattle indicates that venereal transmission may also play a part in the epidemiology of bovine leptospirosis (Radostits *et al.*, 1994).

Table 1. Significant reservoirs of pathogenic leptospires.

Leptospira interrogans serovar	Major reservoir(s)	Alternative reservoir(s)
Autumnalis	Wildlife	
Ballum	Wildlife	
Bratislava	Pigs, horses, cattle	
Canicola	Dogs	Cattle, pigs, rodents
Hardjo	Cattle	Sheep
Icterohaemorrhagiae	Rats	dogs, cattle, pigs
Pomona	pigs, cattle	Dogs, wildlife

Source : Acha and Szyfres (2001).

2.1.2 Etiology

Morphology: The etiologic agent of leptospirosis is *Leptospira interrogans*. It is a thin spiral organism 0.1μm x 3 - 30 μm depending on the number of whorls, with tightly set coils, and it is characterized by very active motility, by rotating ("spinning") and bending. Usually one or both ends of this single-cell organism are bent or hooked, but straight forms also occur that rotate and travel more slowly than hooked forms (Sharma and Kalawat, 2008). The organisms are obligate aerobes that use fatty acids or alcohols as sources of energy (Ricaldi and Vinetz, 2007). Because of their narrow diameter, the leptospires are best visualized by dark-field illumination or phase contrast microscopy and they do not stain readily with aniline dyes. The free living *(L. biflexa)* and parasitic leptospires *(L. interrogans)* are morphologically indistinguishable (Sambasiva *et al.*, 2003).

Leptospira belongs to the bacterial phylum of spirochetes, which has a deep branching lineage in *Bacteria*, as indicated by 16S rRNA analysis (Louvel *et al*, 2006). The genus *Leptospira*

was initially divided into two groups: the pathogenic *Leptospira* referred to as *Leptospira interrogans* and the saprophytic *Leptospira* referred to as *L. biflexa* (Quinn *et al*, 1994). Pathogenic member of the genus *Leptospira* is an anthropozoonosis which is widespread throughout the world (Baron *et al.*, 1994; Seifert, 1996; Ricaldi and Vinetz, 2007). Saprophytic and pathogenic *Leptospira* species were first classified into serovars, with more than 220 serovars defining the pathogens. More recently, DNA-DNA hybridization studies separated *Leptospira* species into 17 genomospecies, 7 of which are pathogenic (Beers *et al.*, 2003; Cullen *et al.*, 2003; Matsunaga *et al.*, 2003).

Pathogenic leptospires were originally classified based on their antigenic characteristics. A single species, *Leptospira interrogans* was divided into taxa called serovars by a classification based on pair wise comparison of the extent of cross-absorption of rabbit antisera against each serovar. Antigenically related serovars constitute serogroups; in total, *L. interrogans* included at least 223 serovars in 23 serogroups. The identification of isolates into serovars requires the maintenance of a comprehensive collection of strains and the corresponding rabbit immunesera (Quinn *et al.*, 1994; Abdollahpour, 2007). The significant reservoirs of pathogenic leptospires are summarized in Table 1and the common *Leptospira interrogans* serovars and their hosts in Table 2.

The organisms are too small to be seen in wet preparations made from fresh blood and they do not cause motion of the erythrocytes. Culturing the organism from body fluid during the first week of illness has been reported to be the most reliable test for detection of leptospirosis. Cultural growth can also be done from a urine sample after a week up to several months. The cultures are incubated at room temperature or 30°C in the dark for up to 6 weeks during which the organisms grow below the surface of the growth medium. Materials collected a few centimeters below the surface of broth cultures should be examined weekly for the presence of the growth using a direct wet preparation under dark field illumination. Leptospires have been known to exhibit corkscrew-like motility (Stanier *et al.*, 1986; Baron *et al.*, 1994).

Serologic diagnosis of leptospirosis is best performed using pools of bacterial antigens containing many serotypes in each pool. Positive results are visualized by examining for the presence of agglutination under dark field examination (Quinn *et al.*, 1994).

Table 2. Common *Leptospira interrogans* serovars and their hosts.

		Occurrence in:						
Serogroup	Serovar	Cattle	Pigs	Dogs	Horses	Sheep	Rodents	Other wildlife
Australis	Australis	+						+
Autumnalis	Bratislava	+	+		+			
	Autumnalis						+	+
Ballum	Ballum						+	+
Bataviae	Bataviae	+					+	
Canicola	Canicola	+	+	+			+	+
Grippotyphosa	Grippotyphosa	+	+	+	+		+	+
Hebdomadis	Hebdomadis							+
	Szwajizak	+						
Icterohaemorrha Giae	Icterohaemoragiae	+	+	+			+	+
	Iopenhageni	+		+			+	
Pomona	Pomona	+	+	+	+	+		+
Sejroe	Balkanica	+						
	Hardjo	+			+	+		
	Saxkoebing	+						
	Sejroe	+					+	+
Tarassovi	Tarassovi		+					

Source: Quinn *et al.* (1994).

2.1.3 Pathogenesis

Leptospires can get entry into the host through mucous membranes or damaged skin from direct or indirect contact (Meites *et al.*, 2004; Slack *et al.*, 2007). After epithelial penetration, there is hematogenous spread with localization and proliferation in parenchymatous organs particularly the liver, kidneys spleen and sometimes meninges. In some cases, large numbers of immune complexes containing leptospiral components form in the blood, kidneys, liver and other organs resulting in severe illness that may be followed by death (Stanier *et al.*, 1986). Acute disease may be observed in several species, especially in man and dog. Hepatitis with a severe jaundice has been often related to acute leptospirosis, because of renal failure or pulmonary hemorrhages. Icteric leptospirosis or Weil's syndrome (in human) is a form of disease characterized by symptoms of hepatic, renal and vascular dysfunction (Sambasiva *et al.*, 2003). Nevertheless, in other species such as cattle, pig and horse, sub acute or even chronic leptospirosis has been generally observed. In these animals, reproduction failure is the

most frequent consequence (Michael *et al.*, 2002). In the kidneys, the organisms reach and localize in the lumen of proximal convoluted tubules.

Penetration and multiplication in the foetus can occur in pregnant animals leading to foetal death and resorption, abortion or delivery of weak offspring (Radostits *et al.*, 1994). If infection occurs in the third trimester, can produce specific antibodies and may overcome the infection. Antibody production in infected animals begins a few days after the onset of leptospiraemia. The leptospires tend to persist in sites such as renal tubules, eyes and uterus where antibody activity is minimal. Leptospires damage vascular endothelium resulting in hemorrhages. Serovars in the serogroups *Autumnalis*, *Grippotyphosa*, *Icterohaemorrhaguae*, and *Pomona* produce a haemolysin that is probably responsible for the haemoglobinuria (red water) in young calves infected with these serovars. Virulence varies between serovars and between two genotypes of *Leptospira interrogans* serovar hardjo known as hardjo bovis and hardjo provitno (Quinn *et al.*, 1994; Feresu *et al.*, 1998).

2.1.4 Clinical Signs

Leptospirosis is a bacterial disease caused by various strains of *L. interrogans* affecting humans and more than 180 species of animals (Levett and Whittington, 1998; Gumussoy *et al.*, 2009). The clinical presentation of leptospirosis varies due to the diversity within the genus *Leptospira* and its complex epidemiology (Ignacio *et al.*, 2002; Sharma and Kalawat, 2008). Several mammalian species may develop leptospirosis mainly in its chronic form and thus act as reservoir for the disease. Human leptospirosis or Weil's disease has a high mortality rate and is caused by *Leptospiora interrogans* serogroup Icterohaemorrhagiae. The vaccination of man and animals has limited effects because vaccines are specific to the serovar and induce only a short term immunity (Ricaldi and Vinetz, 2007).

The clinical findings in leptospirosis have been reported to be similar in each animal and do not vary greatly with the species of *Leptospira* except that infection with *L. interrogans* serovar Icterohaemorrhagiae which usually causes an acute septicemia, following the incubation period of 3-7 days in all animals. In cattle, leptospirosis may appear as acute, subacute or chronic forms and is usually caused by *L. interrogans* serovars Pomona or Hardjo. The disease is manifested by septicemia with high fever of 40.5 - 41.5 °C, (105 -107 °F), anorexia, petechiation of mucosae, depression, dyspnoea and acute hemolytic anemia

with hemoglobinuria, jaundice and pallor of the mucosae (Radostits *et al.*, 1994; Pal 1996, Nassi *et al.*, 2003). The course of the disease is more severe in calves in which stunted growth and varying mortality rates are observed. The case fatality is high and if recovery occurs, convalescence is prolonged. In adult cattle, abortion (20% of which may be followed by death) due to systemic reaction is likely to occur at the acute stage of the disease. It has been indicated that placental retention, prenatal mortality, congenital abnormality, loss in milk production and mastitis are common (Gumussoy *et al.*, 2009). The problem related to udder and milk flow have been attributed to the *L. hardjo* serotype which is associated with the ''milk drop syndrome'' followed by a considerable consequence in dairy and suckler herds (Levett and Whittington, 1998; Oliveira *et al.*, 2001).

Dogs of all ages and both sexes may be affected and the incubation period has been reported to range from 4-12 days. During the time, generalized signs, including fever, depression and anorexia may be seen. Vasculitis, thrombocytopenia and coagulopathy may develop. In horses, the disease has mostly been associated with uveitis or abortion. It has been documented that the serologic prevalence in horses is higher than clinically manifested illness (Aiello *et al.*, 1998).

Sheep have been known to be less susceptible than cattle, possibly due to less intensive husbandry methods and the tendency of sheep to avoid contact with surface water. In the United States, Pomona and Hardjo have been the most common *Leptospira* serovars isolated from sheep (Acha and Szyfres, 2001; Silva *et al.*, 2007).

Although most human leptospirosis infections are self-limited, complications are common, involving hepatonephric failure, pulmonary hemorrhage, and death in 10%–50% of severe cases (Beers *et al.*, 2003; Haake, 2006). Clinically, human leptospirosis is characterized by an acute influenza-like illness, with fever that often reaches 102 °F, chills, severe headache, nausea, vomiting and myalgias (Radostits *et al.*, 1994; Braunwald *et al.*, 2003; Pal, 2007). The eyes usually become red on the third or fourth day. In general, two clinical signs have been distinguished as icteric (the serious form or hepatonephric type that has been known as Weil's disease and less frequent, being estimated to occur in about 10% of cases) and anicteric form, which is less severe and known to occur in 85-90% of cases (LaRocque *et al.*, 2005).

Anicteric leptospirosis

The onset of anicteric leptospirosis is abrupt and is characterized by fever, headache, severe myalgia, chills with rigors, prostration and sometimes, circulatory collapse (Meites *et al.*, 2004). The septicemic (first) phase lasts 3 to 7 days, characterized by high fever and intense headache. Anorexia, nausea, vomiting and abdominal pain occur in most patients (Ignacio *et al.*,2002). The most common physical finding is conjunctival suffusion in the absence of purulent discharge. Other signs include masculopapular skin rash, pharyngeal injection, lymphadenopathy, splenomegaly, hepatomegaly, and muscle tenderness. The symptoms are prominent for 4 to 7 days during the septicemic stage, when defervescence due to lysis occurs. Leptospires can be isolated from the blood and the CSF during this phase (Sambasiva *et al.*, 2003).

The immune (second) stage of anicteric leptospirosis is preceded by an asymptomatic period of 1-3 days. The onset of the immune stage coincides with the appearance of IgM antibodies. Fever, headache and vomiting are less severe at the onset of the immune stage than during the septicemic stage. The duration of the immune stage ranges from 4 to 30 days, and the leptospires are cleared from the blood and the CSF after the first days of this stage. Leptospiruria develops and persists for 1 to 3 weeks (Johnson, 2003; Sambasiva *et al.*, 2003,).

Aseptic meningitis is the hallmark of the immune stage. Mild pleocytosis is present, with or without meningeal signs and symptoms. The CSF cell count is $<500/mm^3$ in most cases. Polymorphonuclear cells may predominate early in the illness, but mononuclear cells predominate later. The CSF protein level ranges from <40mg/dl (normal) to 300 mg/dl and the CSF glucose concentration is generally normal. Uveitis, iritis, iridocyclitis and chorioretinitis may also appear during the immune stage (Jagadishchandar *et al.*, 2003).

Icteric leptospirosis

Icteric leptospirosis or Weil's syndrome is a form of disease characterized by symptoms of hepatic, renal and vascular dysfunction. The clinical manifestations vary in terms of severity and symptomatology. Some patients with jaundice may have no renal manifestation. Supportive therapy has reduced mortality to between 5% and 10%. Any serotype of *L. interrogans* may cause icteric leptospirosis (Sambasiva *et al.*, 2003).

During the leptospiraemic phase of icteric leptospirosis, the symptoms do not suggest leptospirosis until the third to seventh day of illness, when jaundice and azotaemia develops (McKenzie and Shoff, 2008). The biphasic course of the disease is obscured by severe and persistent fever, jaundice and azotaemia. Jaundice appears, but there is no evidence of hepatocellular destruction. Hepatic dysfunction occurs, but it resolves and it is rarely the cause of death. The serum bilirubin level is usually <20 mg/dl, but can be as high as 60mg/dL to 80mg/dL (Silva *et al.*, 2009). Hypoprothrombinemia occurs in a minority of patients and responds to administration of Vitamin K. Serum transaminase levels are mildly elevated, rarely exceeding 100U/L to 200U/L. Serum bilirubin levels peak within seven days and the increase persists for a few days to several weeks (Braunwald *et al.*, 2003).

Renal involvement is common in both anicteric and icteric leptospirosis, but symptoms are present only in patients with icteric disease. Azotemia, oliguria and anuria commonly occur during the second week of illness, but may appear as early as 3 to 4 days after onset (Shah *et al.*, 2003). Blood urea nitrogen levels are below 100mg/dL in most cases, but may occasionally exceed 300mg/dl. Serum creatinine levels are usually 2mg/dL to 8mg/dL, although they may reach 18mg/dL (Meites *et al.*, 2004). Results of urinalysis are abnormal in 70% to 80% of cases; proteinuria, hyaline or granular casts, hematuria and pyuria are typical findings in the urine. The onset of anuria is a poor prognostic sign and diuresis usually signals resolution. Icteric leptospirosis is often associated with infection caused by the serovar icterohaemorragiae (Bovet *et al.*, 1999; Acha and Szyfres, 2001).

2.1.5 Diagnosis

A combination of clinical signs and serologic test results provides the most effective diagnostic technique. According to Michigan DNR, there are three methods used to diagnose leptospirosis in mammals: demonstration methods, serologic methods, and bacteriologic methods. The demonstration methods involve examining body fluids and tissues under dark field microscopy, and histologic examination of biopsy material with special silver stains (Warthin-Starry) or Giemsa stain. The serologic methods consist of fluorescent antibody and microscopic and macroscopic agglutination tests. Depending on the severity or stage of infection, an animal may react serologically to one or all antigens used to determine the serological profile. Using bacteriologic methods, the isolation of leptospires and their subsequent identification provides the most conclusive proof of infection (Romero *et al.*,

2003). Culturing these organisms, however, is difficult. The choice of tissue or body fluid to be cultured depends on the stage of the disease. Isolation of leptospires from domestic animals is most successful during or immediately after the acute stage of the disease (Seifert, 1996).

Different specimens used for the diagnosis of leptospirosis include whole blood or sera and mid stream urine for dark field examination. Kidney sections and foetal abomasal contents, cotyledons and uterine discharges in abortion cases can also be used. Agglutinins and lysins, are used in microscopic agglutination test (MAT) and are found in the serum of infected animals starting from the 10^{th} day post infection (John, 2005).

Under dark field microscopy, the typical motility of leptospires in the clinical specimens (blood, CSF, urine or peritoneal fluid) observed with dark field microscopes, when correlated with clinical parameters, may aid in early diagnosis (Jagadishchandra, *et al.*, 2003). It is a simple method, but it may not be positive if there are few bacteria in the sample (Sharma and Kalawat, 2008). Double centrifugation of the sample at low speed to separate the cellular elements, and then at high speed, help concentrate the leptospires (McKenzie and Shoff, 2008)

Phase contrast microscopy is useful for visualizing leptospires in the laboratory. However, because of its technical limitations in thick suspensions and its optical characteristics, it has less practical purpose whenever dark field microscopy is available (Sambasiva *et al.*, 2003).

As to staining methods, leptospires in smears of tissues or fluids on slides can be stained using silver deposition methods. The variously described procedures are modifications of Warthin Starry's method for staining. The stain is based on chemically reducing surface properties of leptospires and other spirochaetes. Well stained preparations show black spirochaetes in pale yellow or brown tissue elements. This method has the same limitation as dark field procedures, as it is difficult to detect small numbers of organisms in tissue sections and artifacts may be mistaken for leptospires (Quinn, *et al.*, 1994)

Immunofluorescence staining of leptospires is often preferable to silver staining of laboratory, environmental or clinical specimens because it is easier to see leptospires, especially in small numbers, and the serovars or serogroups can be determined presumptively. When a combination of antisera labeled with different fluorochromes is used, more than one serological type of leptospires can be identified in the same preparation. The shortcoming of

this procedure is the need for special fluorescent microscopy equipment and the requirement of specially prepared labeled antisera. A double layer or sandwich method is used with primary specific anti leptospiral antisera and a secondary universal fluorochrome labeled anti rabbit globulin serum (Meites *et al.*, 2004).

The infecting strains can often be isolated in culture that is obtained from samples of blood or cerebrospinal fluid in the early course of illness (leptospiremia) or from the urine during the later stage (leptospiruria). Blood culture of febrile patients has been used especially in Australia and New Zealand, and is recommended for routine diagnosis (Levett, 2003). It is particularly valuable in man, as the serological response can be slow and may be absent altogether if antibiotics are given early. Because serology is usually serogroup specific, isolation is essential to identify the infecting serotype (Venkatesha, 2006).

Serological tests can be used for prognosis and epidemiology, though there is no reaction until a few days after infection. However, the serological reactions persist for months or years once developed. Persistent antibodies allow retrospective diagnosis. The wide range of tests available are broadly divided into genus-specific and serogroup/serotype-specific tests.

Genus specific tests give positive result earlier in the course of illness and are best for a clinical diagnosis. The antigen for these tests is prepared from the non-pathogenic *L. biflexa* Patoc - 1 strain (Levett *et al.*, 2001). These tests are explained as follows;

Macroscopic agglutination test: A rapid macroscopic slide agglutination test can be used to screen human and animal serum samples (Venkatesha, 2006). These tests are carried out with a dense suspension of leptospires, which agglutinate into clumps visible to the naked eye. Strain Patoc 1 may be used in a macroscopic test; this simple test provides a rapid and reliable means of screening human sera for leptospiral-genus-specific antibodies. It allows a provisional diagnosis of acute leptospirosis to be made within a few minutes; it is not suitable for retrospective or survey work. Positive reactions, however, should be confirmed by complement fixation and microscopic agglutination tests (Abdollahpour, 2007).

Sensitized erythrocyte tests: Leptospiral extracts (lipopolysaccharides) and erythrocyte sensitising substance (ESS) are used to sensitize sheep and human red blood cells. Two sorts of reaction occur when ESS-sensitised cells are mixed with sera containing the homologous

antibodies: haemagglutination (HA) or sensitised erythrocyte agglutination (SEA), and hemolysis (HL) or sensitised erythrocyte lysis (SEL) (Abdollahpour, 2007).

Enzyme-linked immunosorbent assay (ELISA) test is now widely used as a genus specific screening test in man. Both peroxidase and urease labeled conjugates have been used satisfactorily. Stable reagents are available and form the basis of bedside tests, which are read visually. The use of computer assisted automated readers and the appropriate controls improves the reproducibility and predictive value of this test (Jagadishchandra *et al.*, 2003; Meites *et al.*, 2004).

Microcapsule agglutination test (MCAT) was developed for serodiagnosis of leptospirosis, based on the passive agglutination of synthetic polymer carriers, sensitized with mixed antigens of sonicated leptospires, by leptospiral antibody. It is simple and can be performed by relatively unskilled personnel with minimum laboratory facilities; it is also very stable and can be kept for long periods without critical storage requirements. However, it may not detect some serovars. In 1997, evaluation of MCAT indicated that the overall sensitivity and specificity of the test in comparison with MAT were 84.7 and 87.0%, respectively. This test also appeared to have a higher sensitivity than MAT during early stages of the disease (75% vs. 58.3%), though the specificity was less than that of MAT (83.3% vs 100%). The sensitivity of MCAT declined to 61%, 3 to 4 weeks after the onset of illness. Thus MCAT appeared to be a useful screening test for early diagnosis of leptospirosis (Abdollahpour, 2007).

Latex agglutination test depends on the sensitization of commercially available latex particles with a leptospiral antigen (Ramasubramaniam, 2000). Antiserum will react with the antigen to cause agglutination of the particles. Antigen prepared from *L. biflexa* serovar Patoc, strain Patoc 1 will cross-react with human convalescent sera to provide a useful screening procedure (Levett and Whittington; 1998, Health Center, 2000).

Lepto dipstick assay for the detection of leptospira-specific IgM antibodies in human sera was compared with ELISA test and evaluated in 1997. The sensitivity of the dipstick assay for sera collected between days 10 and 30 of the disease was 86.8% and that of IgM ELISA was 88.5%. The specificity of the dipstick assay was calculated to be 92.7% and that of IgM ELISA, 94.2%. The dipstick assay revealed cross reactivity with sera from patients with HIV, Hanta virus, *Toxoplasma* infection, Lyme borreliosis, malaria, meningococcal meningitis and

hepatitis A infection. In contrast, no cross reactivity was observed with these sera in IgM ELISA. The highly stable reagents and simple implementation makes this method suitable for use in clinical and field laboratories in tropical countries (Sehgal *et al.*, 1999; Jagadishchandra, *et al.*, 2003).

Microscopic agglutination (MAT), the confirmatory serologic test, is performed only in reference laboratories and requires both acute and convalescent specimens (Red Book, 2003). MAT is carried out with suspensions of living cultures or of cultures killed by the addition of neutralized formaldehyde. Preparations for MAT require meticulous culture of a collection of the strains used alive as antigen suspensions in the tests, their regular subculture and quality control for authenticity, purity agglutination and skilled educated personnel. A recent advance is the use of standardized preparations of dried leptospires available to accredited diagnostic laboratories from a central reference laboratory (Chaudry *et* al., 2002; Gumussoy *et al.*, 2009).

Serotype specific ELISA: Several attempts have been made to develop serotype specific ELISA tests with a variety of extracted antigens. Tests based on boiled whole cell antigens tend to be genus specific but those based on ultrasound-disintegrated or phenol-extracted preparations show considerable serotype specificity (Abdollahpour, 2007).

Molecular diagnosis of leptospirosis includes DNA restriction enzyme analysis (REA), nucleic acid probes and hybridization, polymerase chain reaction (PCR), pulsed field gel electrophoresis (PFGE) and ribotyping (Gumussoy *et al.*, 2009; Hussein *et al.*, 2009; Silva *et al.*, 2009).

Lepto Dri Dot is a new card agglutination test developed by the Dutch Royal Tropical Institute for the rapid diagnosis of leptospirosis. The test does not require special storage or sophisticated equipment and can be performed by relatively low skilled personnel (Vijayachari *et al.*, 2002).

Imaging studies have been included in diagnostic methods, applied in severe cases, where a patchy alveolar pattern may be revealed on lung radiography findings, corresponding to alveolar haemorrhage. Most radiographic changes occur in the periphery of the lower lobes (McKenzie and Shoff, 2008).

Electrocardiographic abnormalities are common during the leptospiremic phase of Weil's syndrome. In severe cases, congestive heart failure and cardiogenic shock may occur (McKenzie and Shoff, 2008).

Table 3. Differential features of the *Leptospira* species.

	Leptospira interrogans	*Leptospira biflexa*
Pathogenicity	+	-
Growth at 13 °C	-	+
Growth inhibited by 8-azaguanine (225μg/ml)	+	-
Conversion of cells to spherical forms by 1M NaCl	+	-

Source: Quinn *et al.* (1994).

2.1.6 Differential Diagnosis

The wide spectrum of clinical symptoms that characterize leptospirosis makes its diagnosis to be easily confused with other febrile diseases (Maria *et al.*, 2002; Romero *et al*, 2003), among which dengue fever (an insect-borne viral disease) has commonly been mentioned to be differentiated (Hernandez *et al.*, 2005; McKenzie and Shoff, 2008; Hussein and Nabi, 2009). Patients with leptospirosis report a slightly longer duration of fever than those with dengue. While most patients with dengue or leptospirosis have continuous fever, an intermittent fever has been said to be more likely with leptospirosis. Reports of rash are more common with dengue fever. Aside from fever and rash, the symptoms of patients with leptospirosis and dengue have been found to be similar: headache, myalgia, nausea, and vomiting are most common (LaRocque *et al.*, 2005). Other conditions to be ruled out include encephalitis, Hantavirus cardiopulmonary syndrome, hepatitis, malaria, meningitis, mononucleosis and typhoid fever (McKenzie and Shoff, 2008).

2.1.7 Postmortem Findings

Postmortem findings vary as much as the clinical signs of disease. In humans, pathological changes occur in the kidney parenchyma (Braunwald *et al.*, 2003). In domestic species, the leptospires cause hepatitis (cellular necrosis and separation of liver cord cells), encephalitis (inflammation of the brain) and nephritis (inflammation of the kidney). The most important

postmortem findings in bovine leptospirosis are anaemia, red urine and icterus (Radostits *et al.*, 1994). The kidneys are swollen with typical signs of red brown patches on the cortex. These red brown patches often are so prominent that they can be seen through the capsule. Enlarged liver covered with numerous small miliary foci of necrosis is common. In the acute course of leptospirosis, petechiae are found on the epicardium and the lymph nodes (Seifert, 1996). Petechiae in other organs are seen in fulminating cases, whereas in more prevalent *L. hardjo* infections, the lesions are primarily restricted to the kidneys (Aeillo, 1998).

2.1.8 Antigens and immune response

Leptospires have a complex antigenic structure. The somatic antigen is genus specific while the surface, a polysaccharide, is serovar specific. The outer membrane has been distinguished as a potent immunogen-lipopolysaccharide in nature, which is the major antigen and the target of antibody and complement-mediated bacterial activity. On the other hand, flagellar antigen is composed of genus and serotype specific antigens (Sambasiva *et al.*, 2003). The specific antibody response of the host can be inhibited by early antibiotic treatment and is difficult to evaluate in areas where leptospirosis is endemic and reinfection can occur (Romero *et al.*, 2003).

2.1.9 Zoonotic importance of leptospirosis

Leptospirosis is a global acute febrile zoonosis. In recent years, it has emerged as an important public health problem in large urban centres in developing countries, although traditionally it has always been considered a disease of farmers, as they are in close contact with domestic animals (Acha and Szyfres., 2001; Matsunaga *et al.*, 2003; Eymann *et al.*, 2007). Humans are accidental hosts of leptospirosis and usually become infected through contact with water or soil contaminated by the urine of infected animals. In urban places, rats and stray dogs predominate as the sources of epidemic human leptospirosis (Jansen *et al.*, 2007)

Generally, the disease is maintained in nature by chronic renal infection of carrier mammals, which excrete the organism in their urine (Table 1) (Levett *et al.*, 2005). It is a very serious disease characterized by a variety of signs and symptoms associated with multi-organ complications with a mortality rate that may exceed 15% (Sehgal *et al.*, 2000). Though the

organism can affect any organ of the body, the kidney and liver are most commonly involved (Ramasubramaniam, 2000). The incubation period varies from two days to four weeks. In the first phase of the illness, the infected person will preset with fever, chills, headache, muscle aches, vomiting and/or diarrhoea (symptoms also associated with other infections like malaria and Dengue fever). In the first phase the patient may recover, feeling well for two to three days before becoming ill again. During the more severe stage, phase two (Weil's Disease) meningitis, renal dysfunction, hepatic necrosis and pulmonary dysfunction are characteristic. Patients are only likely to develop full-blown Weil's Disease if contamination is high and treatment is delayed (Meites *et al.*, 2004).

The long term effect of leptospirosis is that recovery from the infection can be slow. People can have a chronic, fatigue-like illness that lasts for months. In others cases, persistent headache or depression can be observed. Occasionally the bacteria can persist in the eyes and cause chronic eye inflammation (Sambasiva *et al.*, 2003).

People at risk are those who have close contact with animals or who are exposed to water, mud, soil, or vegetation that has been contaminated with animal urine. Some occupations are at higher risk (farmers, veterinarians, abattoir workers, and sugar cane and banana farmers). Some recreational activities that involve contact with contaminated water or soil can also allow leptospirosis to be transmitted (camping, gardening, bushwalking, white water rafting, and other water sports) (Levett *et al.*, 2001). Rarely, leptospirosis can be transmitted from person to person (through sexual transmission, and transmission in breast milk). The organism can be transmitted in urine for months following infection (Johnson, 2003).

Leptospirosis is a classic example of a disease that requires close collaboration of human medical and veterinary medical communities in order to protect the public health. Veterinarians can best participate in this process by encouraging clients to adopt preventive measures (such as vaccination of cattle, sheep, and companion animals) and by taking every opportunity to increase physicians' awareness of leptospirosis as an important zoonosis. Symptoms in humans are influenza-like, high temperature, headache, muscular pain, lethargy and general fatigue. Good practice when handling cows at calving time requires the use of armlength gloves to minimize the potential infection from cows, newly born calves or after births (Silva *et al.*, 2009).

2.1.10 Socio-economic importance

Leptospirosis in domestic animals causes serious financial losses due to abortions, stillbirths, decrease in milk production and infertility, mainly in tropical and sub-tropical countries (Langoni *et al.*, 1999). The disease has been suggested to be of considerable economic significance to animal owners as it can result in high morbidity and mortality (Pal, 1996). Leptospirosis is especially common in low-income areas with poor sanitation and contaminated water (LaRocque *et al.*, 2005; Medical News, 2008).

2.1.11 Treatment

The treatment of all leptospiral infections has primarily been aimed at controlling the infection before irreparable damage to the liver and kidneys occurs. The secondary aim has been to control leptospiruria in carrier animals. The latter aim is effective if the treatment is given as soon as possible after signs appear (Abdollahpour, 2007).

Based on early diagnosis, leptospirosis can be treated successfully with a number of antibiotics especially tetracycline and tetracycline derivatives at a dose of 10mg/kg body weight which has to be applied for many days. Dehydrostreptomycin can be used at a dosage of 25mg/kg body weight (Radostits *et al.*, 1994; Levett *et al.*, 2001; Abdollahpour, 2007).

Medication of cattle feed with chlortetracycline at 400-800g/1000kg for 10 days reduces the number of carriers but does not necessarily eliminate all carriers (Radostits *et al.*, 1994). New stock brought onto clean premises must be held in isolation for two weeks and should be given a single parentral treatment with dihydrostreptomycin in order to eliminate possible carriers.

Blood transfusions (5-10 litters/450kg body weight) are indicated as treatment for the hemolytic anemia in acute leptospirosis in cattle (Radostits *et al.*, 1994; Abdollahpour, 2007). Human leptospirosis can be treated with different antibiotics such as streptomycin, penicillin, erythromycin, tetracycline and doxycycline (Hickey, 2006). Generally, early diagnosis and prompt treatment is important, since both forms of the disease (anicteric and icteric) begin in the same way.

2.1.12 Control and prevention

Control

Control is usually targeted at disease, but in reality what is reduced in most cases is the incidence of infection. The decline in the morbidity of the disease must be monitored and measured. The monitoring results must be continuously documented and reported to the responsible and management bodies such as health care and public health workers and the general population. The success of the control program depends on the support and participation of all participating teams (John, 2005). Generally, leptospirosis control should adhere to:

- A dynamic public health system;
- A functioning disease monitoring and surveillance;
- Preintervention data on disease incidence prevalence
- Appropriate level of adaptation and implementation of policies;
- Laboratory support;
- Scientific evaluation and information networking
- Immunization of animals (decreases the severity of disease but does not prevent leptospiruria, acting as reservoir hosts)
- Reservoir control programs in known endemic areas

Quantifying the presence and determining types of pathogenic *Leptospira* in environmental surface waters would also be an important tool for guiding leptospirosis control efforts in endemic regions.

Prevention

Protection of animals against reservoirs of the leptospires: Whenever leptospirosis is endemic under intensive production conditions, biosecurity to eliminate or to keep rats and other rodents away from the animals should be put in place. Susceptible animals should be separated from potential carriers of infection like dogs and pigs. Furthermore, the accessibility of the animals to potential sources of infection like contaminated water points should be restricted (Pal, 1996).

The provision of clean drinking water would be an effective of preventive measure. Protection of people against contagion by available means for example hygienic methods such as avoidance of direct and indirect human contact with animal urine are recommended as

preventive measures. Workers in flooded fields should be cautioned against direct contact with contaminated water or mud and should be advised to use rubber shoes and gloves. In case of any cuts or abrasion on the lower extremities of the body, the worker should apply an antiseptic ointment like betadine, before entering the field and after exit (Hickey, 2006; Abdollahpour. 2007). Recreational at risk people should avoid swimming in water where there is a possibility of contamination with animal urine (Pal, 2007) or the swimming pools should be disinfected with chlorine.

The main preventive measure for leptospirosis is to create awareness about the disease and its prevention by offering health education to the at risk groups. Various occupational groups should be aware about the source of infection, mode of transmission of leptospirosis and environmental hygiene. This has to be carried out by intensive educational campaigns (Pal, 2007; Ricaldi and Vinetz, 2007).

2.1.13 Future perspective

Leptospirosis is easily overlooked and relatively little has been known about it. Few studies on leptospirosis have been carried out. The lack of momentum in researching this disease may be attributed to the fact that leptospirosis is manifested by a wide variety of clinical presentations, that range from a mild flu-like illness to a serious and sometimes fatal disease (Levett *et al*., 2001). It also mimics many other diseases such as dengue fever and other viral haemorrhagic diseases, in addition to barriers to diagnostic capability in many developing countries (WHO, 2003; Yimer *et al*., 2004).

Funding for leptospirosis research and control efforts is currently haphazard, not organized and not effective for public health efforts, primarily because there are no concerted, ongoing international efforts to assess the impact of leptospirosis on animal and human health (Cachay and Vinetz, 2005). Therefore, both veterinary and medical professionals should combine efforts with the aim of increasing the understanding and creating awareness of the impact of leptospirosis on the health of human and animals.

3 MATERIALS AND METHODS

3.1 Study Area

The current study was undertaken in and around Wonji, East Shoa Zone, Oromia Regional State, Ethiopia. The Wonji-Shoa Sugar Factory and Sugar Estate were established in the 1950's and 1960's by the Dutch Company HVA, in the Wonji plain that lies at the downstream of the Koka Dam in the Awash river Basin. It is located in East Shoa Zone of the Oromia Regional State in the central Rift Valley of Ethiopia, 110km south east of Addis Ababa. It lies at an altitude between 1510 – 1560 meters above sea level. The total human population of the Wonji Shoa Estate including the surrounding villages and towns is estimated to be more than 52,000. Most of the residents of the town are employees of the sugar factory. The bimodal rain fall pattern of the area is about 850mm per year and the average temperature ranges between 17 – 30°C. The Awash river passes through the Wonji plain that is used as a source of irrigation and also water for gardening and washing (CSA, 2008).

3.2 Study Population

The present study focused on cattle and human in Wonji. Apparently healthy cattle that were kept under the smallholder production system were sampled based on accessibility of the sites and convenient situations. Blood samples of human patients attending the Wonji Hospital and a nearby Health Center were collected aseptically. Febrile patients manifesting one or more clinical signs such as fever, headache, abdominal pain, muscular pain, urinary tract infection and conjunctival suffusion were included.

3.3 Sample Size Determination

Patients visiting Wonji local Hospital and a nearby health center (named as seventh camp) were purposively sampled for interviewer administered questionnaire survey and serological study in such a way that as many patients as possible can be included (those who exhibited one or more signs and symptoms suggestive of leptospirosis were sampled).

Moreover, blood sera from bovine were collected based on convenient conditions (when animal owners were willing for sampling and cooperate to handle the animals during veinipuncture and when vaccination campaigns were in place, to follow the animal health

workers of the areas, who facilitated the sampling). Samples were collected so as to include as many individuals as possible.

3.4 Study Design

Cross sectional study design was conducted to assess the prevalence of leptospirosis in bovine and to determine seropositivity of human cases. Blood samples were collected from apparently healthy bovine kept under backyard animal production system in Wonji. Sera of human patients attending the Wonji local Hospital and a Health Center was also studied in collaboration with the respective staffs. Semi-structured questionnaire survey was conducted to gather information about exposure history of the patients to domestic animals or rodents, their water source, the way they manage their animals, and the presence of occupational hazard linked to cane farm. Moreover, the animal owners were interviewed to assess the husbandry condition of their animals, for example, communal grazing, watering points and herd dynamics.

3.5 Sampling Protocol

Serum sample was collected according to the method described by OIE, 2004/08?? Sterile non heparinized vaccutainer tubes with needles were used to collect blood samples from the jugular vein of the selected animals and transported to Addis Ababa University, Faculty of Veterinary Medicine Microbiology Laboratory. The samples were centrifuged to separate the sera from the whole blood, followed by storage under deep freezing temperature (-20°C), until the samples were subjected to serological test. The sera samples collected were accompanied by a record of possible information such as date of sampling, age, sex, herd size, and the likes.

3.6. Sample processing

Following standard procedure described by the manufacturer, the bovine sera samples were tested by *Leptospira interrogans* sv Hardjo antibody ELISA (DRG, Germany).

Patients, who visited Wonji Hospital and Seventh Camp Health Center, having history and clinical manifestations suggestive of leptospirosis were purposely selected for interview to assess the risk factors such as animal contact, area of residence, rat infestation, occupation

that may expose them to the disease and were also sampled for the serological study. Blood samples taken from human patients were centrifuged to collect sera, which were drawn in separate cryovials and kept in clean ice box containing ice packs. The samples were transported to the Microbiology Laboratory of the AAU, FVM, Debre Zeit and stored at deep freezing temperature (-20°C).

Leptospira IgM ELISA patoc 1, (DRG Instruments GmbH, Germany) test was conducted to detect the presence of antibodies against *Leptospira* serovars in human blood sera obtained from the study area according to the test procedure given by the manufacturer (DRG, Germany). *Leptospira biflexa* sv Patoc cross reacts with serovars of *Leptospira interrogans* and it is used as antigen in ELISA tests. This test is not serovar specific.

3.7 Leptospira Microwell Serum ELISA

3.7.1 Assay principle for bovine ELISA test

An antigen solution antobosy mixture was coated to the wells of the microtiter plate. After stabilization and drying, plates were vacuum sealed (DRG Instruments GmbH, Germany). Diluted serum samples were added to the wells. After incubation and appropriate washing a monoclonal anti-bovine conjugate was added and the plates were again incubated. After appropriate washing, substrate was added. Within several minutes, the color reaction was stopped and the plates were immediately read at optical density of 450nm (Appendix II). This test uses a monoclonal antibody which catches a specific *Leptospira interrogans* sv Hardjo sugar antigen.

3.7.2 Assay principle for human ELISA test

The ELISA test was performed according to steps and procedures given by the manufacture (Appendix II). The microwells were coated with purified Leptospira Patoc 1 antigen (DRG Instruments GmbH, Germany). During the first incubation with the diluted patients' sera, antibodies which were reactive with the antigen bound to the coated wells. After washing to remove the rest of the sample, the enzyme conjugate was added. When antibodies bound to the wells, the enzyme conjugate then bound to these antibodies. After another series of washes, a

chromogen (tetramethlybenzidine, or TMB) was added. Presence the enzyme conjugate was followed by the peroxidase catalysis reaction that consumed the peroxide and turned the chromogen from clear to blue. Addition of the stop solution ended the reaction and turned the blue color to a bright yellow color. The reaction was then read with an ELISA reader of 450nm optical density

3.8 Data Analysis and Management

Data entry was performed using Microsoft Office Excel 2007 and was analyzed using the SPSS release 15.0. Cross tabulation of the variables was employed for description part of the categorical variables and ANOVA for continuous variables. Statistical analysis of the association between risk factors (explanatory variables) and outcome variables (status variables) was done by Chi-square test and binary logistic regression (SPSS, release 15.0). In this study, the outcome variable was ELISA test result while animal contact, occupation, age, sex, type of shoes, irrigation and mice presence were explanatory variables (independent). P-values less than 0.05 were considered statistically significant.

4.7 Prevalence of Lpetospirosis in Bovine

A total of 204 blood sera of bovine were analyzed using ELISA test (*Leptospira interrogans* sv Hardjo antibody ELISA, DRG Instruments GmbH, Germany). Of the samples tested, 62 (30.4%) were positive for *Leptospira interrogans* sv Hardjo. Leptospirosis detection was seen to be 16.67% and 13.72% in males and females, respectively with no statistical difference between sexes (Table 9 and Fig. 5).

Table 4. Descriptive statistics for bovine leptospirosis prevalence using cross tabulation of variables

Factor		Proportion (%) within ELISA		p-value
		Negative	Positive	
Area	Bategermam	47(79.7%)	12(20.3%)	
	Kuriftu	62(62.6%)	37(37.4%)	
	Melkahida	33(71.7%)	13(28.3%)	0.074
Sex	Male	78(69.6%)	34(30.4%)	
	Female	64(69.6%)	28(30.4%)	0.990
Total		59.6%	30.4%	

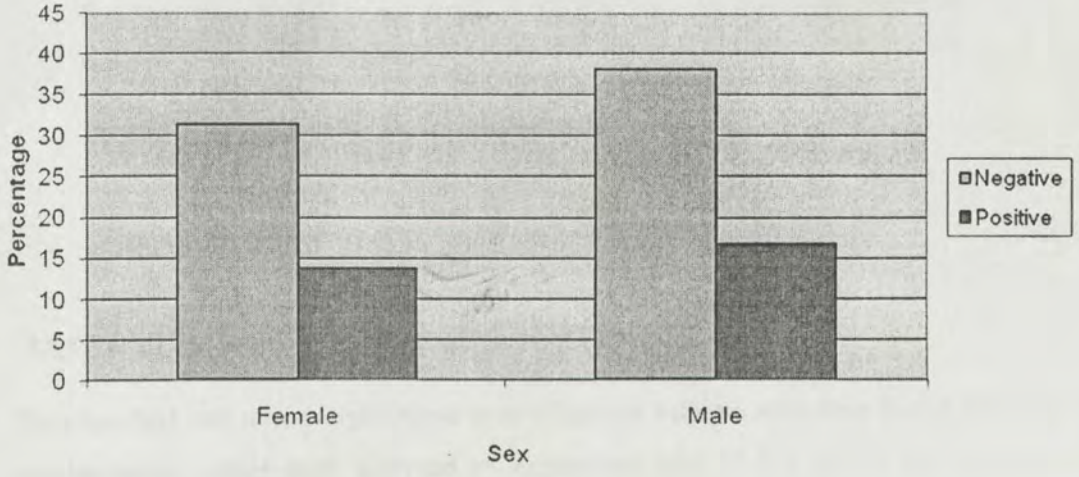


Figure 1. Leptospirosis prevalence in bovine in males and females

The samples were collected from three selected Kebeles in Wonji, where Kuriftu was more at risk (OR=2.34) of bovine leptospirosis infection ($p<0.05$) as compared to the remaining two Kebeles (Table10 and Fig. 6).

Table 5. Analysis of association by area and leptospirosis prevalence in bovine using binarylogistic regression model

Factor	Coeff	OR	p-value	95% CI for OR	
				Lower bound	Upper bound
batigemama			0.079		
Kuriftu*	0.849	2.337	0.027	1.100	4.965
melkahida	0.434	1.543	0.346	0.626	3.803
Constant	-1.365	0.255	0.000		

*$p<0.05$, Presence of association

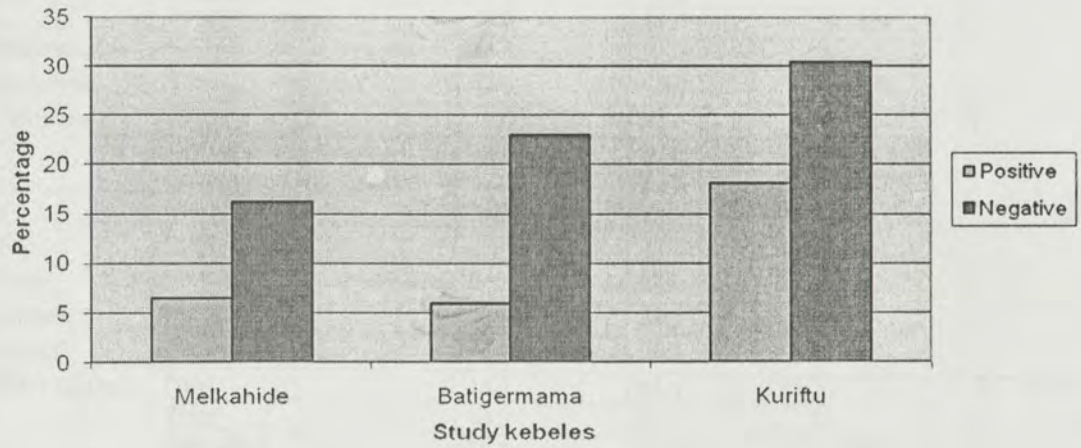


Figure 2. Seroprevalence of bovine leptospirosis in three Kebeles of Wonji

4.8 Overall Positivity Percentage of Human Leptospirosis

Two hundred and ninety eight blood sera of human patients attending Wonji Hospital and a nearby health center were analyzed by *Leptospira* IgM ELISA (DRG Instruments GmbH, Germany) test to detect antibodies against *Leptospira* organisms. Totally, 298 human cases were sampled, 216 of whom were positive. The summary of overall descriptive statistics

(cross tabulation) for the ELISA test result, observed clinical signs/symptoms and the analyzed risk factors is presented in Table 4.

Table 6. Descriptive statistics for categorical variables using cross tabulation of variables

Factor		Proportion (%) within ELISA		p-value
		negative	positive	
Fever	No	20.7	79.3	.106
	Yes	30.1	69.9	
Headache	No	23.7	76.3	.307
	Yes	29.4	70.6	
Urinary tract infection	No	24.7	75.3	.222
	Yes	31.1	68.9	
Abdominal pain	No	26.2	73.8	.451
	Yes	30.4	69.6	
Conjunctival suffusion	No	27.9	72.1	.772
	Yes	25.9	74.1	
Muscular pain	No	24.2	75.8	.082
	Yes	33.7	66.3	
Jaundice	No	27.8	72.2	.588
	Yes	20.0	80.0	
Previous illness	No	30.2	69.8	.412
	Yes	25.8	74.2	
Area*	Wonji	31.8	68.2	.019
	Seventh camp	19.0	81.0	
Irrigation for gardening	No	28.8	71.2	.560
	Yes	25.8	74.2	
Occupation*	House wife	19.0	81.0	.017
	Factory employee	32.9	67.1	
	Government employee	37.9	62.1	
Shoes	Closed	31.7	68.3	.314
	Open	25.8	74.2	
	Bare foot	0	100	
Animal contact*	No	36.8	63.2	.021
	Yes	23.7	76.3	
Water source	Pond	33.3	66.7	.354
	River	42.9	57.1	
	Pipe water	26.4	73.6	
Presence of mice	No	34.7	65.3	.116
	Yes	25.2	74.8	
Cane farm owner	No	29.1	70.9	.632
	Yes	26.5	73.5	
House for animals	No	27.6	72.4	.973
	Yes	27.4	72.6	
Sex	Male	24.1	75.9	.146
	Female	31.6	68.4	

* $p<0.05$, significant difference

Age was analyzed by one ANOVA to see if there is significant difference in ELISA test result. However, no statistical significance was observed ($p>0.05$). Seropositivity was

generally higher in those individuals with the age between 21 and 50 years, though there was no significant difference among different age groups (Table 5 and 7).

Table 7. Descriptive statistics for age and leptospirosis by cross tabulation

Number of samples		Mean	SE	95% CI for mean		Minimum	Maximum
				Lower bound	Upper bound		
Negative	82	33.78	1.178	31.44	36.12	4	60
Positive	216	33.94	0.681	32.60	35.29	7	65
Total	298	33.90	0.589	32.74	35.06	4	65

p=0.907

As indicated in Table 6 below, individuals between 30-39 years age group were most frequently infected by leptospirosis, followed by 20-29 and 40-49 respectively.

Table 8. Frequency of human leptospirosis positivity by different age group

Patients			
Age group	Number sampled	Positive	Percentage
1-9	5	4	80.0
10-19	9	5	55.6
20-29	85	62	72.9
30-39	109	82	75.2
40-49	68	47	69.1
50-59	17	13	76.5
60-69	5	3	60
Total	298	216	72.5

The factors supposed to be associated with occurrence of leptospirosis were found to be widely distributed throughout the study area. However, statistical analysis of the association between the variables shows that only animal contact, occupation and sampling sites were significantly associated ($p<0.05$) with ELISA positivity of human cases. The remaining factors such as age, sex, presence of mice in or around homes, type of shoes (closed, open, or barefooted), presence of irrigation for gardening, water source and sugarcane farm ownership, had no statistical significance ($p>0.05$) (Table 7).

Table 9. Analysis of association between leptospira seroreactivity and risk factors using binary logistic regression model

Factor		Regression coefficient	OR	p-value	95% CI for OR	
Area**	Wonji*	0.688	1.989	0.021	1.111	3.561
	Seventh camp					
	Constant	0.074	1.077	0.852		
Sex	Male	0.377	1.458	0.147	0.875	2.429
	Female*					
	Constant	1.149	3.154	0.000		
Occupation**	House wife*			0.019		
	Factory employee	-0.733	2.079	0.011	0.273	0.846
	Government employ	-0.954	2.597	0.032	0.161	0.921
	Constant	1.447	4.250	0.000		
Irrigation	No irrigation*					
	Irrigation	0.153	1.166	0.561	0.695	1.954
	Constant	0.904	2.469	0.000		
Shoes	Closed shoes*		1.780			
	Open shoes	0.289	1.336	0.283	0.787	2.266
	Bare foot	20.435	7E+008	0.999	0.000	
	Constant	0.768	2,156	0.000		
Animal contact**	No animal contact*					
	Animal contact	0.628	1.873	0.022	1.093	3.212
	Constant	0.542	1.719	0.015		
Water source	Pipe water*			0.420		
	River	-0.405	1.450	0.762	0.048	9.189
	Pond	0.140	1.150	0.799	0.391	3.380
	Constant	0.875	2.400	0.100		
Mice presence	Mice absence*					
	Mice presence	0.044	1.045	0.902	0.519	2.102
	Constant	0.932	2.538	0.004		
Cane farm ownership	No cane farm*					
	Cane farmer	0.016	1.016	0.955	0.594	1.737
	Constant	0.958	2.607	0.000		
Age (years)	1-9*			0.846		
	10-19	0.981	2.667	0.497	0.158	45.141
	20-29	-0.182	1.200	0.872	0.090	7.675
	30-39	0.586	1.797	0.535	0.282	11.453
	40-49	0.705	2.025	0.453	0.321	12.765
	50-59	0.950	1.492	0.674	0.232	9.601
	60-69	1.077	2.167	0.473	0.262	17.892
	constant	0.913	1.500	0.657		

**p<0.05, Presence of association, *Reference category

Of all tested cases, 211 (70.8%) had contact with domestic animals and the remaining 87 (29.2%) individuals did not. Animal contact was significantly associated ($p<0.05$) with ELISA test positivity of human cases (Table 7, Fig. 1).

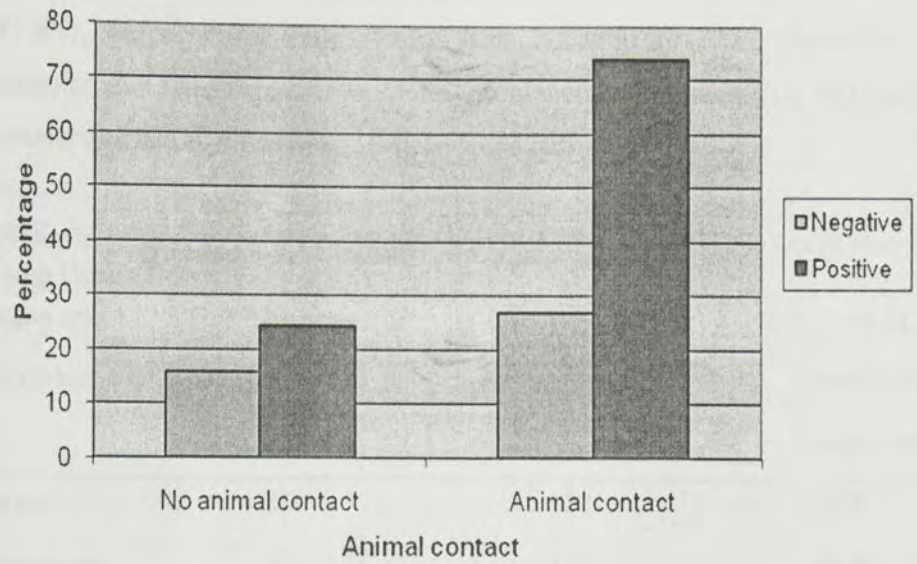


Figure 3. Seroreactivity of leptospirosis by animal contact in human cases

In the present study, occupation was categorized into housewives, sugarcane plantation and factory workers and government employees. The proportion of *Leptospira* IgM ELISA positivity was significantly higher in housewives ($P<0.05$) as compared to other occupational groups (Table 7, Fig.2).

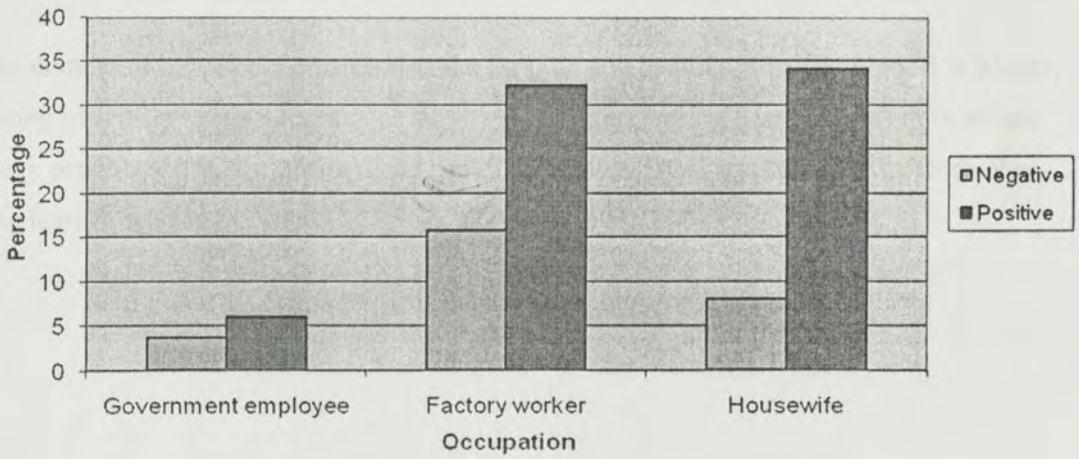


Figure 4. Leptospirosis positivity in different occupational groups

The overall frequency of signs and symptoms out of 298 leptospirosis cases included fever (72.5%), headache (67.4%), urinary tract infection (44.3%), abdominal pain (30.9%), muscular pain (34.9%), jaundice (3.4%), conjunctival suffusion (18.1%) and edema (5%) as summarized in Table 8 below.

Table 10. Frequency by signs/symptoms of leptospirosis patients, Wonji Hospital & Seventh Camp Health Center

Signs and symptoms	Number of patients	Positives	% of ELISA positivity within sign/symptom
Fever	216	151	69.9
Headache	201	142	70.6
UTI	132	91	68.9
Abdominal pain	92	64	69.6
Muscular pain	104	69	66.3
Jaundice	10	8	80.0
Conjuctival suffusion	54	40	74.1
Edema	15	12	80.0

The number of human patients visiting the hospital and health center was highest in March, during which short rain occurred, as shown in the figure below. The next month in which many patients visited the hospital and health center was February, followed by December. The least frequency occurred in January as shown in Fig. 5 below.

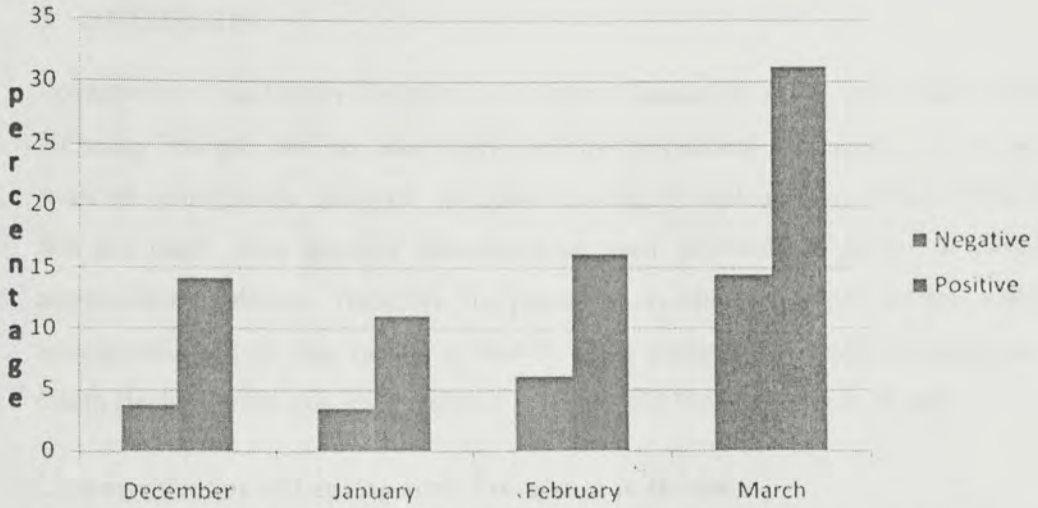


Figure 5. Proportion of seroreactivity of leptospirosis by months in human cases

The proportion of ELISA positivity was significantly different ($p<0.05$) between patients attending Wonji Hospital and Seventh Camp Health Center, as shown in Table 6 and Fig. 4.

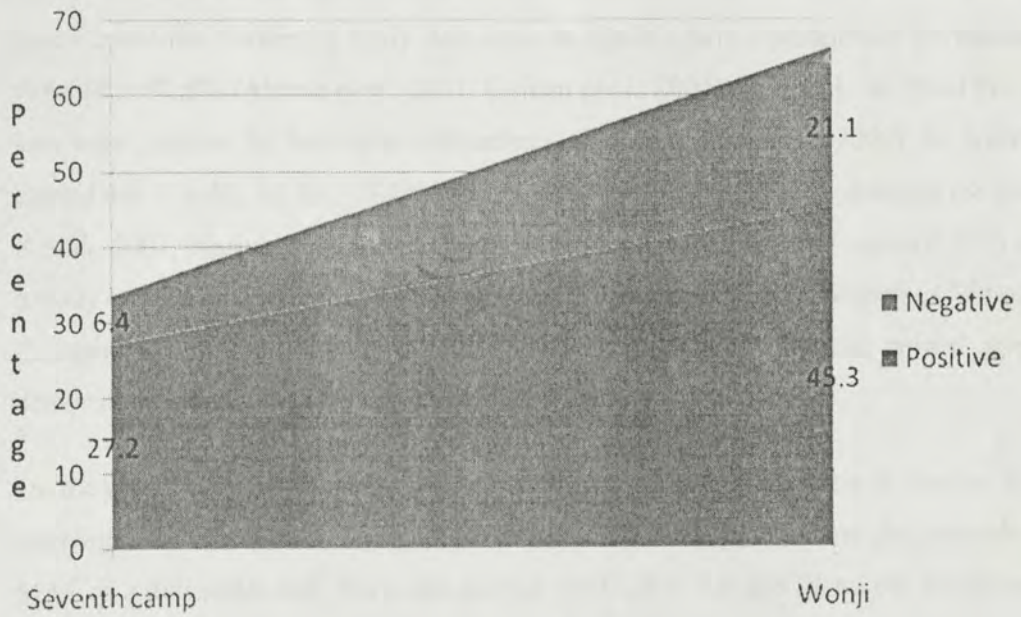


Figure 6. Seroreactivity of human leptospirosis in Wonji Hospital and Seventh Camp

The odds of Seventh Camp is nearly twice that of Wonji, while the reverse was true as to sample sizes taken from these areas (Table 9 and Fig. 6).

5 DISCUSSION

Leptospirosis has largely become a forgotten disease in many countries (WHO, 2009), including Ethiopia, and has been overlooked by the medical community. Since the extensive work on pathogenesis, diagnosis, and epidemiology of leptospirosis of the 1940s and 1950s, this and many other zoonotic diseases have been neglected in favour of pursuing more newsworthy conditions. Therefore, the present study attempts to address the contribution of leptospirosis and its risk factors to human cases visiting the Wonji Hospital and Seventh Camp Health Center, and its prevalence in apparently healthy cattle in Wonji.

5.7 Determination of Leptospirosis Prevalence in Bovine

In the present study, ELISA test was conducted to measure the prevalence of *Leptospira interrogans* sv Hardjo in bovine from Wonji area. Out of 204 sera collected, the prevalence of leptospirosis was found to be 30.4% in apparently healthy cattle. Maria and colleagues (2002) have also reported 43% prevalence of *Leptospira interrogans* sv Hardjo in bovine in Mexico. The seroprevalence of leptospirosis in cattle and dominant serotypes have been shown in many countries. Screening study was done in Spain where leptospirosis prevalence ranged from 18.3- 42.8% (Alonso *et al.*, 2001; Guitian *et al.*, 2001). In Brazil, 46.9% of the examined sera were positive for leptospira antibodies (Lilenbaum and Sauza, 2003). In several studies carried out in India, 15.8%, 18.44% and 21.18% of prevalences were detected (Srivastava and Kumar, 2003; Mariya *et al.*, 2006). On the other hand, Moch and colleagues (1975) conducted a study on the seroprevalence of leptospirosis in domestic and wild animals in Western part of Ethiopia, where the wide occurrence of leptospirosis in different animal species was demonstrated, including 70.7% prevalence in cattle.

On the other hand, in the seroprevalence study of bovine leptospirosis in Turkey, *Leptospira interrogans* sv Hardjo was as high as 63% (out of 500 samples), where the researcher utilized MAT as a diagnostic test. They also carried out ELISA test and found out 26.3% prevalence (Gumussoy *et al.*, 2009). Elles and Thiermann (1999) isolated *Leptospira interrogans* sv Hardjo from the genital tract of 5 out of 11 non pregnant cows which were randomly choosen. The antibodies against leptospires have also been reported in 62.8% of bovine sera in Mexico (Segura-Correa, *et al.*, 2003). These researchers reported that no difference was observed in sex related seroprevalence which is consistent with our findings, where the frequency of ELISA positivity was almost the same both in males and females. However, statistical analysis of leptospirosis prevalence by the sampling sites indicated that one of the three

selected kebeles, showed significantly higher seroprevalence ($p<0.05$) of *Leptospira interrogans* sv Hardjo in bovine (Table 9). This may partly be owed to relatively larger sample size obtained from the Kebele. Regarding age, all of the animals sampled in the current study were adults (the exact age was not known as most owners responded that they frequently sale or purchase adult animals and did not guess the ages).

Detection of *Leptospira interrogans* sv Hardjo in the current study may be related to introduction of animals from other areas of the country. It has been said that this serovar is transmitted when new animals are introduced into a herd. This suggests wide spread of the pathogen wherever the animals move. It may also result from natural disasters (such as forest fire, flood), followed by the migration of wild animals into new areas, creating a new epizootiological environment for transmission of new leptospira serovars.

The result of our study suggests that *Leptospira interrogans* serovar Hardjo can be an important cause of human leptospirosis in Wonji, where our investigation found out 72.5% positivity percentage of leptospirosis in human patients. Similar to our research, Eymann *et al* (2007) have indicated that *Leptospira interrogans* serovar Hardjo was the predominant serovar infecting humans in Australia. In Brazil, 379 cows were examined and 46.9% were positive for leptospirosis, where serovar Hardjo was mainly detected (Lilenbaum and Souza, 2003).

The animal owners were interviewed to assess the management conditions they use for their animals. They replied that all the management conditions are similar (extensive) in the sampled areas.

5.8 Assessment of Positivity of Leptospirosis in Human Cases

A cross sectional study was underway to test blood sera of febrile patients for the presence of antibodies against *Leptospira* serovars in the blood sera of febrile patients. Leptospira IgM ELISA patoc 1 test was utilized to detect antibodies raised to Leptospira serovars. It has been known that IgM antibodies appear first in the early stages of infection and/or illness. However, these antibodies do not persist as long as IgG antibody titres. Therefore, the presence of IgM antibodies is considered as a marker of illness when it is associated to clinical signs and symptoms presented by the patient (Maria *et al*., 2002).

Febrile patients attending Wonji Hospital and seventh camp health center are diagnosed for malaria and typhoid commonly. However, the prevalence of such diseases has been reduced by control and prevention measures taken (personal communication). In most cases, patients become negative for blood film microscopy (malaria) and serological/agglutination test (typhoid), where they are treated by broad-spectrum antibiotics the problem being "fever of unknown origin (FUO)". The physicians working in Wonji hospital explained that the patients well respond to the antibiotic treatment, which supports suspicion of the cases to be related to other bacterial infection.

Out of 298 blood sera screened from patients, 216 (72.5%) were found to be positive for leptospirosis. Similarly, other researchers have conducted a pilot study on blood samples of 59 patients by Lepto Dri dot test in the same area (Yimer *et al.*, 2004). They also found high prevalence (47.5%) of leptospirosis. When comparing the last report of leptospirosis with that of the present study, increased seropositivity is seen that may be attributable to differences in sample size and diagnostic techniques and there may also be increment owed to the dynamics of animal movement in Wonji. Similarly, Koteeswaran (2006) conducted serological study on sera collected from human cases received between 1997 and 2006, where overall 57.5% seropositivity was recorded (highest proportion of positivity being 78.7% during 1998 to 1999 and lowest, 32.8% during 2002 to 2003). On the other hand, Jagadishchandra *et al*, (2003) undertook IgM ELISA test on 733 suspected human patients and reported 11.5% seroprevalence, which is lower than our findings.

Attempt was also made to assess risk factors that may be associated with occurrence of leptospirosis in humans (Table 4). Many of the supposed factors associated with leptospirosis cases commonly occur in Wonji. However, there was no statistically significant association between some of these factors and ELISA positivity of human cases (Table 6).

Animal contact was one of the risk factors associated with the overall seropositivity of the patients with OR value of 1.88 (Table 6). Among the different species owned by the respondents to our questionnaires, bovine were most common. These species harbour *Leptospira interrogans* sv Hardjo which is pathogenic to humans. In this research 30.4% prevalence of this pathogen has been found from blood samples of bovine.

Higher detection of leptospirosis in housewives, whose behaviours or activities involving contact with domestic animals were presumed to play an important role (probably, due to the

fact that they have more contact with animals as they dispose the waste materials including urine which may contain the leptospires. They also milk lactating cows which increases their chance to contract the infection from infected animals).

One of the factors found to have significant association with ELISA positivity in the present study was the sampling sites (Wonji Hospital and Seventh Camp Health Center). These areas are not far away from each other and have similar natural environment, except that the response of most residents of Seventh Camp indicated having animal contact, irrigation access and sugarcane ownership more frequently than residents in Wonji. Similarly, a study on risk factors associated with leptospirosis was undertaken in Barbados, where gardening and presence of pets around homes were significant factors with OR of 4.57 and 7.82, at 95% significance level, respectively (Douglin *et al.*, 1997).

A study conducted in Mexico has demonstrated that largest number of cases was identified from rural areas, where the predominant cultural characteristics of the region such as close co-existence of domestic animals and people and domestic hygiene conditions that attract rodents play an important role in creating propitious environment for transmission of leptospirosis (Ignacio *et al.*, 2002). This condition is also true in the case of Wonji, where animals and their owners live in close contact and may also share same housing.

With respect to leptospirosis distribution and the relationship of age and sex, significant differences were not seen. As summarized in Table 6, most of the patients were in the age category of 20-50 years of age (66.4%), irrespective of statistical insignificance ($p>0.05$). Individuals between 30-39 years of age group were most frequently infected by leptospirosis, followed by 20-29 and 40-49 respectively. This finding is comparable with the report of Meites and colleagues (2004) in California, where the mean seropositivity of leptospirosis was 67% in patients 20 to 40 years old. Romero *et al.*, (2003) have also demonstrated that leptospirosis occurs in all people of all age groups, although middle-aged adults were most frequently infected, in which case, 32.4% of the cases were adults from 20-39 years of age. The finding of a Jamaican researcher (Grant, 1988), that no significances were observed in sex-related seroprevalence is consistent with the result of the present study.

Mexican researchers have indicated that human infection by leptospirosis occurs at early ages facilitated by the pattern of work and recreation (Maria *et al.*, 2008). In agreement with this idea, Wonji residents work in and around sugarcane plantation areas where irrigation

(flooding) is commonly practiced. They also swim in settled water that is collected from irrigated water (personal observation).

We collected the patients' blood samples from December to March, 2009. The leptospirosis ELISA positivity was most frequently observed in March, during which relatively many patients visited the hospital or health center (Fig. 3). This may be due to the fact that short rain took place in March.

In the present study, clinical signs and symptoms considered as suggestive of leptospirosis were fever (72.5%), headache (67.4%), urinary tract infection (44.3%), abdominal pain (30.9%), muscular pain (34.9%), jaundice (3.4%), conjuctival suffusion (18.1%) and edema (5%) (Table 8). In agreement with our study, leptospirosis has been reported from human cases exhibiting fever, headache, myalgia, hemorrhages, jaundice, abdominal pain and also other non specific clinical signs (Silva *et al.*, 2009). In Jamaica, the occurrence of leptospirosis in humans and animals was studied and the highest yearly seroprevalence rate (46%) of leptospirosis was recorded, being high both in humans and animals (Grant, 1988). On the other hand, five leptospirosis cases in California exhibited high fever with uniform renal impairment, mild hepatitis, headache, nausia and myalgia. All of these cases were treated with doxycycline and made complete recoveries after variable levels of severity in the courses of their illness (Meites *et al.*, 2004).

Other risk factors included for statistical analysis were presence of mice in or around homes, type of shoes (closed, open, or barefooted), presence of irrigation for gardening, water source and sugarcane farm ownership. These factors had no statistical significance ($p<0.05$). Though the presence of mice was expected to be correlated with leptospirosis positivity, statistical analysis shows no significance.

6. CONCLUSIONS AND RECOMMENDATIONS

The occurrence of leptospirosis in humans and animals in Wonji is very high. The risk factors predisposing to *Leptospira* infection are also widely distributed. However, leptospirosis is extremely under diagnosed disease in Wonji, where febrile conditions other than commonly occurring bacterial and protozoal diseases (such as typhoid and malaria) are reported as fever of unknown origin. Humans are accidental hosts whereas animals are natural reservoirs of the infection. The serological tests such as ELISA are indicative of the disease occurrence in combination with its signs and symptoms. Leptospirosis is a classic example of a disease that requires combined effort among medical and veterinary professionals in order to protect the public health. Therefore, veterinarians can best participate in this process by encouraging clients to adopt preventive measures. Based on this fact, the following points are forwarded as recommendations:

- Since leptospirosis is a zoonotic disease and its presence in animals is wide spread, there should be a high index of suspicion for this treatable disease.
- Public awareness on the transmission routes, health and economic importance of the disease should be implemented in Wonji in particular and in other similar areas that may support the maintenance of leptospirosis.
- Awareness should also be created to at risk groups such as housewives who are exposed to direct contact with domestic animals, in which case cleaning waste materials and milking cows may pose them to risk of leptospirosis infection.
- People working in sugarcane plantation areas should be provided with protective wears such as plastic gloves and gumboots.
- Veterinarians and physicians should make collaborative efforts on the effective control and prevention of leptospirosis.
- Ultimately, sustainable surveillance for leptospirosis is needed to determine the extent of the disease and this should increase understanding of the evolving epidemiology of leptospirosis.

7. REFERENCES

Abdollahpour, G. (2007): A Review on Leptospirosis, Leptospira Research Laboratory, University of Tehran, Iran: Pp.1-49.

Acha, P. N. and Szyfres, B. (2001): Zoonoses and Communicable Diseases Common to Man and Animals, 3rd Edition, Pan American Health Organization, Washington DC, Pp. 157-168.

Aguiar, D. M., Gennari, S. M., Cavalcante, G. T., Labruna, M. B., Vasconcellos, S. A., Rodrigues, A. A. R., Moraes, Z. M. and Camargo, L. M. A. (2006): Seroprevalence of *Leptospira* species in cattle from Monte Negro Municipality, *Western Amazon, Pesq. Vet. Bras.*, **26**: 102-104.

Aiello, S. E. and Mays, A. (eds.) (1998): Leptospirosis. **In**: The *Merck Veterinary Manual* 8th Edition, Co., Inc., Whitehouse Station, U. S. A. Pp. 474-477.

Alberta Health Services. (2005): Public Health Notifiable Disease Management Guidelines – Leptospirosis, www.albertahealthservices.ca.

Alonso-Andicobererry, C., Pena, F. J. G., Bueno, J. P., Costas, E. and Mora, M. L. O. (2001): Herd level risk factors associated with *Leptospira* species seroprevalence in dairy and beef cattle in spain, *Preventive Veterinary Medicine*, **52**: 109-117.

Baron, E. J., Peterson, L. R. and Finegold, S. M. (1994): Diagnostic Microbiology, 9th Edition, J.B. Lippincott Company, Philadelphia Pp. 449.

Beers, M. H., Fletcher, J. A., Jones, T. V., Porter, R., Berkwits, M. and Kaplan,K. L. (2003): The Merck Manual of Medical Information, 2nd Edition, Merck and Co., Inc. New York, Pp. 998-999.

Bovet, P., Yersin, C., Merien, F., Davis, C. E. and Perolat, P. (1999): Factors associated with clinical leptospirosis: a population based case control study in the Seychelles (Indian Ocean), *International Journal of Epidemiology*, **28**: 583-590.

Braunwald, E., Fauci, A. S., Kasper, D. L., Hauser, S. L., Longo, D. L. and Jameson, J. L. (2003): Harrison's Principles of Internal Medicine, 15th Edition, Vol 1, Pp.1055-1058

Burriel, A. R., Varoudis, L., Alexopoulos, Diplomate ECAR; Kritas, S. and Kyriakis, S. C. (2003) : Serological evidence of *Brucella* species and *Leptospira interrogans* serovars in Greek swine herds, *Journal of Swine Health and Production*, **11**: 186-189.

Cachay, E. R. and Vinetz, J. M. (2005): A Global Research Agenda for Leptospirosis, School of Medicine, University of California symposium, **51**: 174-178.

Center for Disease Control and Prevention. (2009): *Leptospira noguchii* and human and animal leptospirosis, Southern Brazil, *Emerging Infectious Diseases*, **15**, eideditor@cdc.gov

Central Statistical Authority (2008): Federal Democratic Republic of Ethiopia, Sample Survey 2007/08, Vol. 2, Report on Livestock and Livestock Characteristics, Private Peasant Holding, Statistical Bulettin, No 417, Addis Ababa, Ethiopia.

Chaudhry, R., Premlatha, M. M., Mahonty, S., Dhawan, B., Singh, K. K. and Dey, A. B. (2002). Emerging leptospirosis, North India, *Emerging Infectious Diseases*, **18**, http://www.cdc.gov/ncidod/EID

Cullen, P. A., Haake, D. A, Bulach, D. M., Zuerner, R. L. and Adler,B. (2003): LipL21 is a novel surface-exposed lipoprotein of pathogenic *Leptospira* species, *Infection and Immunity*, **71**: 2414-2421.

Diagnostic Automation, Inc. (2001): Leptospira IgM Microwell Serum ELISA, *www.rapidtest.com*

Douglin, C. P., Jordan, C., Rock, R., Hurley, A., and Levett, P. N. (1997): Risk factors to sever leptospirosis in the Parish of St. Andrew, Barbados, *Emerging Infectious Diseases*, **3**: 1-3

Ellis, W. A. and Thiermann, A. B. (1999): Isolation of leptospirosis from the genital tract of Lowa cows, *American Journal of Tropical Medicine and Hygiene*, **61**: 399-404.

Esen, S., Sunbul, M., Leblebicioglu, H., Eroglu, C. and Turan, D. (2004): Impact of clinical and laboratory findings on prognosis in leptospirosis, *Swiss Medical Weekly*, **134**: 347-352.

Eymann, J., Smythe, L. D., Symonds, M. L., Dohnt, M. F., Barnett, L. J., Cooper, D. W. And Herber, C. A. (2007): Leptospirosis serology in common brushtail possum (*Trichosurus vulpecula*) from urban Sydney, Australia, *Journal of Wild Life Diseases*, **43**: 492-497

Feresu, S. B., Bolin, C. A. and Korver, H. (1998): A New *Leptospira* serovar, *ngavi*, in the Tarassovi serogroup isolated from Zimbabwe oxen. *International Journal of SystematicBacteriology*, **48**: 207-213.

Gracey, J. F., Collins, D. S. and Huey, R. J. (1999): Meat Hygiene, 10th edition, Vol. 2, W.B. Sounders Company LTD, London, Pp. 243-244.

Grant, G. H. (1988): A Sero-epidemiological Survey of Leptospirosis Among Jamaican Human and Animal Populations, Kingston maps, 10 :76.

Guitian, F. J., Pena, F. J. G, Oliveira, J., Sanjuan, M. L. and Yus, E. (2001): Serological study of the frequency of leptospiral infectons among dairy cows in farms with suboptimal reproductive efficiency in Galicia, Spain, *Veterinary Microbiology*, **80**: 275-284.

Gumussoy, K. S., Ozdemir, V., Aydin, F., Aslan, O., Atabek, E., Ica, T., Dogan, O., Dumaz, Z. and Ozturk, A. (2009): Seroprevalence of bovine leptospirosis in kayseri, Turkey and detection of leptospires by polymerase chain reaction, *Journal of Animal and Veterinary Advances*, **8**: 1222-1229.

Haake, D. A. (2006): Molecular epidemiology of leptospirosis in the Amazon. PLoS Med **3**: e302. doi:10.1371/journal.pmed.0030302

Hernandez, M. G., Diaz, J. V. P., Garcia, S. G., Liorens, G. B. and Pena, F. J. G. (2005): Comparison of the prevalence of the infection by *Leptospira* Species, *Leishmania infantum* and *Ehrlichia canis* in dogs in the Comunidad Valenciana, Spain, *Epidemiol. et Sante Anim.*, **45**: 83-86.

Hickey, P.W. (2006): Leptospirosis, *e*Medicine, www.emedicinehealth.com.

Hirsh, D. C. and Zee, Y. C. (1999): Veterinary Microbiology, Blackwell Science ltd, Pp. 185-189.

Hussein, M. F. and Nabi, A. R. G. (2009): Serological evidence of leptospirosis in camels in Saudi Arabia, Journal of Animal and Veterinary Advances, **8**: 1010-1012

Ignacio V. S., María F. Marrufo, C., Bertha, J. D., Alejandro A. L., Hugo, L. M., Víctor, S. Z. & Jorge, E. Z.V. (2002): Clinical-epidemiological study of leptospirosis in humans and reservoirs in Yucatan, Mexico, *Review of Institute of Tropical Medicine, Sao Paulo* **44**:335-340.

Jagadishchandra, K., Prathb, A. G. and Rao, S. P. (2003): Clinical and epidemiological correlation of leptospirosis among patients attending KMCH, Manipal, *Indian Journal of Medical Sciences*, 57: 101-104.

Jansen, A., Luge, E., Guerra, B., Wittschen, P., Gruber, D., Loddenkemper, C., Schneider, T., Lierz, M., Ehlert, D., Appel, B., Stark, K. and Nocklert, K. (2007): Leptospirosis in urban wild boars, Berlin, Germany, *Emerging Infectious Disease*, www.cdc.gov./eid, **13**: 739-742.

John, T. J. (2005): The Prevention and Control of Human Leptospirosis. The Kerala State Institute of Virology and Infectious Diseases, Alappuzha, Kerala, India Symposium, **51**: 205-209.

Johnson, R.C. (2003): Leptospira, Medmicro.

Koteeswaran, A. (2006): seroprevalence of leptospirosis in man and animals in Tamilnadu, Indian Journal of Medical icrobiology, **24**: 329-331.

Krawczyk, M. (2006): Serological evidence of leptospirosis in animals in Northern Poland, *Veterinary Record* **39**: 87-100.

Langoni, H., Sauza, L. C., Silva, A.V., Luvizotto, M. C. R., Paes, A. C. and Luchies, S. B. (1999): Incidence of Leptospiral abortion in Brazilian dairy cattle, *Preventive Veterinary Medicine*, **40**: 271-275.

LaRocque, R. C., Breiman, R. F., Ari, M. D., Morey, R. E., Janan, F. A., Hayes, J. M., Hossain, M. A., Brooks, W. A., and Levett, P. N. (2005): Leptospirosis During Dengue outbreak, Bangladesh, Emerging Infectious Diseases: www.findarticles.com

Levett, P. N. (2003): *Leptospira* and *Leptonema* In: Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, pp 929-936.

Levett, P. N. and Whittington, C. U. (1998): Evaluation of the indirect hemagglutination assay for the diagnosis of acute leptospirosis, *Journal of Clinical Microbiology*, **36**: 11-14.

Levett, P. N., Branch, S.L., Whittington, C. U., Edwards, C. N. and Paxton, H. (2001): Two methods for rapid serological diagnosis of acute leptospirosis, *Journal of Medical Microbiology*, **8**: 349-351.

Levett, P. N., Morey, R. E., Galloway, R. L., Turner, D. E., Steigerwalt, A. G. and Mayer, L. W. (2005): Detection of pathogenic leptospires by real-time quantitative PCR, *Journal of Medical Microbiology*, **54**: 45-49.

Lilenbaum, W. and Sauza, G. N. (2003): Factors associated with bovine leptospirosis in Rio de Janeiro, Brazil, *Research and Veterinary Science*, **75**: 249-251.

Lopes, A. A., Costa, E., Costa, Y. A., Sacramento, E., Ribeiro, A. R., Jonior, O., Lopes, M. B. and Lopes, G. B. (2004): Comparative study of the in-hospital case-fatality rate of leptospirosis between pediatric and adult patients of different age groups, *Rev. Inst. Med. Trop. S. Paulo,* **46** (1): 19-24.

Louvel, H., Bommezzadri, S., Zidane, N., Boursaux, C., Creno, S., Magnier, A., Rouy, Z., Medigue, C., Girons, I. S., Bouchier, C. and Picardeau, M. (2006): Leptospirosis, *Journal of Bacteriology*, **188**: 7893 – 7904.

Maria, L. A., Valverde, J., Bernal, L., Lizeth, T. And Kirsten, V. (2002): Development of a Lepto-IgM EIACR test to diagnose leptospirosis in Costa Rican patient samples, *Investigacion Clinica*, **48**: 1-3.

Mariya, R., Chaudhary, P., Kumar, A. A., Thangapandian, E., Amutha, R. and Srivastava, S. K. (2006): Evaluation of recombinant LipL41 antigen of *Leptospira interrogans*

Koteeswaran, A. (2006): seroprevalence of leptospirosis in man and animals in Tamilnadu, Indian Journal of Medical icrobiology, **24**: 329-331.

Krawczyk, M. (2006): Serological evidence of leptospirosis in animals in Northern Poland, *Veterinary Record* **39**: 87-100.

Langoni, H., Sauza, L. C., Silva, A.V., Luvizotto, M. C. R., Paes, A. C. and Luchies, S. B. (1999): Incidence of Leptospiral abortion in Brazilian dairy cattle, *Preventive Veterinary Medicine*, **40**: 271-275.

LaRocque, R. C., Breiman, R. F., Ari, M. D., Morey, R. E., Janan, F. A., Hayes, J. M., Hossain, M. A., Brooks, W. A., and Levett, P. N. (2005): Leptospirosis During Dengue outbreak, Bangladesh, Emerging Infectious Diseases: www.findarticles.com

Levett, P. N. (2003): *Leptospira* and *Leptonema* In: Manual of Clinical Microbiology, American Society for Microbiology, Washington, DC, pp 929-936.

Levett, P. N. and Whittington, C. U. (1998): Evaluation of the indirect hemagglutination assay for the diagnosis of acute leptospirosis, *Journal of Clinical Microbiology*, **36**: 11-14.

Levett, P. N., Branch, S.L., Whittington, C. U., Edwards, C. N. and Paxton, H. (2001): Two methods for rapid serological diagnosis of acute leptospirosis, *Journal of Medical Microbiology*, **8**: 349-351.

Levett, P. N., Morey, R. E., Galloway, R. L., Turner, D. E., Steigerwalt, A. G. and Mayer, L. W. (2005): Detection of pathogenic leptospires by real-time quantitative PCR, *Journal of Medical Microbiology*, **54**: 45-49.

Lilenbaum, W. and Sauza, G. N (2003): Factors associated with bovine leptospirosis in Rio de Janeiro, Brazil, *Research and Veterinary Science*, **75**: 249-251.

Lopes, A. A., Costa, E., Costa, Y. A., Sacramento, E., Ribeiro, A. R., Jonior, O., Lopes, M. B. and Lopes, G. B. (2004): Comparative study of the in-hospital case-fatality rate of leptospirosis between pediatric and adult patients of different age groups, *Rev. Inst. Med. Trop. S. Paulo,* **46** (1): 19-24.

Louvel, H., Bommezzadri, S., Zidane, N., Boursaux, C., Creno, S., Magnier, A., Rouy, Z., Medigue, C., Girons, I. S., Bouchier, C. and Picardeau, M. (2006): Leptospirosis, *Journal of Bacteriology*, **188**: 7893 – 7904.

Maria, L. A., Valverde, J., Bernal, L., Lizeth, T. And Kirsten, V. (2002): Development of a Lepto-IgM ElACR test to diagnose leptospirosis in Costa Rican patient samples. *Investigacion Clinica*, **48**: 1-3.

Mariya, R., Chaudhary, P., Kumar, A. A., Thangapandian, E., Amutha, R. and Srivastava, S. K. (2006): Evaluation of recombinant LipL41 antigen of *Leptospira interrogans*

serovar Canicola in ELISA for serodiagnosis of bovine leptospirosis, *Comparative Microbiology and Immunology of Infectious Diseases*, **29**: 269-277.

Matsunaga, J., Barocchi, M. A., Croda, J., Young, T. A., Sanchez, Y., Siqueira, I., Bolin, C. A., Reis, M. G., Riley, L. W., Haake, D. A. and Ko, A. I. (2003): Pathogenic *Leptospira* species express surface-exposed proteins belonging to the bacterial immunoglobulin, *Superfamily, Mol Microbiol*. **49**: 929-945.

McKenzie, J. G. and Shoff, W. H. (2006??): Leptospirosis in humans. *E*Medicine, www.emedicinehealth.com

Medical News Today. (2008): New Species Found that Causes Leptospirosis, pressrelease@medicalnewstoday.com

Meites, E., Jay, M. T., Deresiniski, S., Shieh, W. J., Zaki, S. R., Tompkins, L. and Smith, D. S. (2004): Reemerging Leptospirosis, California, *Emerging Infectious Disease*, http://www.cdc.gov/ncidod/EID

Michael, V., Brangerl, C. and Fontainel, G.A. (2002): Epidemiology of leptospirosis. *Rev. Cubana Med.Trop.* **54**:10-12.

Moch, R. W., Ebner, E. E., Barsoum, L. S. and Botros, B.A. (1975): Leptospirosis in Ethiopia: a serological survey in domestic and wild animals. *Journal of Tropical Medicine and Hygiene*, **78**: 38-42.

N S W Public Health Bulletin 2003, **14**: 230–231.

Nassi, F., Seixas, F. K., Jouglard, S. D. D., Simionatto, S., Silva, E. F., Seyffert, N., Brod, C. S., Dellagostin, O. A. (2003): Leptospirosis diagnosis using nested-PCR, *Brazilian Journal of Microbiology* **34**: 90-92.

Oliveira, A. A. F., Morta, R. A., Pereira, G. C. A., Langoni, H., Souza, M. I., Navegantes, W. A. And Sa, M. E. P. (2001): Seroprevalence of bovine leptospirosis in Garanhuns Minicipal District, Pernambuco State, Brazil. *Onderstepoort Journal of Veterinary Research*, **68**: 275-279.

Pal, M. (1996): Leptospirosis: A contemporary zonoosis. *Veterinarian*, **20**: 11-12

Pal, M. (2007): Leptospirosis: Zoonoses, Satyam Publishers, Jaipur, India, 2nd editon, Pp 116-117.

Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (1994): Clinical Veterinary Microbiology, Mosby, Spain Pp 292-299.

Radostits, O. M., Blood, D. C. and Gay, C. C. (1994): Veterinary Medicine: A text Book of Diseases of Cattle, Sheep, Pigs, Goats and Horses, 8th Edition, Bailliere Tindal London, Pp. 884-898

Ramasubramaniam, V. (2000): Leptospirosis, WebHealthCenter.com

Red Book. (2003): Leptospirosis in: Summaries of Infectious Diseases, American Academy of Pediatrics, http://www.aapredbook.aappublications.org/cgi.

Ricaldi, J. N. and Vinetz, J. M. (2007): Leptospirosis in the tropics and in travelers. *Current Infectious Reports* **8**: 51-58.

Romero, E. C., Bernardo, C. C. M. and Yasuda, P. H. (2003): Human leptospirosis: A twentyi-nine years serological study in Sao Paulo, Brazil, *Rev. Inst. Med.Trop. Sao Paulo*, **45**: 245-248.

Sambasiva, R.R., Naveen, G., Bhalla, P. and Agarwal, S. K. (2003): Leptospirosis in India and the rest of the world, *Brazilian Journal of Infectious Diseases*, 7: 23-25

Segura-Correa, V.M., Solis-Calderon, J. J. and Segura-Correa, J. C. (2003): Seroprevalence and risk factors for leptospiral antibodies among cattle in the State of Yucatan, Mexico, *Tropical Animal Health and Production*, **35**: 293-299.

Sehgal, S. C., Vijayachari, P., Sharma, S. and Sugunan, A. P. (1999): LEPTO dipstick: a rapid and simple method for serodiagnosis of acute leptospirosis, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **93**: 161-164.

Sehgal, S. C., Vijayachari, P., Smythe, L. D., and Norris, M. (2000): Lai-like *Leptospira* from the Andaman Islands, *Indian Journal of Medical Research*, **3**: 1-2.

Seifert, H. S. H. (1996): Leptospirosis. **In**: Tropical Animal Health, CTA, Kluwer Academic Publishers Dordrecht Pp. 384-386.

Senthilkumar, T. M. A., Subathra, M. and Ramadass, P. (2007). Evaluation of recombinant leptospiral antigen LipL41 in enzyme-linked immunosorbent assay and latex agglutination test for serodiagnosis of canine leptospirosis, *Veterinariki Archiv*, **77**: 475-484.

Shah, I., Warke, S., Deshmukg, C. T. and Kamat, J. R. (1999): Leptospirosis- an under-diagnosed clinical condition, Journal of Postgraduate Medicine, **45**: 93

Sharma, K. K. and Kalawat, U. (2008): Early diagnosis of leptospirosis by conventional methods: One-year prospective study, Indian Journal of Pathology and Microbiology, **51**: 209-211.

Silva, E. F., Brod, C. S., Cerqueira, G. M., Bourscheidt, D., Seyffert, N., Queiroz, A., Santos, C. S., Ko, A. I. and Dellagostin, O. A. (2007): Isolation of *Leptospira noguchii* from sheep, *Veterinary Microbiology*, **121**: 144-149.

Silva, E. F., Cerqueira, G. M., Seyffert, N., Seixas, F. K., Hartwig, D.D., Athanazio, D. A., Pinto, L. S., Queiroz, A., Albert, I. K., Brod, C. S. and Dellagostin O. A. (2009): *Leptospira noguchii* and human and animal leptospirosis, Southern Brazil, Center for Disease Control and Prevention, *Journal of Epidemiology and Infectious Diseases*,**15** (4): 1-5.

Slack, A. A., Symonds, L., Dohnt, M. F., Corney, B. G. and Lee, D. (2007): Epidemiology of *Leptospira weilii* serovar Topaz infections in Australia, *Communicable Diseases Intelligence*, **31**: 216-222

Srivastava, S. K., and Kumar, A. A. (2003): Seroprevalence of leptospirosis in animals and human beings in various regions of the country, *Indian Journal of Comparative Microbiology and Immunology of Infectious Diseases*, **24**: 155-159.

Stanier, R. Y., Ingraham, J. L., Wheelis, M. L. and Painter, P. R. (1986): General Microbiology. 5th Edition, Macmillan Education LTD, Houndmills, Pp. 643.

Travel Health News (2005): Leptospirosis, Pro-MED-Mail, www.promedmail.org

Venkatesha, M. D. (2006): Laboratory techniques in diagnosis of leptospirosis, *Intas Polivet*, 7: 332 – 336.

Vijayachari, P., Sugunan, A. P. and Sehgal, S. C.(2002): Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis, *Epidemiology and Infection*, **129**:617-621.

Vinetz, J. M., Glass, J. E., Flexner, C. E., Mueller, P. And Kaslow, D. C. (1999): Sporadic urban leptospirosis, *Weekly Epidemiological Record*, 74: 234-242

Web Health Center, 2000, WebHealthCenter.com

WHO. (2003): Human Leptospirosis: Guidance for Diagnosis, Surveillance and Control.

WHO. (2009): Leptospirosis: Water Related Diseases.

Wiwanitkit, V. (2006): A note from a survey of some knowledge aspects of leptospirosis among a sample of rural villagers in the highly endemic area, Thailand, the *International Electronic Journal of Rural and Remote Health Research, Practice and Policy*

Yimer, E., Koopman, S., Messele, T., Wolday, D., Newayeselassie, B., Degefe, B. and Sanders, E.J. (2004): Human leptospirosis in Ethiopia: a pilot study in Wonji *Ethiopiopian Journal of Health Development*, **18**: 48-51.

8. APPENDICES

Appendix 1 Color plates showing ELISA test result

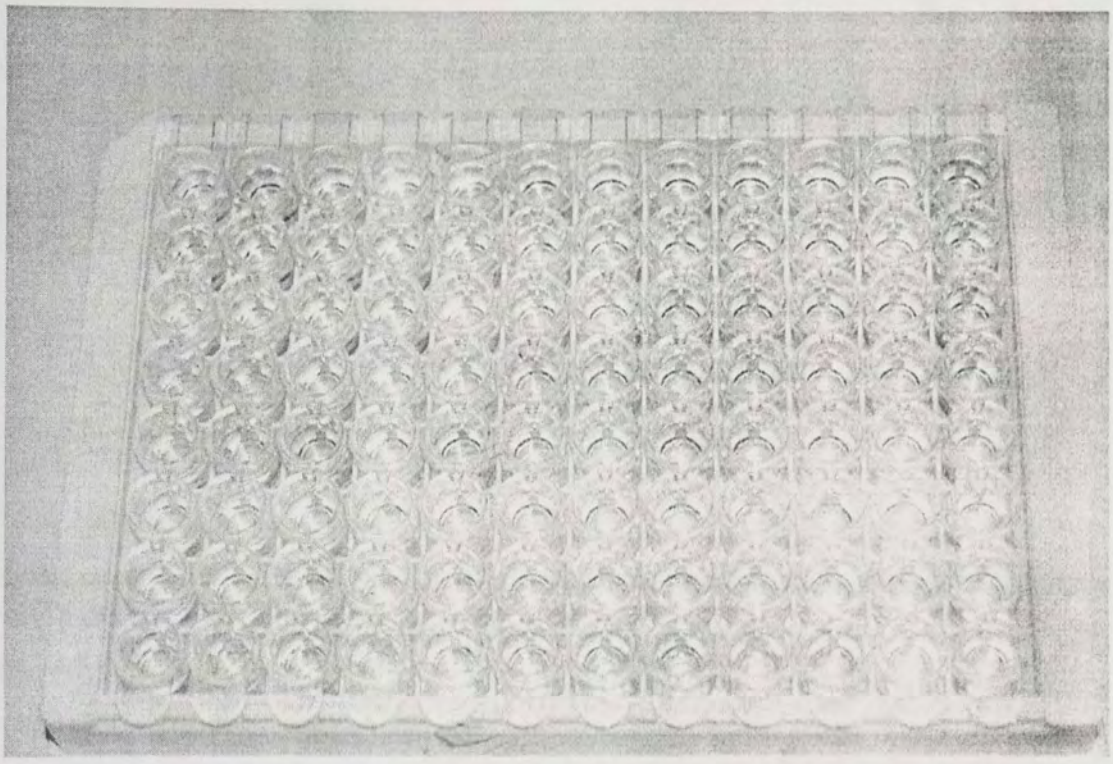


Plate I. ELISA test result of human case samples, positive samples appear as yellow

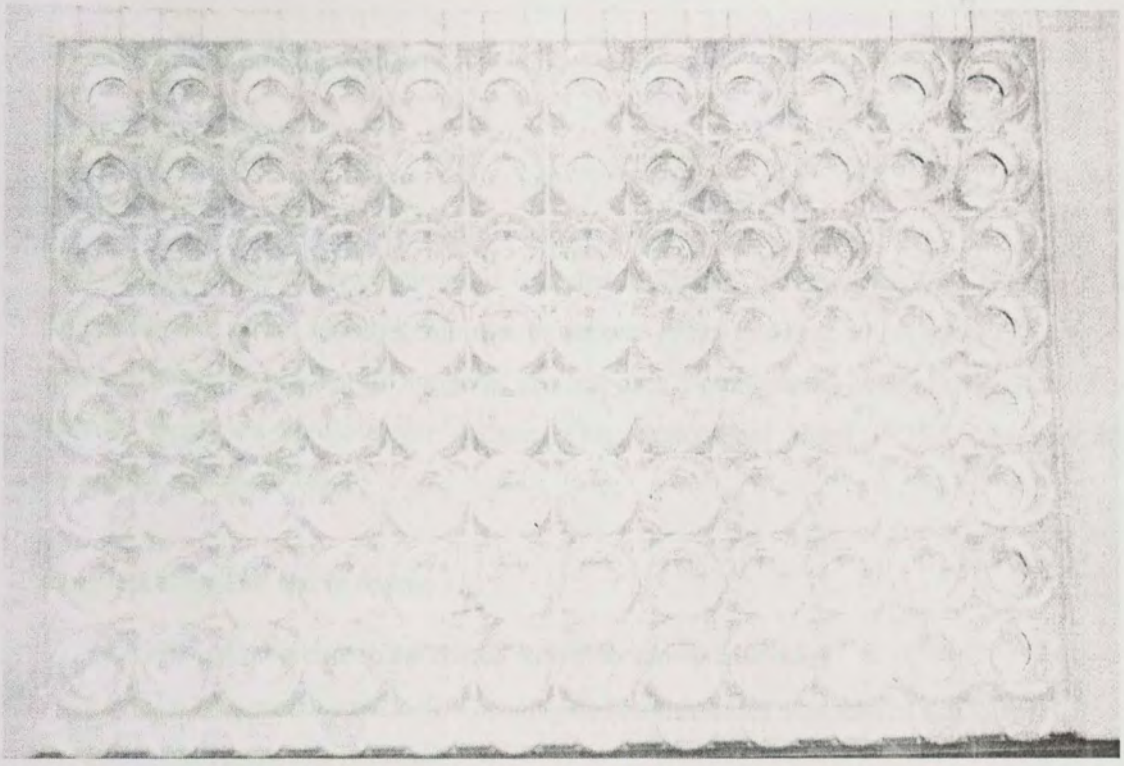


Plate II. ELISA test result of human and bovine sera on the same plate, from fourth rows onwards, bovine sera were dispensed to all wells. Since the kit was specific to human sera, all bovine sera were nonreactive on this plate.

Appendix 2. ELISA kit contents

ELISA kit for bovine

Intended use of the kit

This diagnostic test for leptospirosis is intended to identify antibodies against sugar antigens of leptospira in serum and milk samples. In contrast to test systems, which make of unpurified non-specific leptospira hardjo antigens, this test uses a monoclonal antibody which catches a specific leptospira hardjo sugar antigen. This monoclonal based ELISA has very high sensitivity and specificity.

Reagents of ELISA test in bovine

- 1x96 well microtiter plate coated with monoclonal antibodies
- 1vial 100xconcentrated HRPO conjugated (anti-bovine) monoclonal antibody
- 1x13ml conjugate diluents
- 1x1ml inactivated negative control (freeze-dried)
- 1x1ml inactivated positive control (freeze dired)
- 1x20ml wash-solution 200xconcentrated, must be diluted in deionized water before use
- 1x20ml ELISA buffer
- 1x7ml substrate buffer A
- 1x7ml substrate buffer B
- 1x8ml stop solution
- 1xplastic cover seal

ELISA test procedure for bovine sera

- Wash the microtiter plate with washing solution according to the wash protocol (Annex II). The washing solution provided has to be diluted 200x. Reconstitute positive and negative control with 1ml bidistilled water, divide in aliquots and store the not immediately used controls at -20°C.
- Predilute the test sera to be tested 1:10 (10µl control+90µl buffer) in a round bottomed microtiter plate, also predilute the positive and negative controls 1:10 (10µl control+90µl buffer).

- Dispense 100µl ELISA buffer to wells A1 and B1 (blanks) of the leptospira hardjo coated plate.
- Transfer 10µl of the prediluted samples to the wells of the coated microtiter plate already filled with 90µl ELISA buffer.
- Seal and incubate for 60 minutes at 37 °C
- Wash as in 1
- Dilute the 100x concentrated HRPO conjugated monoclonal antibody 1:100 in conjugate diluents.
- Seal and incubate for 60 minutes at 37°C
- Wash as in 1
- With gentle shaking mix equal parts of buffer A and B together. Prepare immediately before use. Dispense 100µl substrate solution to each well, incubate for 10-15 minutes at room temperature (21°C)
- Add 50µl stop solution to each well
- Read the absorbency values immediately (within 10 minutes) at 450nm. Use as a reference wave length 620nm.

Reagents of ELISA kit for human serum

- Test strips: Microwells containing leptospira antigen - 96 test wells in a test strip holder.
- Enzyme Conjugate: One (1) bottle containing 11 ml of anti-human IgM antibody conjugated to peroxidase.
- Positive Control Serum: One (1) vial containing 1 ml of diluted positive IgM human serum.
- Negative Control Serum: One (1) vial containing 1 ml of diluted negative human serum.
- Chromogen: One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
- RF Absorbent: One (1) bottle containing 5 ml of goat anti-human IgG.
- Wash Buffer Concentrate (20X): Two (2) bottles containing 25 ml of concentrated buffer and surfactant.
- Dilution Buffer: One (1) bottle containing 30 ml of buffered protein solution.
- Stop Solution: One (1) bottle containing 11 ml of 1 M phosphoric acid.

ELISA test procedure to test human serum

Wash Buffer - Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling. Avoid generating bubbles in the wells during the washing steps.

Coagulate blood and remove serum. Freeze sample at -20° C or lower if not used within five days.

Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:40 dilution of patient's sera using the dilution buffer (e.g. 10 µl sera and 390 µl dilution buffer).

To perform tests (Diagnostic Automation, Inc., 2001):

- Break off number of wells needed (two for controls plus number of samples) and place in strip holder.

Note: Negative and positive controls are supplied pre-diluted. Do not dilute further.

Add 40µl of RF Absorbent in a tube and add 100µl of positive control and negative to each tube respectively (#1, #2). Transfer all 140µl mixture to well (#1, #2) after ten minute incubation.

Dilute patient sera 1:40 in dilution buffer. To 100 µl of diluted serum add 40 µl of RF Absorbent. Mix well. Incubate in tube for 10 minutes. Transfer all 140 µl of test samples to the remaining wells (#3-#96).

- Incubate at room temperature (15 to 25° C) for 10 minutes.
- Shake out contents and wash 3 times with the diluted wash buffer.
- Add 2 drops of Enzyme Conjugate to each well.
    - Incubate at room temperature for 10 minutes.
    - Shake out contents and wash 3 times with wash buffer.
    - Slap wells against paper towels to remove all liquid.
    - Add 2 drops of the Chromogen to every well.

    Incubate at room temperature for 5 minutes.
    - Add 2 drops of the Stop Solution and mix by tapping strip holder.
    - Read within one hour of adding Stop Solution.

Reading results

Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.

ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

Annex 3. Sample collection format for bovine

Date _____

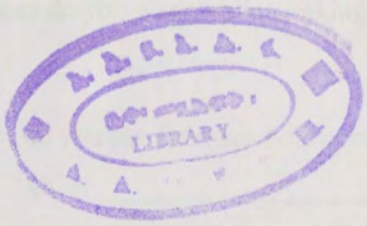
Owner's Name _____ Code _____

Address: Kebele/PA _____ House No. _____

Age _____ Sex _____

Husbandry condition _____

Remark _____



Appendix 3: Questionnaire format used to assess risk factors associated with human leptospirosis

Date _____

Patient's Name _____ Sex _____ Age ____ Occupation _____ Code _____

Address: Kebele/PA _____ House No. _____ Tel. No. _____

1. What have you felt? Acute fever_____ Headache _____ Pain in the urinary tract _____ Pain in the abdomen _____ Conjunctival suffusion _____ Edema _____ Muscular pain ____ Jaundice _____ Other (specify)_____

2. Have you ever got similar sickness before? Yes _____ No _____

   a. If yes, did you take medicine? Yes_____ No_____

   b. If yes, how was the response? Better _____ No change _____

   c. How long have you been ill before you visit the hospital?

3. What type of shoes do you wear while working? Closed_____ Open _____ Bare foot_____

4. What source of water do you use for household consumption? Pond _____ River ____ Pipe water _____ Other _____

5. Are there mice around your home? Yes _____ No _____

   a) If yes, how often do you see them? One per day ____ Two per day _____ More than two per day _____

6. Is occurrence of the disease related to some seasons in the year? Yes _____ No ____

   a) If yes, in which season do you see wider disease occurrence? Dry season _____ Rainy season ____Other

7. Do you have contact with sugar cane/farm? Yes _____ No _____

   a) If yes, what type of contact? Factory employee _____ Farmer _____

8. Do you participate in animal slaughtering? Yes _____ No _____

9. Do you use irrigated water for such activities as horticulture/gardening? Yes _____ No _____

10. Do you have direct contact with domestic animals? Yes _____ No _____

a) If yes, with which animal species? Bovine _____Ovine ____Caprine _____ Canine _____ Other_____

CURRICULUM VITAE

Personal detail

Name	Roman Yilma Gere Selassie
Sex	Female
Date of birth	December 19, 1977
Place of birth	South East Shoa, Ethiopia
Marital status	Married
Contact address	Addis Ababa
	Tel: 251911313719 .
	251911386543
	P.O.Box 2143 (code 1250)

Education and qualification

1987-1993	Primary school education at Kelemework Tiruneh Junior Secondary School, Addis Ababa
1994-1997	Secondary school education at Entoto Academic, Technical and Vocational School, Addis Ababa, Award: Ethiopian Secondary School Leaving Certificate
1998-2004	Higher education at Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia Award: Degree, Doctor of Veterinary Medicine

Other trainings

- Training on computer knowledge of windows,
- Training on management of Scientific laboratory equipments
- Training on gender and HIV mainstreaming in Agricultural research institutes

Language ability

- Amharic Spoken and Written
- Oromifa Spoken and Written
- English Spoken and Written

Work experience

- 2003-2004: Externship student, working in clinic, abattoir and microbiology laboratory, diagnosing and treating animals. In Addis Ababa abattoir, ante and postmortem examination of the slaughtered animals. In the microbiology laboratory of FVM, processing clinical case specimens and food samples collected for the purpose of DVM thesis research.
- 2004-2005 Lecturer of Veterinary Gross Anatomy and Embryology at the Faculty of Veterinary Medicine, Gondar University.
- 2005-2007 Researcher of Animal Health in Amhara Regional State Agricultural Research Institute, Debre Birhan Agricultural Research Center.

Research works

- Occurrence of *Listeria* Species in Retail Meat and Milk Products in Addis Ababa, Ethiopia
- Evaluation of Community Based Foot rot Control options in Sheep at Angolela, North Shoa, Ethiopia
- Investigation of Causes of Sudden Death in Kewet, North Shoa, Ethiopia
- Serological Studies on Bovine and Human Leptospirosis in Wonji, East Shoa Zone, Oromia Regional State, Ethiopia

Publication

- Occurrence of *Listeria* Species in Retail Meat and Milk Products in Addis Ababa, Ethiopia

Professional Membership

- Member of Ethiopian Veterinary Association and
- Biological Society of Ethiopia

References

1. Dr Fikadu Regasa (DVM, PHD, Associate professor), FVM, Addis Ababa University, P.O.Box 34, Tel 251114338917, 251114338533, Debre Zeit, Ethiopia
2. Dr Tsegaw Fentie (DVM, MSc, Asst. Prof.), Dean of FVM, Gondar University, P.O.Box 196, Tel 251581141276, Gondar, Ethiopia
3. Dr Moses N. Kyule (BVM, MSc, MPVm, PhD, Associate Prof.) FVM, Addis Ababa University, P.O.Box 34, Tel 251114338917, 251114338533, Debre Zeit, Ethiopia

Signed Declaration Sheet

I, the undersigned, declare that the thesis is my original work and has not been presented for a degree in any University.

Name _____

Signature _____

Date of submission _____

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

Advisor

Name 1. DR. Moses N. Kyule

2. PROF. M. PAL