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Graz, am 23.09.2013 *Carina Wallner*

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ZUSAMMENFASSUNG

Hintergrund

Im Gegensatz zu Deutschland und anderen europäischen Ländern treten Fälle von Leptospirose in Österreich gehäuft auf. Speziell in Süd-Ost Österreich werden immer wieder Infektionen gemeldet. Bisher sind die Gründe dafür und etwaige Risikofaktoren noch nicht bekannt.

In der vorliegenden Studie wurden retrospektiv Patienten mit Leptospirose (autochthon erworben oder importiert) hinsichtlich Risikofaktoren, in Freizeit, Beruf und häuslicher Umgebung, Symptome, Laborwerte und *Leptospira* Serovare evaluiert.

Methoden

In einer retrospektiven Studie wurden Risikofaktoren für eine Infektion mit *Leptospiren* in Süd-Ost Österreich erhoben. Eingeschlossen wurden Patienten (Alter ≥18) die im Zeitraum von 2004-2012 positiv auf *Leptospiren* getestet wurden (Serologie: IgM). Alle Daten wie Risikofaktoren (bis 3 Wochen vor Beginn der ersten Symptome), Symptome, klinischer Verlauf und Laborwerte wurden mittels eines telefonischen Fragebogen und/oder dem Kommunikations- und Informationsnetzwerk für die steirischen Landeskrankenhäuser (MEDOCS) erhoben. Die verschiedenen Leptospira Serovare wurden mit Hilfe der AGES Mödling (Österreichische Agentur für Gesundheit und Ernährungssicherheit) erfasst.

Ergebnisse

Insgesamt wurden 128 Patienten (79 männlich, 49 weiblich; Durchschnittsalter 42.7 Jahre) eingeschlossen. 120/128 (93.75%) aller Patienten hatten eine autochthon erworbene Leptospirose, nur 8 (6.25%) eine wahrscheinliche importierte Infektion (5 Asien, 1 Südamerika, 2 Afrika; innerhalb 3 Wochen vor Krankheitsbeginn).

Die am häufigsten aufgetretenen Symptome waren Fieber 83/128 (64.8%), Myalgie/Arthralgie (35.2%), Bauchschmerzen/Diarrhö (32%), Müdigkeit, Mattigkeit, Abgeschlagenheit /–MMA; 28.1%), Ikterus (24.2%), Kopfschmerzen (21.7%), Übelkeit/Erbrechen (20.3%) und akutes Nierenversagen (11.7%).

Die Laborwerte am Tag der Aufnahme zeigten in 20.3% der Fälle eine Thrombozytopenie, in 13.3% eine Leukopenie und in 21.9% eine Leukozytose. Auffällig war auch die Erhöhung von der Alanin- Aminotransferase (ALT) ≥90 U/l in 42.2% und Serumkreatinin (39.1%).

Mit Hilfe der AGES Mödling konnten wir von 59 Patienten die *Leptospira* Serovare erheben.

Dabei waren *L. Bratislava* (30.5%), *L. Sejroe* (23.7%) und *L. Ballum* (22%), *L. Australis* (16.9%) und *L. Grippotyphosa* (13.6%) die am häufigsten nachgewiesenen Serovare.

Die Risikofaktoren wurden anhand der telefonisch beantworteten Fragebögen evaluiert (n=82; wobei 4 Fälle möglicherweise importiert gewesen sein könnten).

Als Risikofaktoren im Freizeitverhalten stellten sich vor allem Aktivitäten im Wald oder feuchten Gebieten (58.8%), Gartenarbeit/Jagd (46.3%), Reinigung oder Renovierung von Hütten, Dachböden, Kellern oder Garagen (28%) und schwimmen/schnorcheln/tauchen in naturbelassenen Gewässern heraus. Risikofaktoren in häuslicher Umgebung waren Nagetiere (v.a. Ratten und Mäuse) in unmittelbarer Umgebung (73.2%), Haustiere (Katzen und Hunde, 61%), Bauernhof/Nutztierhaltung (28%) und eigener Obst und/oder Gemüseanbau (24.4%).

Auf Grund eines relativ hohen Frauenanteils wurden auch die Unterschiede zwischen den Geschlechtern bei Risikofaktoren und Klinik erhoben.

Deutlich mehr Frauen gaben an Obst und Gemüse auf Bauernmärkten oder direkt bei Bauern zu kaufen, und im Garten zu arbeiten. Männer hingegen verbrachten ihre Zeit häufiger im Wald oder sumpfigen Gebieten und arbeiteten/lebten öfter auf Bauernhöfen und hielten Nutztiere. Auch bei Laborwerten fanden wir Unterschiede.

Während ALT öfters bei Frauen (46.9%) als bei Männern (39.2%) erhöht war, zeigten deutlich mehr Männer (45.6%) als Frauen (28.6%) ein erhöhtes Serumkreatinin. Männliche Patienten klagen häufiger über gastrointestinale Beschwerden, im Gegensatz dazu litten mehr Frauen unter MMA.

Schlussfolgerung

Autochthone Infektionen stellen den Großteil aller Leptospirosen in Süd-Ost Österreich dar.

Hauptrisikofaktoren für die Infektion mit *Leptospiren* sind Aktivitäten im Wald oder feuchten Gebieten, sowie Haustiere und Nagetiere in der häuslichen Umgebung.

ABSTRACT

Background

In contrast to Germany and other European countries autochthonous leptospirosis occurs frequently in Austria. In particular high rates are found in south-east Austria, the reasons for that as well as associated risk factors, however, have not been identified yet. The present clinical study analysed patients with leptospirosis regarding origin of infection (autochthonous versus imported) common risk factors for acquiring the infection, clinical symptoms and *Leptospira spp.*.

Methods

We retrospectively analysed risk factors for leptospirosis in South-East Austria. Adult patients (age ≥18 years) that had been tested positive for leptospirosis (serology: IgM) within a 9-years period (2004-2012) were included. Data concerning clinical course of disease as well as recreational or residential risk factors (up to 3 weeks before onset of infection) were collected via telephone questionnaires and/or electronic patient databases.

Results

128 patients (79 male, 49 female, median age 42.7 years) were included. 120/128 (93.75%) of patients had acquired leptospirosis within Austria and 8 (6.25%) had possible imported infections (5 Asia, 1 South America, 2 Africa). Symptoms reported most frequently included fever 83/128 (64.8%), myalgia/arthralgia (35.2%), abdominal pain/diarrhoea (32%), general weakness (28.1%), jaundice (24.2%), headache (21.7%), nausea/ vomiting (20.3%) and acute kidney injury (11.7%).

Lab results at admission showed thrombocytopenia in 20.3% of cases, 13.3% had leukopenia and 21.9% leucocytosis. Elevated serum creatinine (39.1% of cases) and liver transaminases (>2 times normal value; 42.2%) were frequent.

L. Bratislava (30.5%), *L. Sejroe* (23.7%), *L. Ballum* (22%) *L. Australis* (16.9%) and *L. Grippotyphosa* (13.6%) were the most frequently identified serotypes.

Risk factors were evaluated in cases that completed the telephone questionnaire (n=82, 4 possibly imported cases).

Concerning recreational risk factors activities in woods/wet areas were reported by 58.5%, followed by gardening/ hunting (46.3%), tidying up basement/ attic/ hut (28%), and swim/snorkel/dive (17.1%). Rodents in surroundings (73.2%) was the most important residential risk factor, followed by contact to pets (61%), farm/farm animals (28%) and eating fruit/vegetable grown in own garden (24.4%).

Because of the relatively high number of infections in females we tried to figure out differences in risk factors and clinical presentation.

Infected females were more likely to have eaten food from own garden/farmer/farmers market or have worked in a garden. On the contrary activities in woods/wet area and farm/contact to farm animals were risk factors more often present in males. With regard to lab results we detected differences in elevation of alanine transaminase (female 46.9%; male 39.2%) and serum creatinine (male 45.6%, female 28.6%). Male patients were more likely to present with gastrointestinal symptoms, while a higher percentage of females complained about prostration.

Conclusion

Autochthonous infections represent the vast majority of leptospirosis cases in South-East Austria. Because of its rarity and broad clinical spectrum the burden of leptospirosis is certainly an underestimated disease in Austria. The main risk factors for acquiring leptospirosis were activities in woods and wet areas as well as contacts to pets and rodents living in the surroundings.

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1 GLOSSARY AND ABBREVIATIONS

AGES	Österreichische Agentur für Gesundheit und Ernährungssicherheit
ALT	alanine aminotransferase
AIN	acute interstitial nephritis
AKI	acute kidney injury
AP	alkaline phosphatase
ARDS	acute respiratory distress syndrome
ARF	acute renal failure
ATN	acute tubular necrosis
CDC	Centers for Disease Control and Prevention
CRP	C-reactive protein
CSF	cerebrospinal fluid
DIC	disseminated intravascular coagulation
ELISA	Enzyme-linked Immunosorbent Assay
GBS	Guillain- Barré syndrome
GFR	glomerular filtration rate
HD	hemodialysis
HUS	hemolytic-uremic syndrome
ICU	intensive care unit
IgG	Immunogloblulin G
IgM	Immunoglobulin M
LPS	lipopolysaccharide
MAT	microagglutination test

MEDOCS	Kommunikations- und Informationsnetzwerk für die steirischen Landeskrankenhäuser (electronic patients database)
MMA	müde, matt, abgeschlagen
MODS	multiple organ dysfunction syndrome
PCR	Polymerase Chain Reaction
POCT	Point- of- Care- Testing
SD	standard deviation
SIRS	systemic inflammatory response syndrome
SPHS	severe pulmonary hemorrhagic syndrome
TTP	Thrombotic thrombocytopenic purpura
VHF	viral hemorrhagic fever
ZAMG	Zentralanstalt für Meteorologie und Geodynamik (Division for Meteorology and Geodynamics)

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2 Introduction

2.1 History

Leptospirosis (λεπτός "thin" and σπείρα "coil") is one of the world's most common zoonotic infectious diseases, and occurs mostly in tropical or subtropical areas, but also in temperate regions. It has gained attention as a very important emerging infectious disease over the last few years. The disease was probably already recognized in ancient China as an occupational hazard of rice harvesting, and the Japanese name "*akiyami"*, or autumn fever, is still used in modern medicine (1). It´s thought that *L. Interrogans* came to Europe in the 18th century by extension of Rattus norvegicus from Eurasia (12). The disease is also known by other names like Canicola fever, 7- day fever, Fort Bragg fever or Rat Catcher´s yellow. Leptospirosis has been first characterized by Landouzy in 1883, when some canal workers showed symptoms of the disease. In 1886 Adolf Weil described the most severe form of leptospirosis presenting with fever, jaundice, renal failure and haemorrhage, now known as Weil´s disease (2). In the years thereafter, the icteric infectious disease was known to occur in humans after the exposure to water, mud, wet soil or marshy land. It´s been also known that certain circumstances like rainy season, and activities like swimming or working in wet soil are risk factors for acquiring the disease. In those years leptospirosis was thought to be "the yellow fever of the temperate zones". In 1915 *leptospires* were discovered by Japanese scientists in rats and field mice, which are known to be the most important vectors for the disease (3).

2.2 Aetiology

Spirochetes are helical shaped, aerobe, gram-negative bacteria. Although we know some types of spirochetes that are not pathogenic, there are some that cause different diseases in humans. For example *Treponema pallidum* is causing syphilis, *Borrelia burgdorferi* (Lyme disease), *Borrelia recurrentis* (relapsing fever) and also *Leptospira* species causing leptospirosis.

Leptospira is a genus of spirochete bacteria of only 0,1-0,2 µm in diameter, 6-20 µm length, with a wavelength of about 0,5 µm and one or both ends are hooked. Because of their size, *Leptospira* are best observed by dark field microscopy.

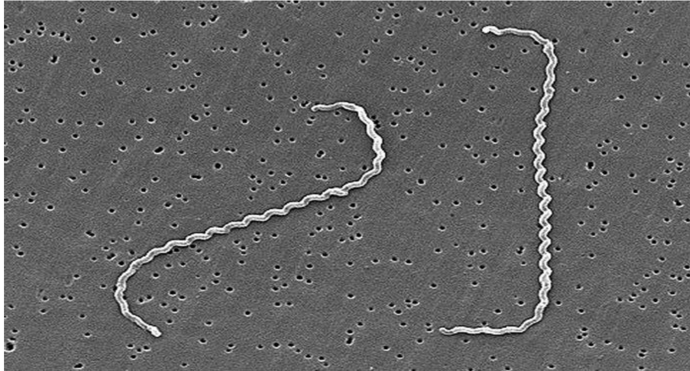


Figure 1: Leptospira interrogans strain; electron micrograph (4)

Leptospira can be divided into 3 species: *L. Interrogans*, which is pathogenic, and *L. Biflexa* and *L. Parva* which are saprophytes. *L. Interrogans* can be sub classified in 24 serogroups and over 200 serovars by micro agglutination. Also more than 60 serovars of *L. Bifelxa* have been described- to date.

Although a high number of serovars are known by now, there are only a few that cause illness of any severity. Table 1 outlines some serogroups and associated serovars. Not all of them can be found in every part of the world, some are related to tropical areas, some to temperate zones, but they all can cause leptospirosis in humans.

The most common human pathogenic serovares are *L. Icterohaemorrhagiae* causing Weil´s disease, *L. Canicola* (Canicola fever), *L. Australis* (Bouget- Gsell- disease or swineherd´s disease), *L. Batavia* causing rice- field fever and *L. Autumnalis* (Fort Bragg fever). Also *L. Ballum*, *L. Bratislava* and *L. Grippotyphosa* are detected frequently. All mentioned diseases are summarised as leptospirosis (2)

Serogroup	**Serovar(s)**
Australis	Australis; Bratislava
Autuumnalis	Autuumnalis; Fortbragg; Bim
Ballum	Ballum; Arborea
Bataviae	Bataviae
Canicola	Cannicola; Portlandvere
Grippotyphosa	Grippotyphosa
Hebdomadis	Jules
Icterohaemorrhagiae	Icterohaemorrhagiae; Copenhageni; Lai
Lyme	Lyme
Pomona	Pomona
Pyrogenes	Pyrogenes
Sejroe	Sejroe; Hardjo
Tarassovi	Tarassovi

Table 1: Important leptospira serogroups and serovars (adapted from (1) and (5))

2.3 Epidemiology

Leptospirosis is a worldwide zoonotic disease traceable in domesticated and non-domesticated animals, mostly in rodents, dogs, cattle and pigs. Different host species can be reservoir for one serovar. In general rats are host for serovar *Icterohaemorrhagiae and Copenhageni*, mice for serovar *Ballum, Arborea and Bim*, dairy cattle may harbour *Hardjo* and *Pomona*. Dogs are often infected with *Canicola* and *Grippotyphosa* may be carried by marsupials or racoons. In sheep we also find *L. Hardjo*, in bats the rare serovars *Wolffi* and *Cynopteri* (Depicted in table 2).

Resrvoir host	**Serovar(s)**
Pigs	Pomona; Tarassovi
Cattle	Hardjo; Pomona
Horses	Bratislava
Dogs	Canicola
Sheep	Hardjo
Racoon	Grippotyphosa
Rats	Copenhageni; Icterohaemorrhagiae
Mice	Ballum; Arborea; Bim
Marsupials	Grippotyphosa
Bats	Wolffi; Cynopteri

Table 2: Typical reservoir hosts of common leptospiral serovars (adapted from(6))

Usually humans are dead end hosts, as transmission between humans is very rare. The incidence of infection is higher in tropical countries than in temperate zones, *Leptospira spp.* can survive longer in warm, humid conditions. The peak of infections in temperate zones is seen in summer and fall, and during rainy season in tropical regions (7).

Geographical distribution is shown on the chart below (8).

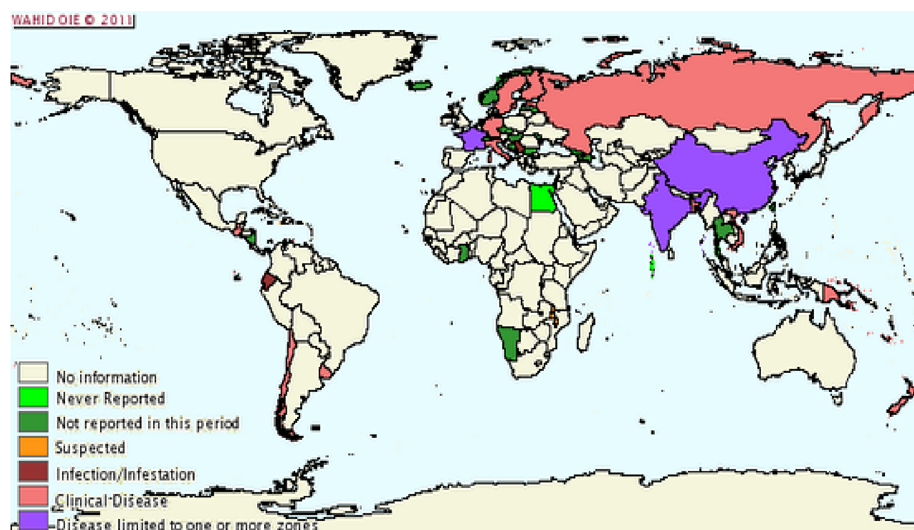


Figure 2: Geographical distribution

In contrast to Germany and other European countries autochthonous leptospirosis occurs more frequently in Austria. In particular high rates are found in south-east Austria, the reasons for that, as well as associated risk factors, have not been identified yet.

Because of the rarity of the disease, varying of symptoms and frequently mild clinical manifestation, it´s sometimes hard to find the right diagnose.

2.3.1 Animals/Reservoirs

The most common ways of acquiring the disease are contact with soil, water, or directly with contaminated urine of infected animals (rodents, wild animals, domestic animals) through skin lesions or animal bites. Animals, in particular rodents, may become asymptomatic carriers as *Leptospira* can survive in the proximal renal tubules of a host life-long. *Leptospira* are then released continuously with the urine of the infected animal.

These can transfer the infection to livestock, wild animals, domesticated animals like dogs, and humans, most likely via direct contact with urine, or infected water.

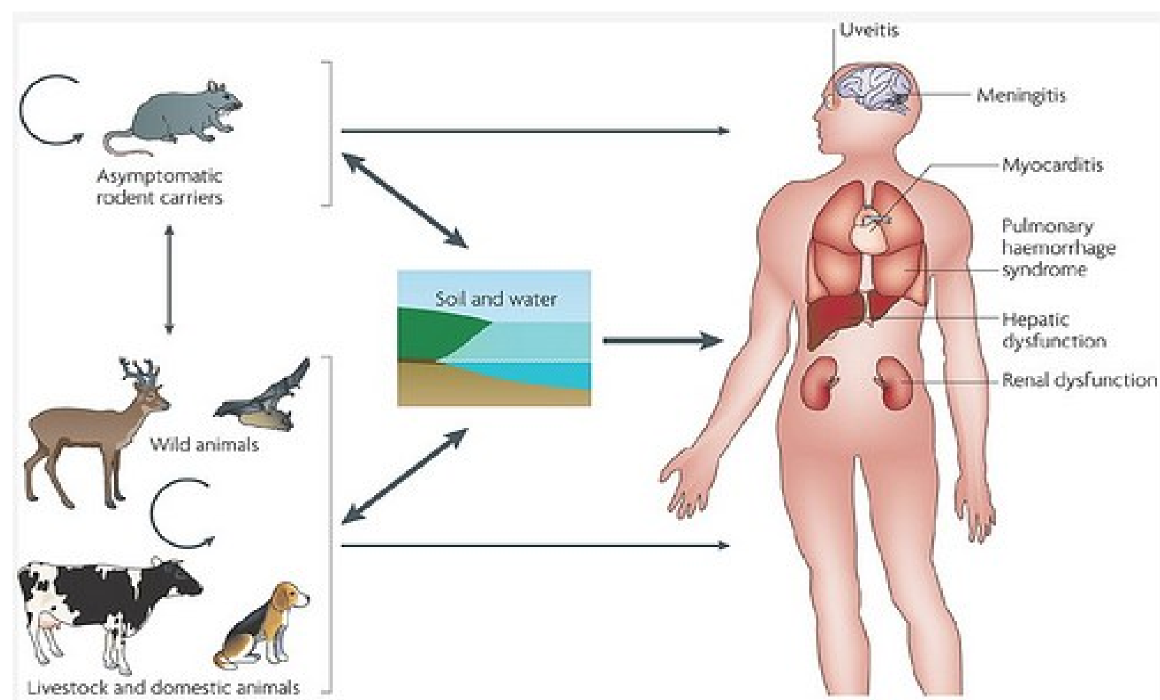


Figure 3: Path of transmission (9)

Leptospira can persist over years at the proximal tubule of the host animal and are continuously eliminated by urine. Those animals can be symptom- free eliminators, but they also can be symptomatic and finally die of nephritis (2).

2.3.2 Environment

The survival of pathogenic *Leptospira* in the environment depends on various factors, like pH and temperature. Most studies only used single serovars and different methods, but some general findings are evident. *Leptospira* can survive for several months under laboratory conditions in water at room temperature at pH 7.2 to 8.0. In rain water flooded soil serovar *Australis* survived for about 3 weeks, and in soil that was infected with urine from rats *Leptospira* survived 2 weeks (10).

2.3.3 Incidences

The incidence in central Europe varies. From 1998-2003 the incidence in Germany was about 0.06/ 100.000, in 2011 it was about 0.1/ 100.000. In France the incidence is about 0.39/ 100.00. In Italy the incidence is about 0.07/ 100.000, and in the Netherlands 0.25/100.000. Countries in temperate zones with higher incidence of leptospirosis are Croatia, Russia (both countries 0.17/ 100.000) and Ukraine with an incidence of approximately 0.15/ 100.000. Worldwide there are approximately 350.000-500.000 infections every year. In fact prevalence is probably even higher, because of mild forms of the disease that are not recognised and diagnosed as leptospirosis infections (11-14).

2.3.4 Risk factors

In temperate zones an infection is most likely related to outdoor activities, whereas in developing countries or (sub) tropical areas it´s mostly an occupational disease (e.g. rice fields) or related to insufficient hygiene and low life standard.

Walking barefoot, water wells or washing dishes or clothes in contaminated water, living in slums and bathing in stagnant water are high risk factors for

acquiring leptospirosis. Especially during rainy season there are reported outbreaks from time to time. In July to August 2001, during monsoon in Mumbai causing flooding, 30 out of 93 children (32%) with suspected leptospirosis were confirmed positive. They all lived in slums and were tested for antibodies by *Leptospira* genus- specific latex agglutination assay, dipstick assay, or MAT (15).

Over the last few years, due to the rise of outdoor activities like canoeing, swimming, rafting, caving and adventure travelling, leptospirosis has also been increasingly acquired during recreational activities (6)

The relation between social-economic standards and infection rates is exhibited by data from all over the world, demonstrating a higher risk for infection in lower life standard areas (e.g. slums). The main reason is that low income countries, are associated with higher rates of contacts to rodents, especially rats.

2.3.5 Outbreaks

From time to time there are flood related outbreaks of leptospirosis occurring worldwide, like in Brazil (1983, 1988 and 1996), Nicaragua (1995), Russia (1997), USA (1998), India (1999), Philippines (2009), Indonesia (2011), and Thailand in 2000; and 2012 (16, 17).

The most likely reason for such outbreaks is that the environmental changes (water, humidity) are increasing the vector population, and therefore the number of infections.

Contaminations of water supplies and natural waters have also led to infections, for example: Italy 1984, drinking from water fountain, causing 34 infections, or Illinois and Wisconsin 1998, swimming in a lake during a triathlon, causing 74 infections. The source of infection could not be identified in Wisconsin and Illinois. In Italy a dead hedgehog in a header tank was identified as carrier of leptospirosis (12).

A number of other outbreaks after recreational activities have been reported. An interesting example is the outbreaks after the ECO Challenge 2000 in Malaysia (7).

In 2000 the ECO Challenge took place in Borneo from August 21st to September 1st. It is a 500km course including disciplines like trekking, white-water canoeing, sea kayaking, swimming, scuba diving and mountain biking.

During the Challenge the participants faced various risk factors for acquiring leptospirosis, including contact with the water of the Segema River, caving and trekking in the jungle, where they could easily get skin lesions.

There were also heavy rain falls in the area where the race took place. All in all 304 athletes from 26 countries were taking part. The CDC (Centres for Disease Control and Prevention) was notified by the GeoSentinel Network, the Los Angeles County department of Health services and the Idaho department of Health, of at least 20 cases of an acute febrile illness, including symptoms like high fever, myalgia and headache after the race. 189 participants were contacted afterwards and were asked to answer a standardized questionnaire. 80 patients met the case definition for leptospirosis, out of those 38 serum samples were collected. 26 were tested positive for leptospirosis.

The screening for leptospirosis was performed by ELISA, Dip- S- Ticks enzyme-linked immunodot assay, or both. 20 of the 26 samples (77%) were positive for anti-leptospiral antibodies by MAT (18-20).

2.3.6 Occupational risk factors

The majority of patients who have been tested positive for leptospirosis are male patients as certain occupations (e.g. canal worker, field worker, meat inspectors, abattoir workers) that are related to a higher risk for acquiring the infection are more likely to be done by males (2). The majority of patients who have been tested positive for leptospirosis are male individuals. According to the World Health Organization, in Germany the incidence ratio between male and female has been reported 5:1, in Italy and France even 10:1 (21).

Miners and sewers belonged to the first occupational risk groups that were recognized in the 1930´s. This led to the first approach of a rodent control program and the use of protective clothing.

After that the incidence of infections has decreased, but remained stable on a low level.

A serological survey in workers who spent long times in water tanks soaking rice grains showed that 68,3% were seropositive for *L. Interrogans* (serovar *Autuumnalis* and *Icterohaemorrhagiae*), tested by MAT (18).

There are also cases reported that got infected by contaminated food, or while working on fields in temperate zones (2). In July 2007 a leptospirosis outbreak among strawberry harvesters from Eastern Europe (Poland, Romania, Slovakia), who were all working in the same area of Germany, was recognized. After an investigation it was discovered that out of 153 strawberry harvesters 13 had confirmed leptospirosis (clinical signs and antibodies against *Leptospira Interrogans*, serogroup *Grippotyphosa*), and another 11 cases had clinical signs of leptospirosis.

The serum samples were tested by MAT and ELISA. Also the rodent population (voles) of the affected area in North Rhine- Westphalia was tested. The clinical course was mild in most cases. This had probably been the largest number of infections within an epidemic happening in Germany since the 1960´s.

The warm winter in 2006/2007 was presumed to facilitate the vole population. The risk of infection was given because of contact with soil and rodents during the harvest for over 1 month (22).

2.4 Pathogenesis

There are still many unknown facts about pathogenicity of leptospirosis. *Leptospira* are mostly acquired through small cuts or abrasions on the skin, but they are also able to invade the body through mucosa, lungs or conjunctiva. Usually they cannot penetrate undamaged skin, except when it´s been exposed to water for a long time and swollen significantly. For entrance via the lungs it has to be an aerosol inhalation, not only bacteria alone. After entering the bloodstream and lymphatic system they spread in the whole organism and replicate in renal tubules, blood and liver, best at body temperature. Typical doubling time is about 8 hours.

The bacteria of virulent strains cannot be attacked sufficiently by the human innate immune system.

As it takes time until the adaptive immune system can select and replicate cognate antibodies, the bacteria are able to replicate rapidly (10).

Hyaluronidases are thought to be partly responsible for penetrating intact mucous membranes and damaging capillary endothelium.

In experiments also lipopolysaccharides, certain membrane proteins and secretory proteins are indicated to be virulence factors.

Leptospira do not cause an early inflammatory process. In a host without adapted immunity chances for replication are therefore high. Despite the absence of early inflammation there may be early hepatocellular damage, causing jaundice, and renal- tubular defects, being responsible for acute kidney failure at early stages of the disease. Liver damage can vary from no pathological findings or only single-cell necrosis, to multiple necrosis areas. The kidneys may enlarge; other findings could be oedema, sub capsular haemorrhage and tubular necrosis.

Due to the endothelial damage internal bleedings can occur. Bacterial products cause the response of the innate immune system, which releases cytokines and mediators.

This causes changes in the hemodynamic system, decreasing systemic vascular resistance, hypotension, increased renal vascular resistance and compromised microcirculation.

Cytokines and mediators also determine inflammation, which is responsible for intravascular coagulation, haemolysis and haemorrhage, cytoadherence, activation of the complement system, increased blood viscosity and vascular permeability. Then also the humoral immune response as part of the defence system steps in to combat against the infection. It is responsible for production of auto antibodies, bacterial clearance and immune complex deposition. All aforesaid reactions lead to cell damage. The further progress of the disease depends on the immune reaction of the host, including immune complex formation, and resulting endothelial damage and glomerulonephritis (2).

The pathomechanism of cell damage is shown below.

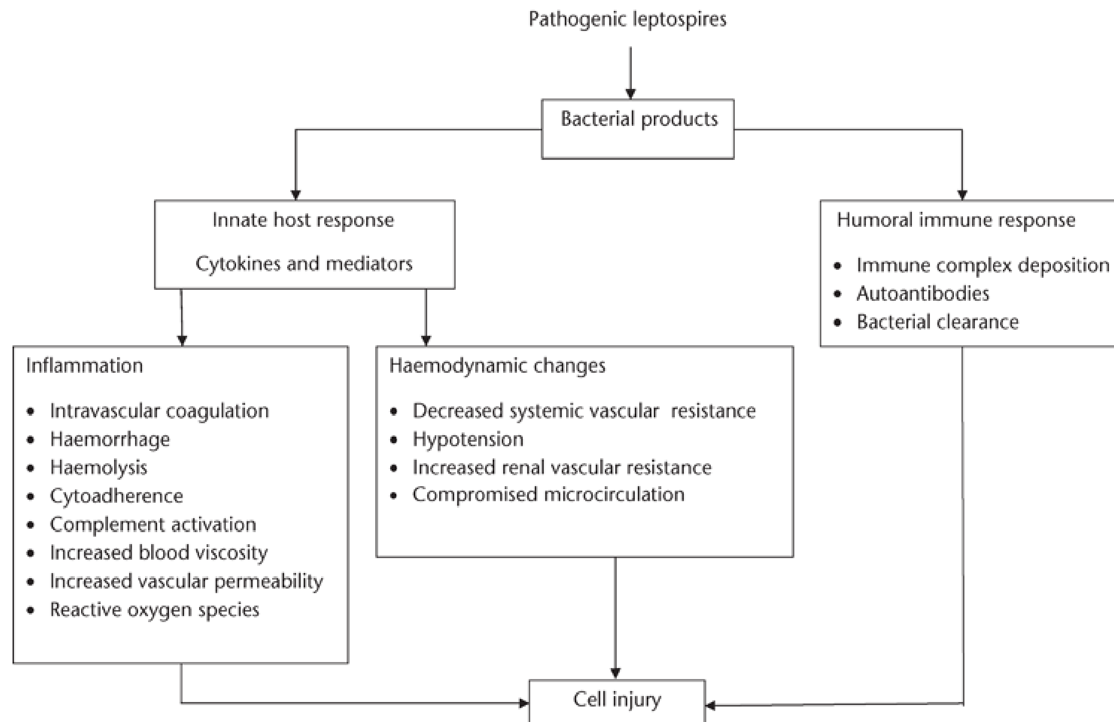


Figure 4: Pathophysiology of leptospirosis (23)

The leptospiral lipopolysaccharide (LPS) is immunogenic and responsible for serovar specificity. It is similar to other lipopolysaccharides of gram-negative bacteria, but less endotoxic. It also stimulates adhesions of neutrophil granulocytes to endothelial cells and platelets, causing aggregation and is suspected of playing a role in thrombocytopenia (7).

2.5 Clinical picture

Leptospirosis is an acute systemic infection. After an incubation time of about 2-20 days the patient shows first symptoms. In many cases the non-specific symptoms of the infection are self-limiting and present like the flu, with fever, headache, myalgia and maybe gastrointestinal problems, like nausea or diarrhoea, sometimes also with milder clinical symptoms. Nonetheless there can be a dramatic clinical course, for example Weil´s disease with acute renal failure (ARF), jaundice, acute respiratory distress syndrome (ARDS), systemic inflammatory response syndrome (SIRS), severe pulmonary haemorrhagic syndrome (SPHS), multiple organ dysfunction syndrome (MODS) or haemorrhages (2).

For long time it was considered that some clinical symptoms were associated with certain serogroups, but recent research and studies showed no relations between the serogroups and clinical manifestations. Nonetheless *L. Icterohaemorrhagiae* is known to cause severe leptospirosis in humans frequently (7).

Usually the course of disease is biphasic, as shown on chart (differentiation between anicteric leptospirosis and Weil´s disease).

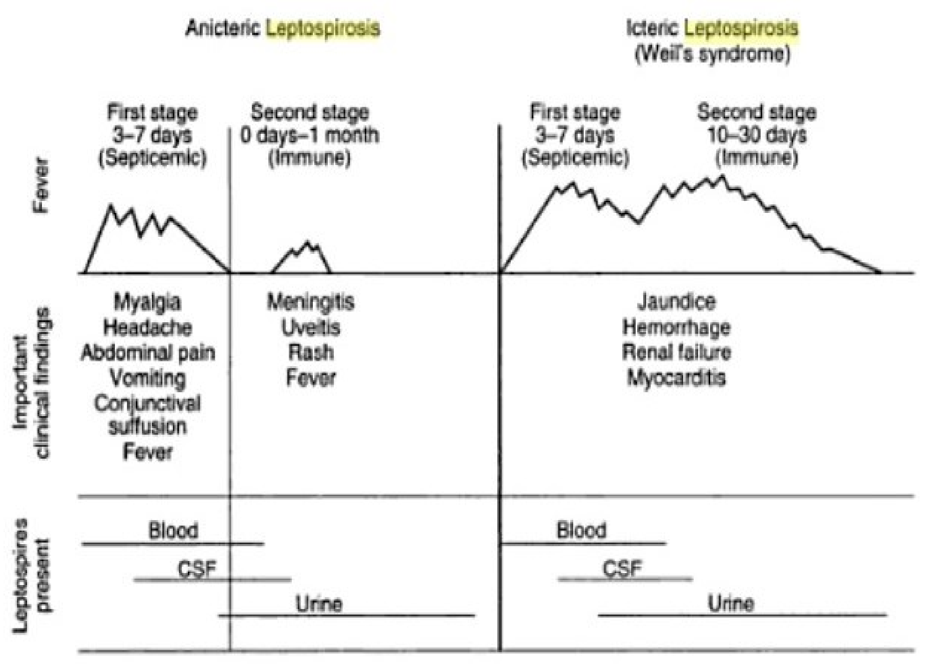


Figure 5: Clinical course of leptospirosis (24)

2.5.1 Anicteric leptospirosis

Phase 1 (bacteraemia): The majority of infections with leptospirosis show a mild clinical course. Many patients probably don´t seek medical care. After an incubation period of averagely 4-12 days (2-20 d) the first symptoms are sudden onset of fever, chills, myalgia- especially calf pain, headache, nausea, vomiting, sometimes skin rash and conjunctivitis.

The rash is often transient and disappears within 24 hours. In most cases the patients are anicteric. Sometimes thrombocytopenia can lead to bleeding.

This period takes from 3-7 days, the bacteria is verifiable in the patients´ blood and also in the CSF, but not in the urine.

There are also no antibodies found in the blood in the first week of the disease. If it´s a mild clinical course recovery starts after that. In approximately 50% of cases phase 1 continues after a time interval of 1 to 3 days to phase 2 (bacteriuria). Mostly the symptoms are similar to phase 1 but less intense. It´s characterized by fever, rash, uveitis and sometimes meningitis (2, 7). It can last up to 30 days.

About 15-25% of the patients develop aseptic meningitis at this time, which usually improves after a few days, but in a few cases meningitis may be lasting over a number of weeks. At this time *Leptospira* can be found in the urine, and antibodies in the patients' blood. Results of a study on 626 patients by Alston and Broom showed that aseptic meningitis occurs more frequently in children (62%), whereas only 31% of young adults and 10% of patients over 30 years old developed aseptic meningitis (6, 25).

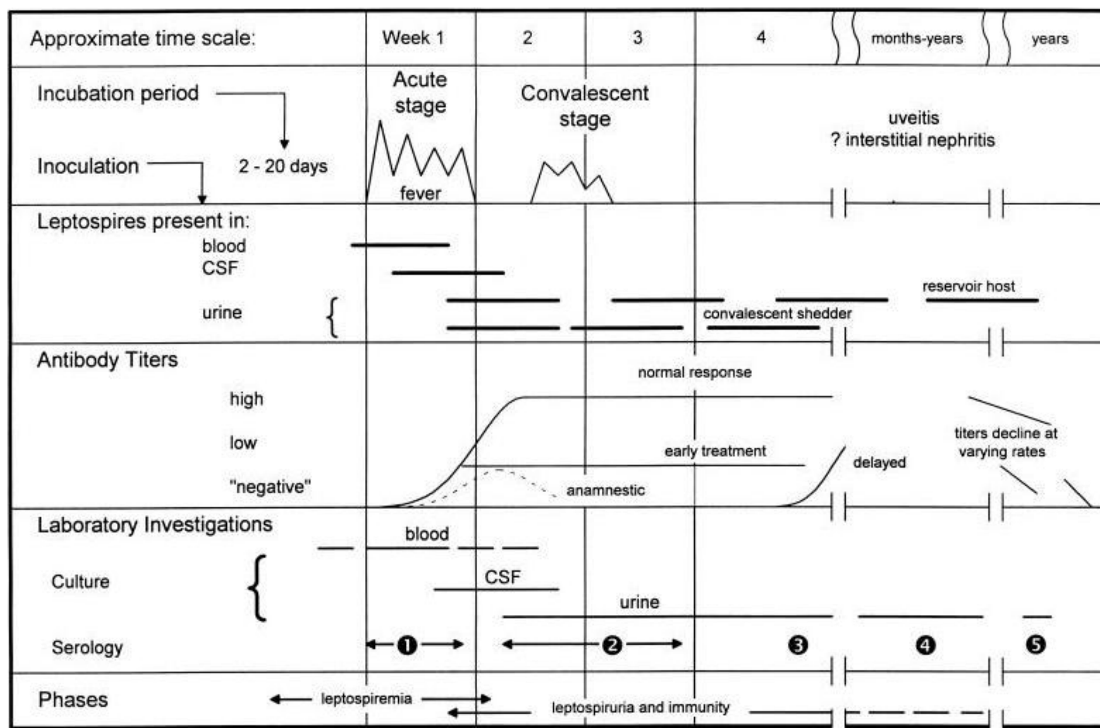


Figure 6: Clinical course (1) (5)

2.5.2 Weil´s disease

The "typical" triad of Weil´s disease is jaundice, acute renal failure and haemorrhages. Symptoms may, however, also be unspecific. Usually there are also 2 phases in Weil´s disease that are merging into each other.

Patients in phase 1 typically have high fever, chills, gastrointestinal problems, headache and myalgia. The disease is often progressive. Sometimes patients already show a severe clinical course in phase 1. That severe course is characterized by persisting high fever, dyspnoea, or haemoptysis. In others the conditions may worsen after a few days of the disease. Typical signs in the latter are hepatorenal involvement, like hepatomegaly, jaundice and oliguria.

The kidney is damaged via direct toxicity of the bacteria and it´s endotoxins, by products of cell damage like myoglobin, by hypotension cause by vasodilatation or the loss of blood volume due to haemorrhages, or dehydration because of fever, diarrhoea and vomiting (26). In 16-40% of cases leptospirosis causes acute renal failure.

The reason for the development of jaundice is suspected to be liver cell damage and haemolysis that lead to an increase of serum bilirubin (2, 7).

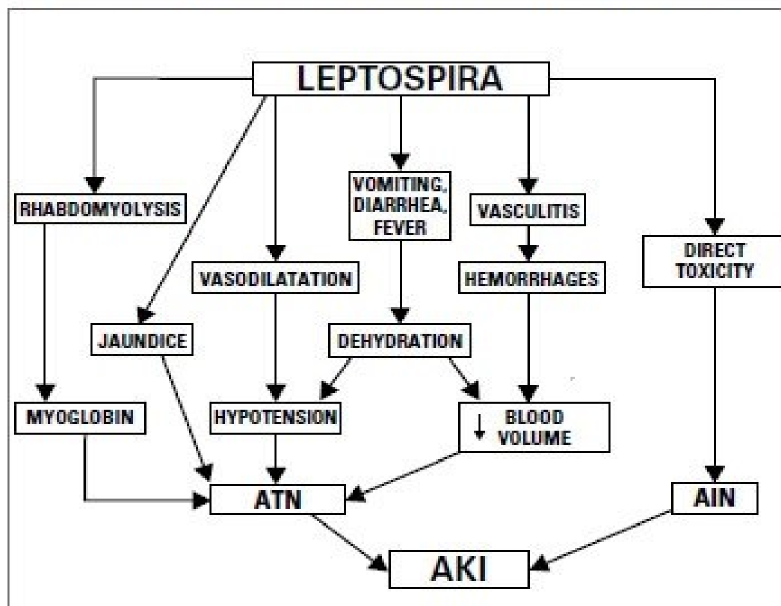


Figure 7: Physiopathology of AKI in leptospirosis (26)

2.5.3 Mortality of Weil´s disease

If not treated, Weil´s diseases mortality is about 20-30%. Patients usually die between day 9 and 16 of the infection, as a result of liver or multi organ failure, uraemia or shock.

2.5.4 Other manifestations

Weil´s disease is not the only severe form of leptospirosis, but there may occur also other clinical courses that may affect other organ systems. Patients may develop petechiae, internal bleeding and shock.

In certain cases of anicteric forms myocarditis and pulmonary oedema, SPHS or ARDS may occur. In many patients intra- alveolar haemorrhage was detected, although patients had presented no pulmonary symptoms.

2.5.5 Mortality

If myocarditis occurred in severe cases, a mortality rate of up to 54% was reported. Mortality rates of severe anicteric forms with pulmonary manifestations can also be up to 50%. In many cases thrombocytopenia occurs, but it´s not a result of DIC, rather an immunological phenomenon (1, 27)

Jaundice was thought to be a strong indicator for severity of the disease for many years, but couldn´t be proven in studies (2).

2.5.6 Complications and long-term effects

Conjunctivitis is very often seen in severe and mild cases. There also have been reports of abortion in pregnancy and foetal death because of the infection. Some rare complications are rhabdomyolysis, TTP, cerebrovascular events, GBS or reactive arthritis. There is also a possibility of long- term ocular effects after infection with leptospirosis, e.g. iridocyclitis, uveitis, iritis or chorioretinitis, which may develop a few weeks after the infection and can last up to years (2, 7).

2.6 Routine laboratory findings

In cases of leptospirosis white blood count (WBC) is often in normal range. However, leukocytes may be either elevated or reduced too. In severe cases usually leucocytosis with left-shift is found. In the study by Hoffmeister et al. median of WBC at time of admission was 9250 cells/µl, ranging from 2.800-20.600 cells/µl (28)

Thrombocytopenia is seen in more than 50% of all cases, however this is transient and does not result from disseminated intravascular coagulation (DIC) (1, 29). A moderate increase of C-reactive protein (CRP) is also frequently seen in patients with leptospirosis.

In many cases liver function is slightly affected, but depending on the severity of the infection, we may find high elevation in alkaline phosphatase (AP) and bilirubin. Alanine aminotransferase (ALT) often is only moderately elevated. A study by Andrade et al. from 2008 showed a mean level of 88.5 U/l, while direct bilirubin levels mean ± standard deviation (SD) was 13.5± mg/dl.

In severe cases a combination of high creatinine kinase and bilirubin without highly elevated transaminases and AP is frequently seen and parameters for kidney function like creatinine and GFR worsen. Hypokalaemia is more frequent seen than hyperkalaemia. Andrade et al. found potassium levels mean ± SD 3.8 ±1.2 mmol/l (30).

Because the routine laboratory findings may not be very specific, further microbiological tests are necessary to confirm the diagnosis.

2.7 Differential diagnoses

In most cases the patient presents with symptoms that are not very specific, like fever, myalgia, headache and mild gastrointestinal symptoms. It´s common to think of the flu, rather than of leptospirosis. That´s the reason why the prevalence of the disease is probably higher worldwide than recognized.

If the patient presents with fever and jaundice the first differential diagnosis is acute viral hepatitis. In severe cases of leptospirosis you´ll find also damage of the kidneys and thrombocytopenia.

Differentials could be malaria, Dengue or Yellow fever, Rickettsial disease, Hanta- Virus infections and leishmaniosis. If petechiae occur you must think of hemolytic- uremic syndrome (HUS), caused by *E. Coli*, *shigella* or meningococcal- sepsis.

Differentials for abdominal disorders and diarrhoea may include enteritis, and if the patient suffers from meningism, headache and fever, viral meningitis has to be excluded.

Even typhoid fever, Lupus, mononucleosis or HIV seroconversion can show similar symptoms. Despite a number of differentials the combination of fever, conjunctivitis, renal failure and jaundice is highly suspicious for leptospirosis. An efficient travel-, and exposition anamnesis is very important. The patient has to be asked about contact with rodents or potentially contaminated water, wet soil, other animals and stays in basic accommodations (2).

2.8 Clinical diagnosis

Because of its rapidity and easy operability, IgM quick test (e.g. Leptocheck) is one of the world´s most common tests for leptospirosis. This test uses the principle of immunochromatography and detects *Leptospira* -specific IgM antibodies, and can be read in 15 minutes. In a study from 2001-2012 performed by Goris et al., overall sensitivity and specificity of this test were 78%, respectively 98%. Lower sensitivities could be possible if the test is conducted at a very early stage of the disease, when IgM antibodies are not present at an detectable level (31).

Another common diagnostic test is micro agglutination test (MAT) but specific antibodies cannot be found until day 6 of infection. This test is serovar specific, so a huge amount of antigens has to be tested, and the test is very time consuming. A study by Limmathurotsakul et al. shows that the test is limited by the low specificity of only 50% for diagnosis of the infection. A reason for that could be that *Leptospira* antibodies may take several weeks to become detectable by MAT (32).

For cultivation on agars blood samples have to also be taken in an early stage of the disease (day 1-4). On day 5 only 50% of blood cultures are positive, during the following days a blood culture will be almost a 100% negative.

After about 10 days, in the leptouric phase, it´s also possible to cultivate urine samples. This method also requires a specialized laboratory.

The study by Limmathurotsakul also demonstrated that culture and MAT represent unexpected poor results compared to other diagnostic tests (33).

PCR is a very sensitive and quick method to detect *Leptospira* in patients' blood. The sample has to be taken within the first 4-7 days of infection. Unfortunately PCR is not available everywhere around the world, and if the burden of *Leptospira* organisms in the sample is low, the test may be negative.

Another way of proof is dark-field microscopy. It has to be made in an early stage of the disease, with blood, urine or liquor. Specificity and sensitivity are low when analysing blood, because membranes of the erythrocytes can be misinterpreted as spirochetes. The motility of the spirochetes is the most important fact in diagnosing leptospirosis via dark-field microscopy.

In the last few years also ELISA established in the diagnosis of leptospirosis to proof specific IgG and IgM antibodies, although specificity is because of cross reactions lower than with other tests (2, 33).

2.9 Therapy

Therapy of leptospirosis depends on the symptoms of the patient. The most important action is to start antibacterial treatment as soon as possible. In 2000 a systematic review on different therapies was published on Cochrane database. The first choice for severe leptospirosis is penicillin and ampicillin (34), 6-8 mega benzylpenicillin i.v. every 6 hours. Also cephalosporins of the 3rd generation like ceftriaxone (1-2 g/d) or cefotaxim are effective against the infection. If there is an allergy to these antibiotics, also doxycycline, (100mg oral every 12 hours), or erythromycin (500mg oral every 12 hours) can be given. Aminoglycosides should be well considered because of their nephrotoxicity (2).

Studies also found doxycycline to be effective in prevention of leptospirosis in patients with high risk exposure.

WHO guidelines recommended doxycycline only in less severe courses of diseases. Because of its toxicity for children and pregnant woman the drug should not be used in these groups of patients (34).

A rare complication of antibiotic treatment of infections with spirochetes can be the Jarisch- Herxheimer- reaction. It´s caused by endotoxins that are released when a large amount of bacteria disintegrate. That causes vasoconstriction with hypertension, fever, chills and paleness.

After that, symptoms may change to hypotension and flush because of vasodilatation, the process is similar to an anaphylactic shock. Patients also may develop cephalea, myalgia, arthralgia or a rash. Treatment is symptomatic, substitution of volume and cortisone are key factors.

If the patient complains about severe headache, a lumbar puncture often allays the symptom. If the patient shows acute renal failure, ARDS or MODS, transfer to an ICU is necessary.

Patients with prerenal azotaemia have to be rehydrated with observation of their kidney function. If renal failure requires HD, low coagulation parameter are no contraindication (2).

2.10 Prevention

There is a vaccine against leptospirosis for livestock and dogs that lowers, but doesn´t eliminate the risk for human infection. Because of the variety of animal reservoirs it´s not possible to eradicate leptospirosis. The risk of infection during occupational exposure can be reduced by wearing suitable protective clothing (e.g. gloves and shoes) that prevent skin lesions and hygiene measures. Education about the disease and the latter would also lower the risk of infection in countries with lower life standard, but this is often not possible due to costs. Individuals should also avoid swimming or wading in water that may be contaminated with infected urine. Until now the search for a human vaccine has not been successful. The immunity to leptospirosis is for the most part humoral and serovar specific. So immunity persists only for the homologous serovar (2).

In high risk activities like contact with lots of water in tropical areas, prophylaxis with Doxycycline shows acceptable protection against acquiring leptospirosis (35).

3 Methods

Leptospirosis is, by the epidemic act of 1950, a notifiable disease in Austria. The aim of the study was to retrospectively analyse the risk factors for acquiring leptospirosis, symptoms and laboratory findings, as well as the different *Leptospira spp.*.

3.1 Study setting

The study took place at the Section of Infectious Diseases and Tropical Medicine at the Medical University of Graz between the years 2011-2013.

3.2 Patients

All leptospirosis cases that presented over a 9- year period, from the 1st of January 2004 until 31st December 2012, were included. All patients were either transferred from other hospitals or general practitioners, or had directly sought medical help at the University Hospital Graz, which has a catchment area of about 1.6 million people.

3.3 Inclusion criteria

- male and female patients age ≥ 18 years

- tested positive for leptospirosis (Leptocheck-WB; rapid test for IgM antibodies to *Leptospira*)

- positive POC (Point- of- Care; Leptocheck- WB) test; retesting if only slight positive the first time

- clinical symptoms suspecting leptospirosis

3.4 Exclusion criteria

- POC (Leptocheck-WB) test negative at the first time

- POC test negative at retesting, if only slightly positive at the first time of testing

- odd symptomatology

3.5 Data

Data concerning age and sex of the patient, history of travel (up to 3 weeks before onset of infection) clinical course of the disease, symptoms, date of diagnosis, laboratory results and length of hospitalization were collected via the electronic patients database (MEDOCS) of Styrian hospitals.

Risk factors like recreational, occupational, nutritional, accidental and residential risk factors, were evaluated by a self-composed questionnaire via telephone.

Because we know of the path of transmission of the bacteria and possible reservoirs, the focus of leisure behaviour was on outdoor related activities within two to three weeks prior to onset of the first symptoms.

The questionnaire included:

Recreational risk factors

- fishing, swimming in lakes, ponds,

- camping, trekking, canyoning

- rafting, surfing, caving

- hunting, gardening

- activities or stay in wet areas

Residential/Occupational risk factors

- contact to pets
- contact to farm animals
- rats, mice or water within the living area
- farms
- excavation/canal work
- cleaning/ renovating basements, attics or huts
- own fruit or vegetable growing
- buying food at farmer/ farmers market

In lab findings we concentrated on white blood count, thrombocyte count, C-reactive protein (CRP), potassium, serum creatinine and ALT levels. We chose the standard values from the references of the laboratory of the LKH Graz.

Because in some cases I could not find further information of the infecting serovar in MEDOCS, we contacted the AGES Mödling to get this data. This institution analyses the patients´ blood samples with microagglutination test (MAT) for the different serovars of *Leptospira* and collects the data of all cases in Austria. Because only data until 2007/2008 were available on their computer system, I went to Mödling and looked up all cases from 2004-2006 by hand to get the results.

3.6 Statistical analysis

The study was performed retrospectively. All data were entered electronically in a case report form and evaluated with Microsoft Excel.

We calculated occurrence of the various risk factors, symptoms and distribution between gender, as well as median, arithmetic average, standard variance and quartiles of laboratory parameters.

A p-value <0.05 was defined as statistically significant, chosen tests were Chi-square test and Fisher exact test.

4 RESULTS

Data were collected over a period of 9 years, from 2004 to 2012. All patients were seen and treated at the LKH Graz, either as in- or outpatients. All together we found 128 patients tested positive with leptospirosis by quick test. 82 (64.1%) patients were reached by telephone and finished the questionnaire. Additional data of these patients (e.g. lab results) were collected via MEDOCS. 46 (35.9%) patients could not be reached by telephone. Therefore information was collected only with MEDOCS only and included laboratory results and clinical data, but risk factors only if they were written down in the anamnesis of the medical report.

79 (61.7%) of all patients were male, 49 (38.3%) female.

4.1 Age

The median age of all included patients was 43 years. Age ranged from 18 to 89 years at time of diagnosis. Female median age was distinctly higher with 46 years than male with 41 years.

The graph below shows the age distribution of all patients.

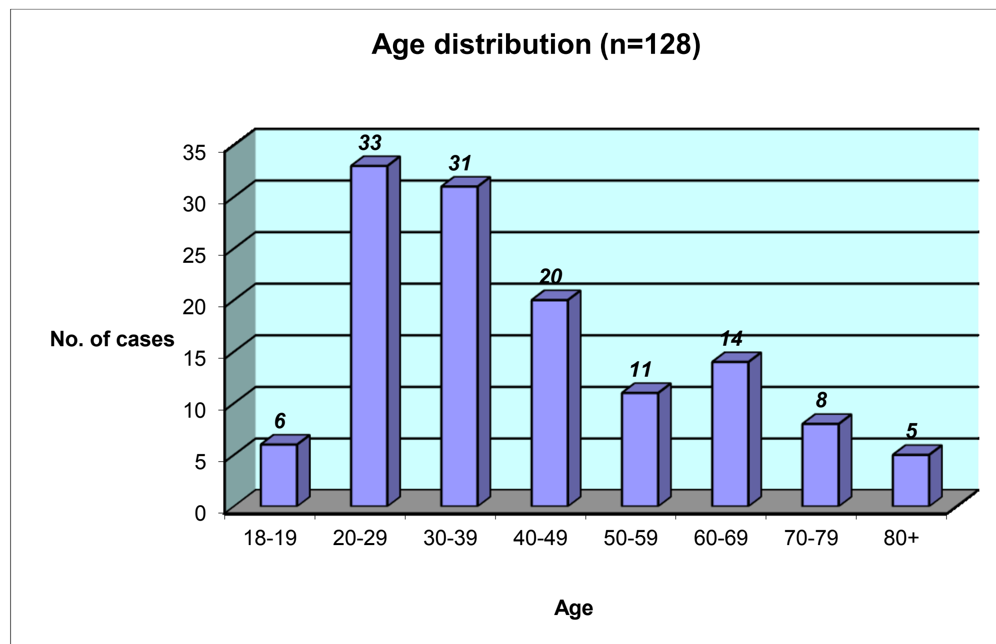


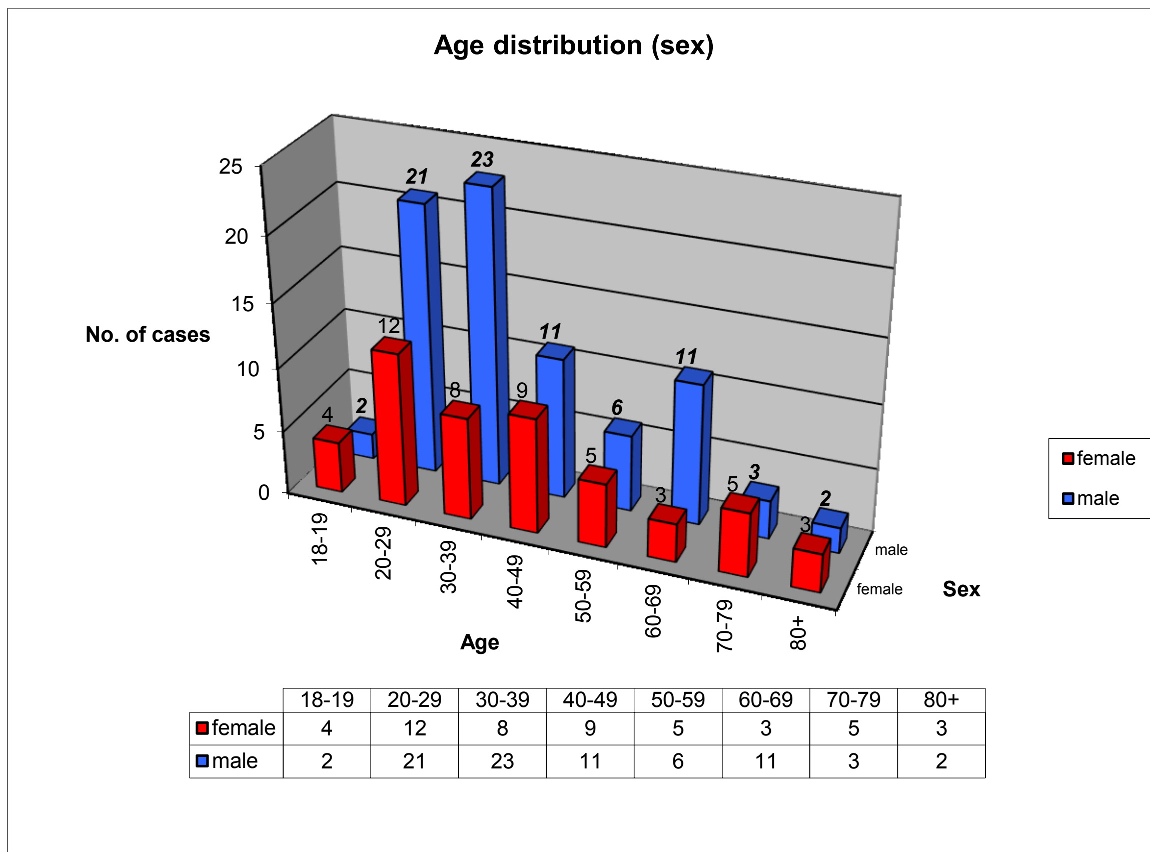
Figure 8: Age distribution

A peak of infections occurred at the age of 20-39 years. Overall 64 infections occurred in patients at his age group, which is exactly 50%. The highest number of infections was seen at the age of 20-29 with 33 patients (25.8%), and from 30-39 with 31 infections (24.2%). 20 patients (15.6%) were between 40-49 years at time of infection. Only 11 cases (8.6%) occurred in patients from 50-59, and 14 (10.9%) between 60- 69. 13 patients (10.2%) were older than 70 years, 5 of them (3.9%) over 80 years. 6 patients (4.7%) were between 18 and 20 years.

4.1.1 Age distribution by gender

Age distribution subdivided in females and males showed the highest peak of infections in males between the age of 30-39 and in females at 20-29 years. 23 of 79 males (29.1%) were between 30-39 years, and 12 out of 49 females (24.5%) were from 20-29 years at time of infection.

55.7% of all male patients and 40.8% of all female patients had acquired leptospirosis between 20-39 years. 11 men (13.9%) and 9 women (18.4%) were at age 40-49. Another peak at age 60-69 occurred in male patients only (13.9%). At the age over 70 the percentage of females was 16.3%, while only 6.3% of male infections occurred at this age.



	18-19	20-29	30-39	40-49	50-59	60-69	70-79	80+
female	4	12	8	9	5	3	5	3
male	2	21	23	11	6	11	3	2

Figure 9: Age distribution split by gender

4.2 Symptoms

Symptoms were determined with MEDOCS and, if possible, by questionnaire. Patients showed the first symptoms, if cause and time of infection were rememberable, at average 4-10 days after infection. Following symptoms, as shown on chart below (n= 128), were the most recorded ones.

Fever >38.5°C and chills were the most common symptoms and occurred in 83 patients (64.8%) at the very beginning of the disease. More than one third of all patients (35.2%) complained about myalgia and/or arthralgia, especially calf and back pain. 41 patients suffered from abdominal pain/diarrhoea (32%), 36 from weakness, exhaustion or fatigue (28.1%) and 31 patients developed jaundice (24.2%) during the infection. Headache was seen in 27 patients (21.1%) and nausea and/ or vomiting in 26 patients (20.3%).15 patients developed acute kidney failure (AKF; AKI) (11.7%) either present at time of admission, or developing during their stay at the hospital.

6 patients (4.7%) showed a hyposphagma or abnormal bleeding (petechiae, nose bleeding).

There were also other symptoms like meningism, cough, dyspnoea, exanthema, iridocyclitis, conjunctivitis, ascites and cardiac symptoms like myocarditis and arrhythmia, reported, but only in a small number of cases.

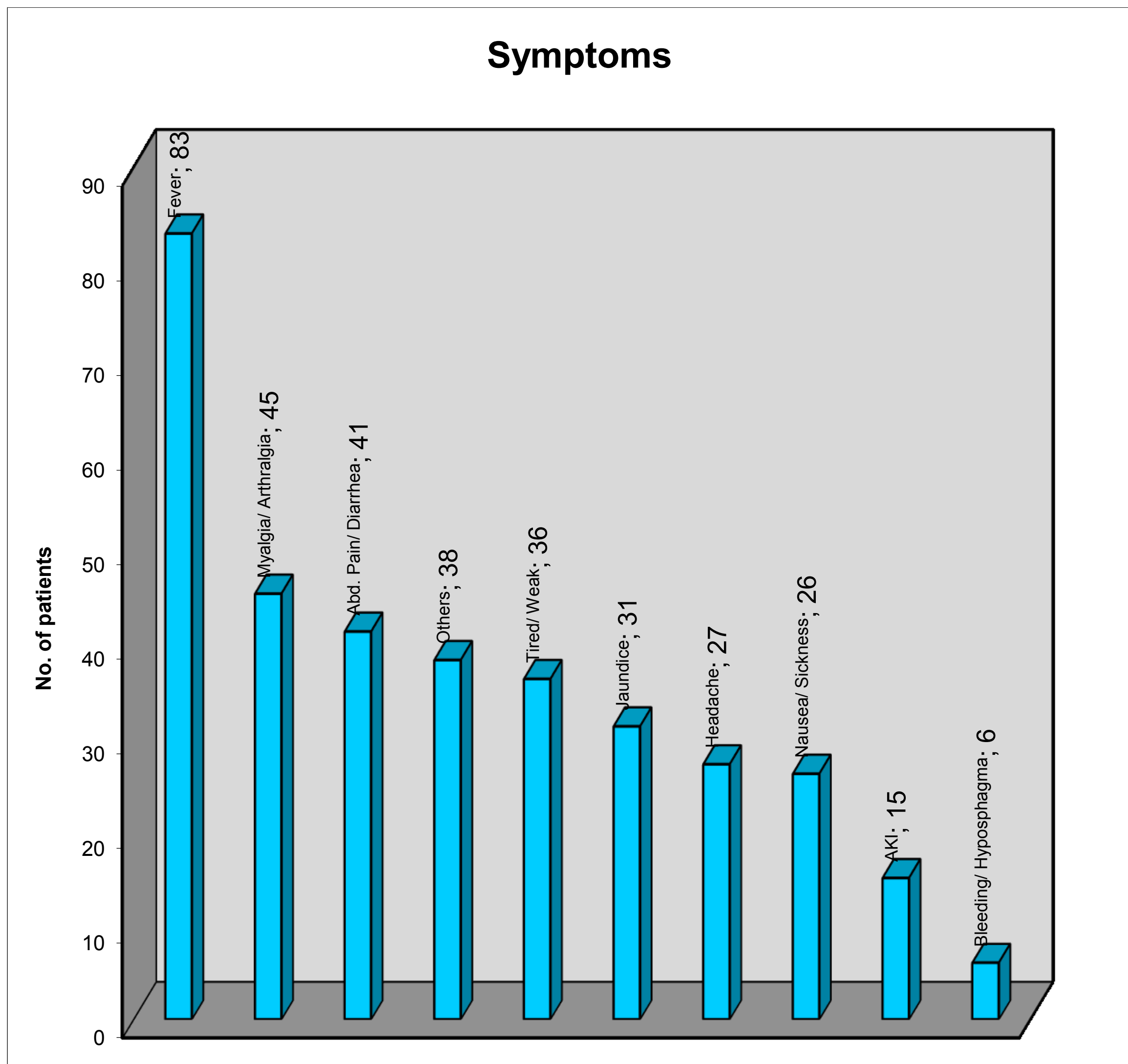


Figure 10: Most frequently reported symptoms

4.2.1 Distribution of symptoms by gender

Because of the relatively high number of female patients (38.3%), we tried to compare symptoms between male and female patients. We found significant differences in the symptoms abdominal pain/diarrhoea and headache.

Male patients showed abdominal pain/diarrhoea in 37%, female patients in only 20.4%, p=0.037. 30.6% of all females, but only 15.2% of male patients suffered from headache, p=0.045

Differences were also found with regard to nausea/sickness, 28.6% females and only 15.2% males presented with this symptom, but they were not statistically significant (p=0.079).

Also no significant differences were found for the symptoms fever (p=0.456), myalgia/arthralgia (p=0.640), ARF (p=0.675), jaundice (p=0.428), bleeding or hyposphagma (p=0.405) and general weakness (p=0.370).

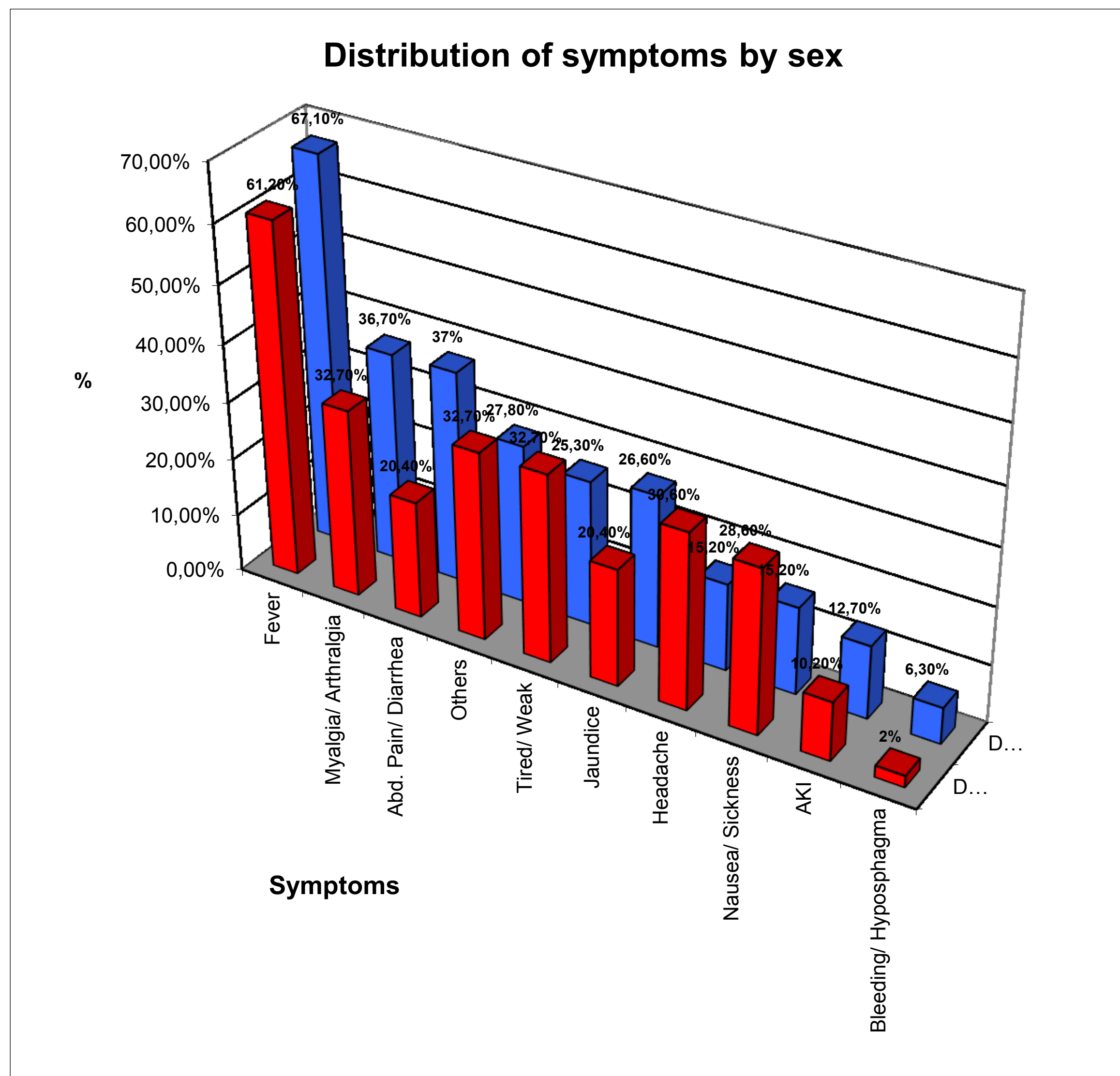


Figure 11: Distribution of symptoms split by gender

7 patients, 4 males and 3 females, suffered from a severe course of leptospirosis. 3 patients were septic (2.3%), and each one patient (0.8%) suffered from meningoencephalitis, ARDS, and SPHS.

One male patient, working as a GSM technician in Afghanistan, had imported leptospirosis and suffered from Weil´s disease.

4.3 Risk factors

One of the main objectives of this study was evaluation of potential recreational and residential risk factors for acquiring leptospirosis. 82 patients out of 128 answered the questionnaire and could be asked for every risk factor.

4.3.1 Recreational risk factors

In the category "*recreational risk factors"* patients were asked for unexceptional daily activities.

48/82 patients (58.5%) were doing different *activities in woods or wet areas*, like hiking, or walking with their dogs, or looking for mushrooms. Out of 38 patients (46.3%), 37 patients were *gardening*, only 1 patient has been *hunting* (deer) where he probably got infected with leptospirosis. 28% of patients were *tidying up or renovating an attic, basement or hut*. Every patient saw mice or excrements of rodents. None of them wore gloves, masks or other protective clothing.

14 patients (17.1%) were doing water related activities, like *swimming or diving* in a pond, lake or river and another 11 questioned individuals (13.4%) were *trekking or camping* in rural areas. 9 patients (11%) were working on a field, in a stall or canal within 2-3 weeks before onset of the first symptoms. 1 patient reported that he had been *surfing* in a river (Mur) and another was climbing in a *cave*.

Recreational risk factors are depicted in the figure below.

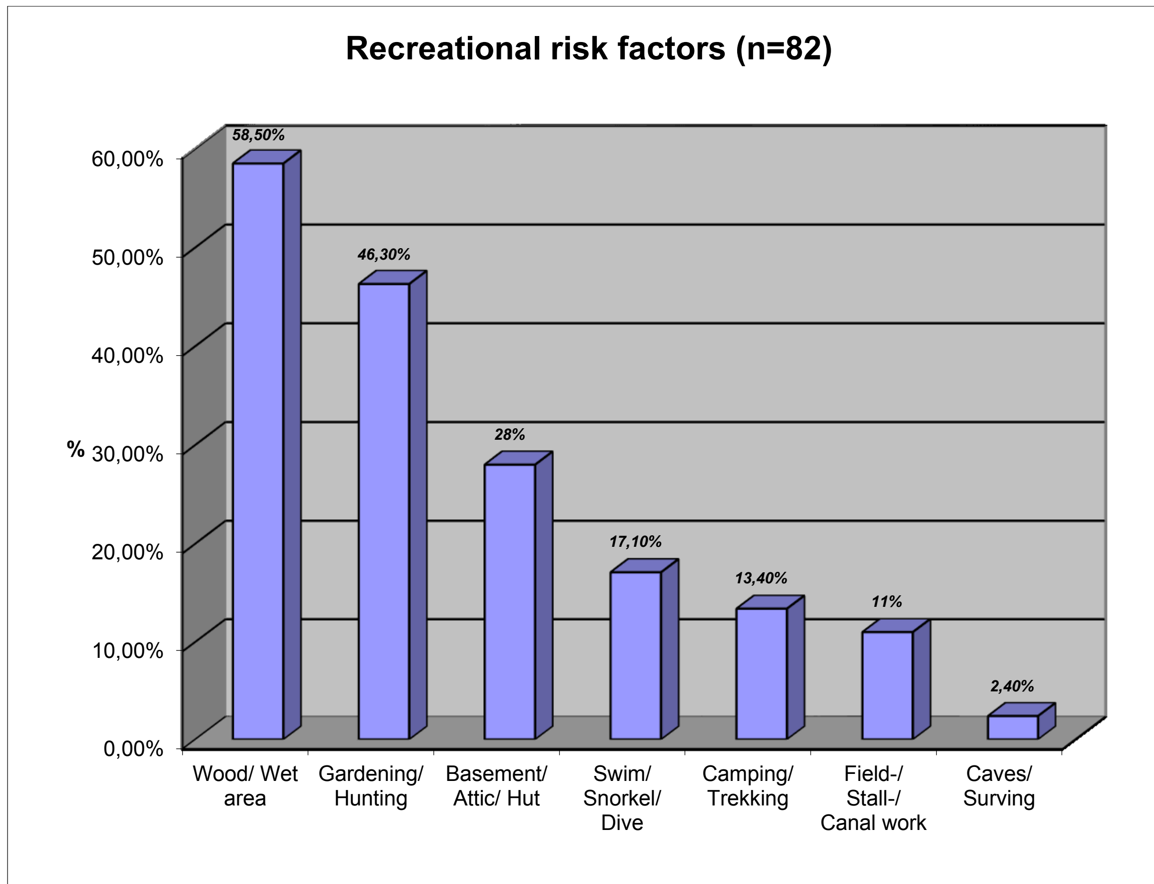


Figure 12: Recreational risk factors

7 interviewees were *working with wood* (e.g. wood chopping and stacking),4 patients had close *contact to mammals* (feeding nutrias, handling a dead mole and disposing dead mice without any protection) and 3 had lots of *contact with soil* (two persons working in a nursery and one as a landscaper). The 2 patients who worked in the canal system, didn´t wear protective gloves and one of them suffered from a cut on his hand, while the other one got canal water in his face and mouth. 1 veterinarian got his face accidentally splashed with pig urine during work in a slaughterhouse.

One patient worked in a shutdown sewage plant, with lots of mice in surroundings, and reported that he did not wear a protective mask and inhaled a lot of dust only a few days before onset of the first symptoms. One patient described putting a golf-T in his mouth, while playing golf right next to the woods.

Also one single person remembered, while working in a club, drinking out of a can that was not wrapped in plastic and stored in a stockroom, which also served as living environment for mice.

Another patient ate some blueberries while collecting them from a natural grown blueberry field in the woods, which was the patients' only risk factor for acquiring the infection.

46/128 patients have not been reached by telephone to complete the questionnaire. Recreational risk factors of those patients were collected via MEDOCS. Four patients (8.7%) were doing different activities in woods/wet area, 3 (6.5%) were tidying up an attic/basement od hut, 1 patient reported gardening and 1 patient canal work as a risk factor. Because we could not find more information on MEDOCS of patients that did not complete the questionnaire, the number of known recreational risk factors is very low.

4.3.2 Differences in recreational risk factors by gender

Because of the relatively high percentage of infections in females, 32/82 (39%), recreational risk factors were split up by gender. There were no significant differences in risk factors *caves/surfing* (male 4%, female 0%), *trekking* (m 12%, f 6.3%) and *camping* (m 2%, f 6.3%). Also differences in *swim/ snorkel/dive* (m 16%, f 18.8%) were not noticeable. We found, however, differences with regard to *activities in woods/wet area*, which was reported by 32 out of 50 male patients (64%), but only 15 of 32 female patients (46.9%). Contrarily we found that the risk factor *gardening/hunting* (only one patient was hunting), was more frequently present in females. 56.3% (18 patients) of all females, but only 40% (20 patients) of all male patients reported working in the garden within 2-3 weeks before onset of the first symptoms (Table 8).

The differences between genders were not statistically significant ($p<0.05$).

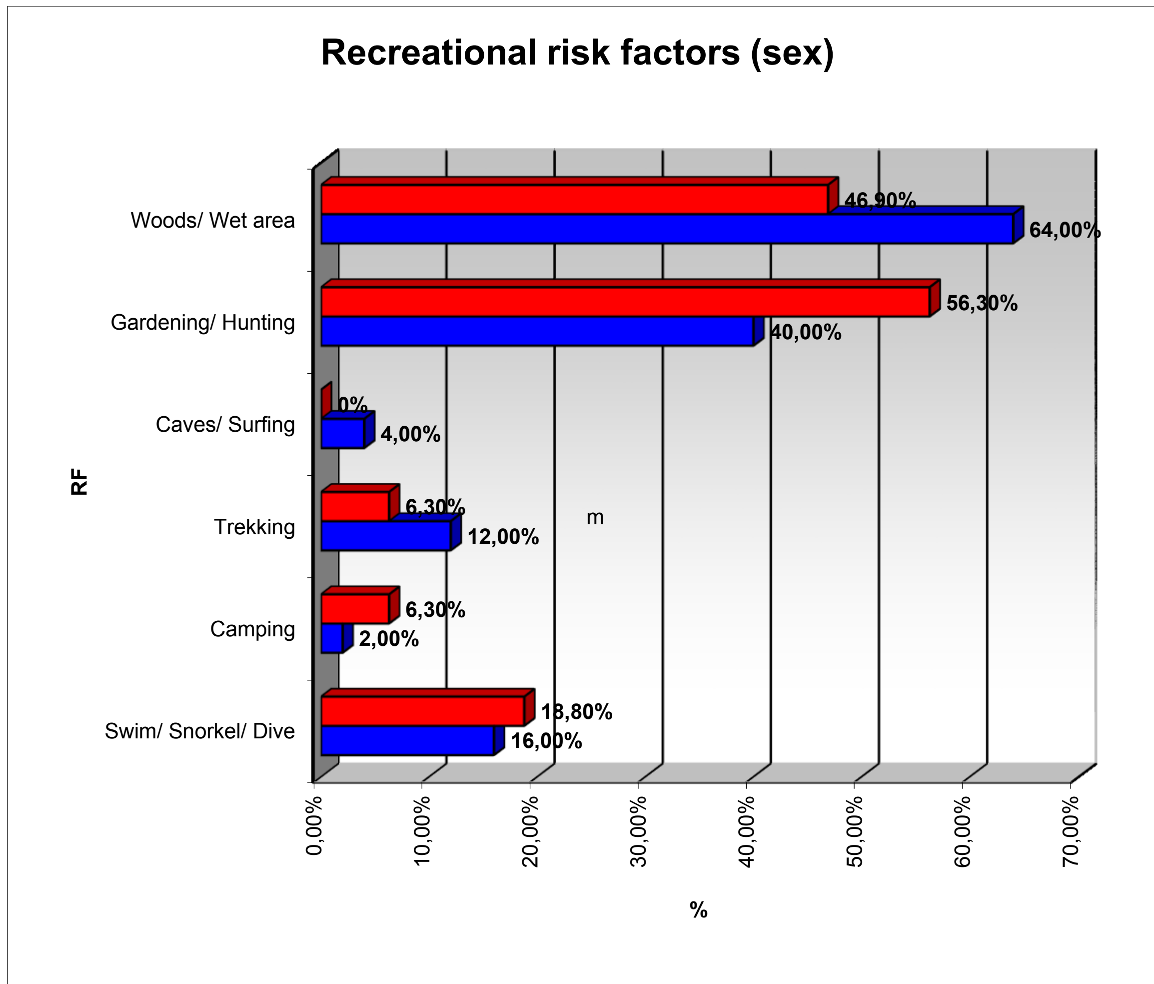


Figure 13: Recreational risk factors split by gender (red- female; blue- male)

4.3.3 Residential risk factors

Also residential risk factors were analysed of patients that answered the questionnaire (n=82). The most important *residential risk factor* for acquiring leptospirosis was shown to be *rats or mice in surroundings* (around the house, in the attic, basement or backyard) occurring in 73.2% (60 patients). 41 persons (50%) owned *pets* (21 dogs, 29 cats), and 19 (23.2%) had eaten fruit or vegetables *grown in their own garden*. 15 patients (18.3%) had *water in their surroundings* (they were asked for a pond, biotope, well or creak). 12 patients (14.6%) had *farm animals* like pigs, cows, horses and chicken, while 13.4% (11 patients) were *living and/or working on a farm* summarized to *farm/farm animals* (28%). Out of 82 patients, 7 (8.5%) did excavation work, within 2-3 weeks before onset of the first symptoms.

Two patients who lived right next to a wood reported an *animal bite* (tick) only a few days before onset of the first symptoms, Lyme disease and tick- borne encephalitis had been excluded.

Data are shown on the graph below:

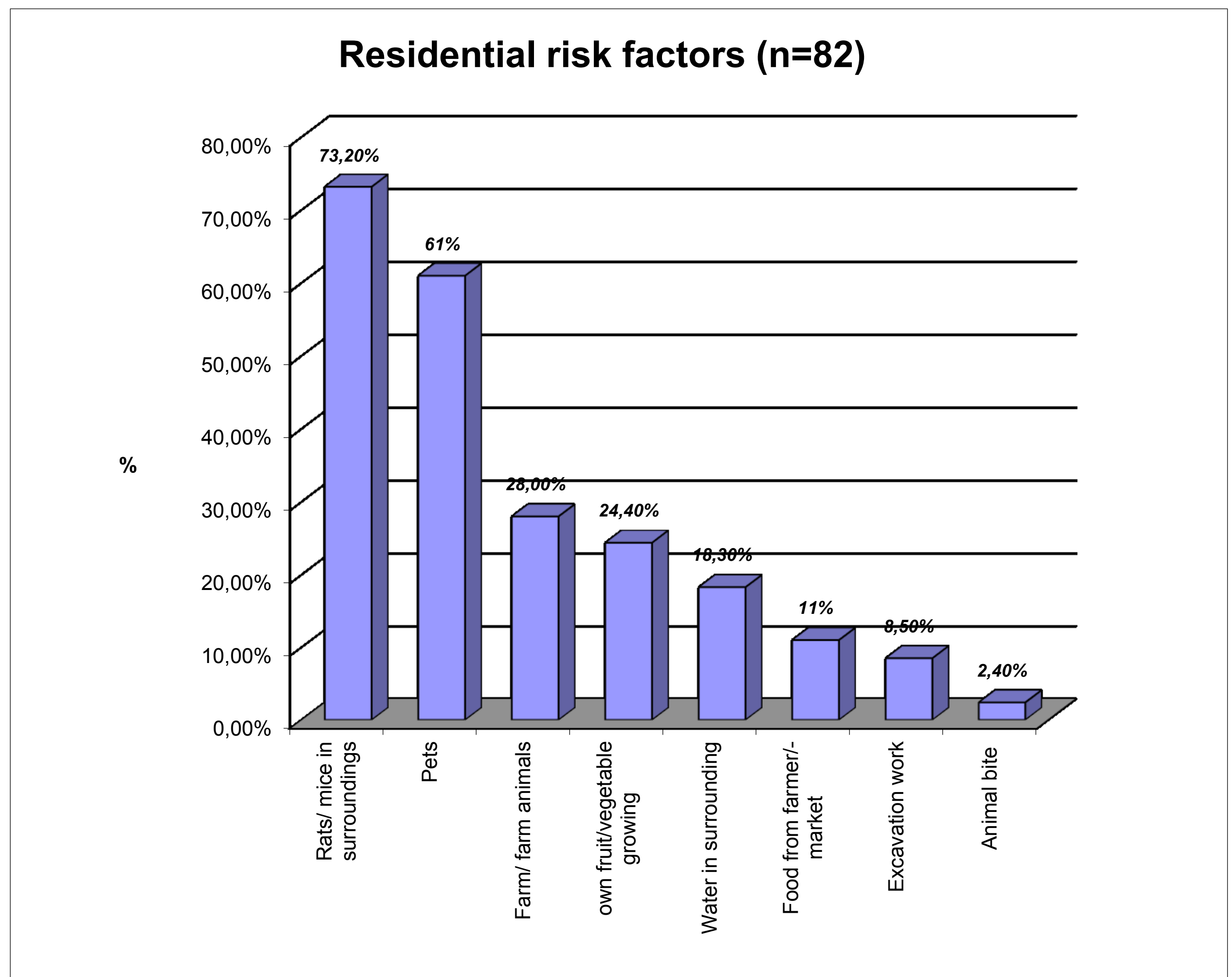


Figure 14: Residential risk factors

As for recreational risk factors, we also got the data for residential risk factors of 46 patients, who did not answer the questionnaire, out of MEDOCS. 9 patients (19.6%) owned a pet (2 cats, 8 dogs), 3 (6.5%) reported rats/mice in surroundings and 1 patient (2.2%) had water in surroundings.

4.3.4 Differences in residential risk factors by gender

Residential risk factors of interviewed patients (n=82; 50 males, 32 females) were split up by gender too. In the risk factor *excavation work*, males 10% (n=5) and females 6.3% (n=2) and *growing own fruit and/or vegetable*, 8 females (25%), 11 males (22%), there was no meaningful difference seen.

Also *rats and/or mice in surroundings* was denoted in equal parts, 74% (n=37) of males and 71.9% (n=23) of females reported this risk factor. Occurrence of the risk *factors food directly from a farmer or farmers market, living and/ or working on a farm, water in living surroundings, farm animals, pets* and *growing own fruit/vegetables*, however, differed between genders.

65.6% (n=21) of all female patients held at least one *pet* that was allowed to go outside of the domicile, and so had the possibility of getting infected with leptospirosis, while 58% (n=29) male patients owned a pet. 22% of male patients (n=11) had *water in their living surrounding*, in female patients only 12.5% (n=4) answered this question positively.

The most significant difference was found in the risk *factor farm and farm animals*. Only 4.2% females (n=2) lived or worked on a farm and 4.2% (n=2) held farm animals, while 11.4% males (n=9) worked/lived on a farm and 12.7% (n=10) held animals, like chicken, pigs, sheep or cattle. We also found differences in buying *food (vegetable and fruits) directly from farmers or on a farmers market*. 18.8% of female patients (n=6) and only 6% of male patients (n=3) did so.

None of the results however showed to be statistically significant.

Differences are depicted in the figure below.

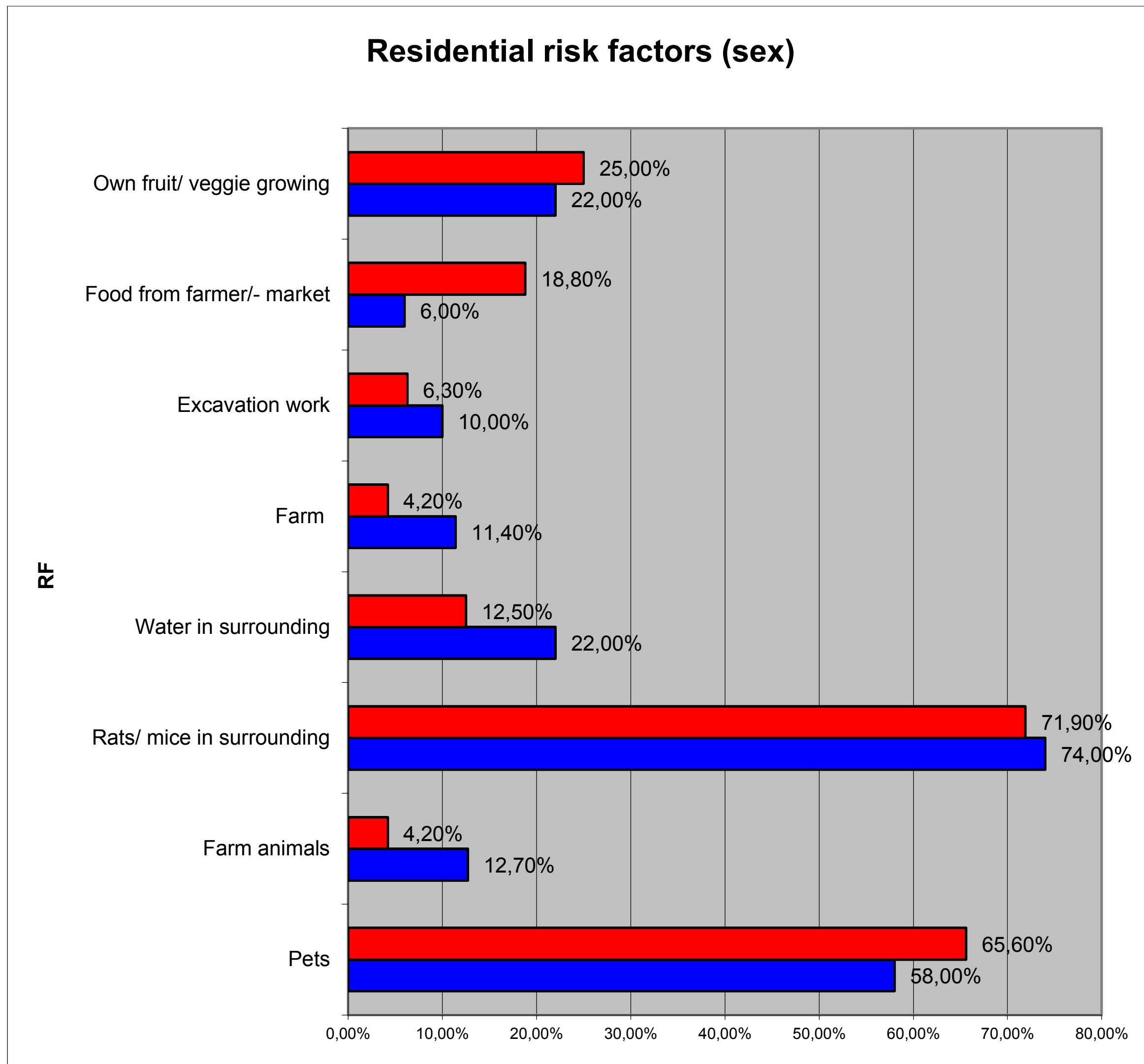


Figure 15: Residential risk factors split by gender (red- female; blue- male)

4.4 Seasonal distribution

Another point of interest was the seasonal distribution of infections with leptospirosis. All collected data of the 9- year observation period were put together (n=128).

The first peak rises in March with 10 infections (7.8%). The highest rate of infections occurs between May and August, with altogether 63 infections, which means almost half of all infections (49.2%), happened in those 4 months. The very highest peak appears in July (17 infections), followed by June, August and October (each month 16 infections).

Lower rates were found in September- 10 cases- (7.8%), November and April (each month 7 cases; (5.5%), and January and December- each month 6 cases, 4.7%.

In February only 3 cases (2.3%) were detected in the 9 year period, as shown on the graph below.

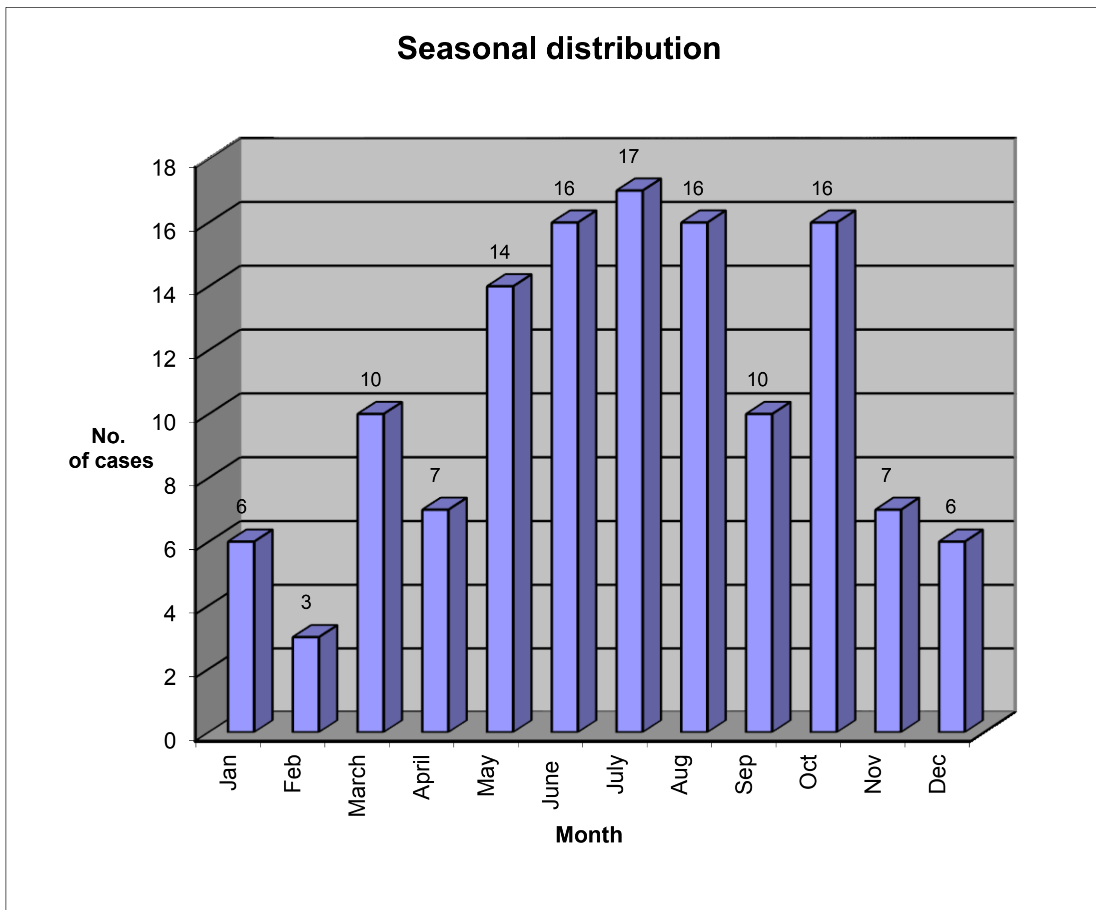


Figure 16: Seasonal distribution of leptospirosis

4.5 Case distribution over years

Analysis of the number of cases over the 9 years shows an interesting distribution. In the 9 years period under review an average of 14.2 reported cases per year occurred. The highest number of infections occurred in 2007, with a total number of 29 cases. 25 infections were reported in 2008 at the Microbiology Laboratory, Medical University of Graz, followed by 2006 with 21 cases, and 2005- 17 infections,14 infections were reported in 2004.

In the years from 2004 until 2008 all in all 106 (82.2%) cases of leptospirosis were recognized. The lowest rates of infection were found in the last 4 years, from 2009-2012.

Altogether 22 infections occurred in this time period. In 2009 and 2012, each year 6 infections were recognized; in 2010 and 2011 only 5 cases occurred each year. In those 4 years only 17.8% of all infections were reported.

Out of all, 8 cases were probably imported, 4 patients reported having been in Asia, 3 in Africa and 1 in South America, within 3 weeks before onset of the first symptoms, but they also had potential sources of infection back home in Austria (gardening and pets). One patient remembered drinking out of a not originally sealed bottle in India and one patient described swimming in a small river and sleeping in a dusty hut in South America.

The figure below shows the distribution of all cases (imported and autochthonous) from 2004 until 2012.

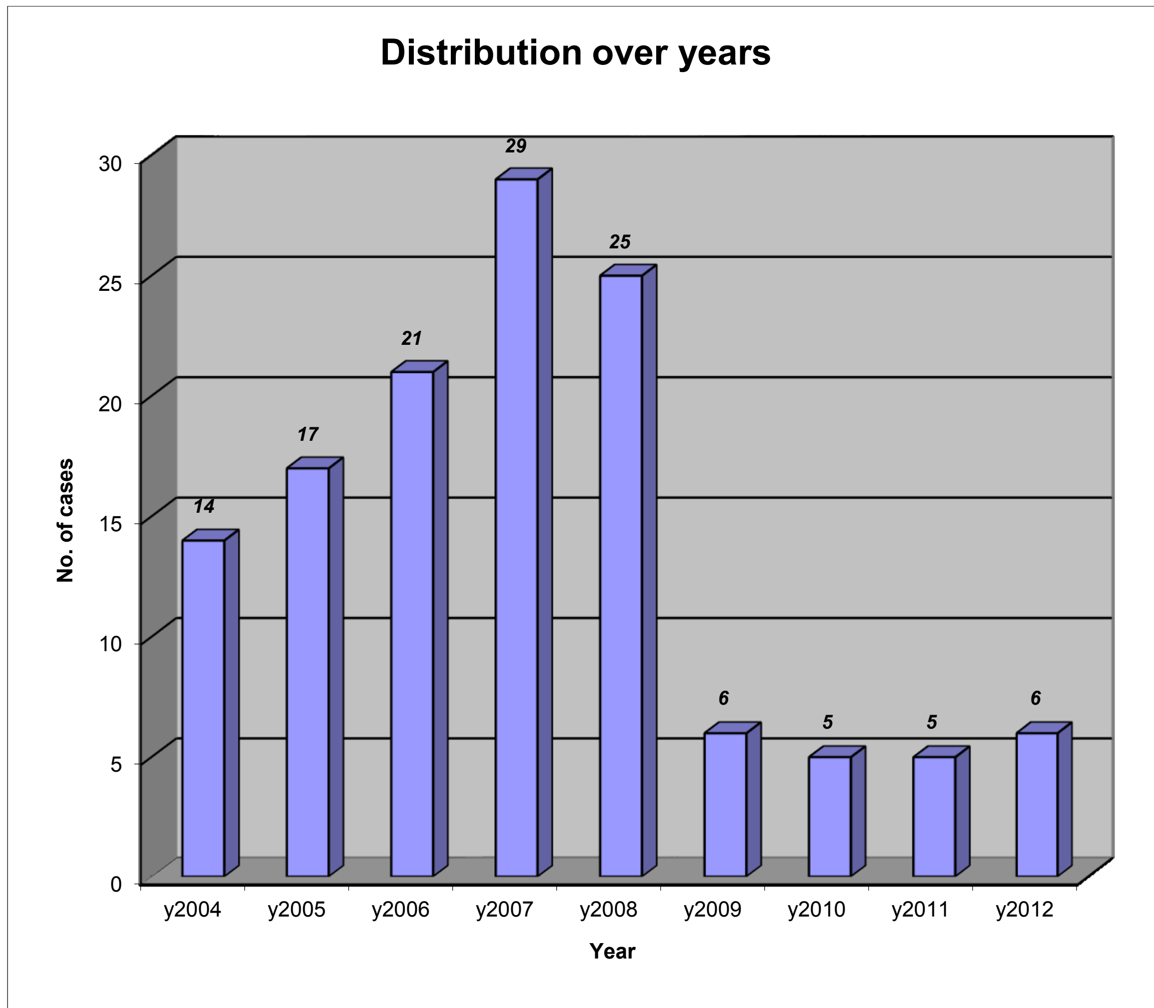


Figure 17: Distribution over years

4.6 Laboratory findings

Laboratory findings described were from blood samples, taken the day of the first contact with the patient at the LKH Graz. Because all data could be found in MEDOCS we were able to get the laboratory results of all 128 patients.

We focused on CRP (C- reactive protein) and white blood cell count, they were chosen as signs on an acute inflammation. With regard to leucocytes the range between 4400 and 11300 cells/µl was defined as normal, CRP above 5 mg/l was determined as sign of the acute inflammation. Liver involvement was rated by ALT (GPT) ≥2 times normal value (males ≥90 U/l, females ≥70 U/l), and involvement of the kidneys by the level of potassium, either hypokalaemia (≤3.5mmol/l) or hyperkalaemia (≥5.5 mmol/l), and the level of serum creatinine (males ≥1.2 mg/dl, females ≥1.0 mg/dl).

Because the infection with leptospirosis may also be accompanied with thrombocytopenia, this parameter was involved in statistics too. Levels below 140.000 cells/μl were set as thrombocytopenia.

White blood count

33 patients (25.8%) showed leucocytosis (≥11.300 cells/μl), the highest level we found was 32710 cells/μl at time of admission to the hospital. In 17 patients (13.3%) we found leukopenia (≤4400 cells/μl), median of all leucocyte results was 7660 cells/μl. We calculated the interquartile range (IQR), Q1 was 5660 cells/μl and Q3 11.400 cells/μl.

Thrombocytes

46 patients (35.9%) suffered from thrombocytopenia (≤140.000 cells/μl), median 185.000 cells/μl, first quartile 115.000 cells/μl and third quartile 245.000 cells/μl. Only 2 out of the 26 patients with thrombocytopenia suffered from abnormally epistaxis, no other bleeding complications were recorded.

CRP

In 109 patients (85.2%) CRP was higher than 5 mg/l, median 38.6 mg/l, first quartile 10.1 mg/l, third quartile 115.3 mg/l and the highest finding 418 mg/l.

ALT

The liver parameter alanine aminotransferase (ALT) was increased (two times normal value: ≥ 90 U/l in males; ≥70 U/l in females) in 57 patients (44.5%). The median was 66 U/l, first quartile 26 U/l, third quartile 269 U/l, whereas the range was from 10 U/l to 9944 U/l.

Serum creatinine

Pathologic serum creatinine indicating that the kidney function is affected was determined at ≥1.2 mg/dl for males and ≤1.0 mg/dl for females.

This was found in 54 of all 128 patients, which are 42.2%. Median was 1.03mmol/l, first quartile 0.89 mmol/l, and third quartile 1.9 mmol/l. The highest level, 15.51mmol/nl, we found in a male patient who showed signs of a severe course of disease already at time of admission.

Potassium

Hypokalaemia (potassium levels below 3.5 mmol/l) was detected in 23 patients (18%), hyperkalaemia (levels above 5.0 mmol/l) was found in 7 blood samples (5.5%). Median in potassium levels was 4 mmol/l, first quartile 3.7 mmol/l and third quartile 4.3 mmol/l.

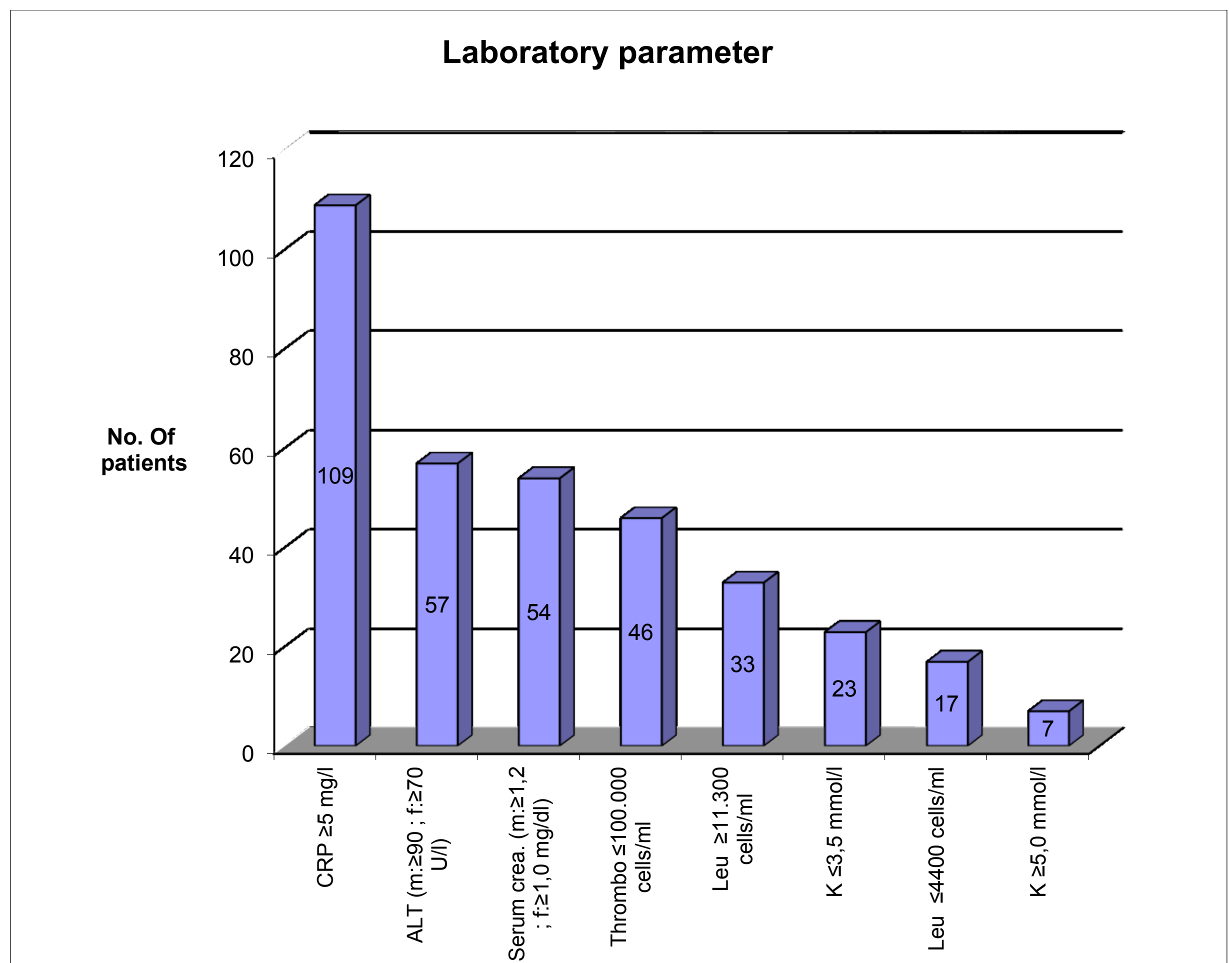


Figure 18: Laboratory parameters

4.6.1 Distribution of laboratory parameters by gender

We also analysed differences in laboratory parameters between female and male patients.

White blood count

In 22 (27.8%) of all male patients and 11 (22.4%) of all female patients we found leucocytosis as sign of infection, and 11 (13.9%) of all male patients and 7 (14.3%) of all female patients suffered from leukopenia.

Thrombocytes

Thrombocytopenia was found in 28 (35.4%) of all male patients, and 18 (36.7%) of all female patients.

CRP

67 out of 79 (81.7%) male patients and 42 out of 49 female patients (85.7%) showed increased CRP values.

ALT

In 26 female patients (53.1%), and 31 male patients (39.2%) ALT was increased two times normal value.

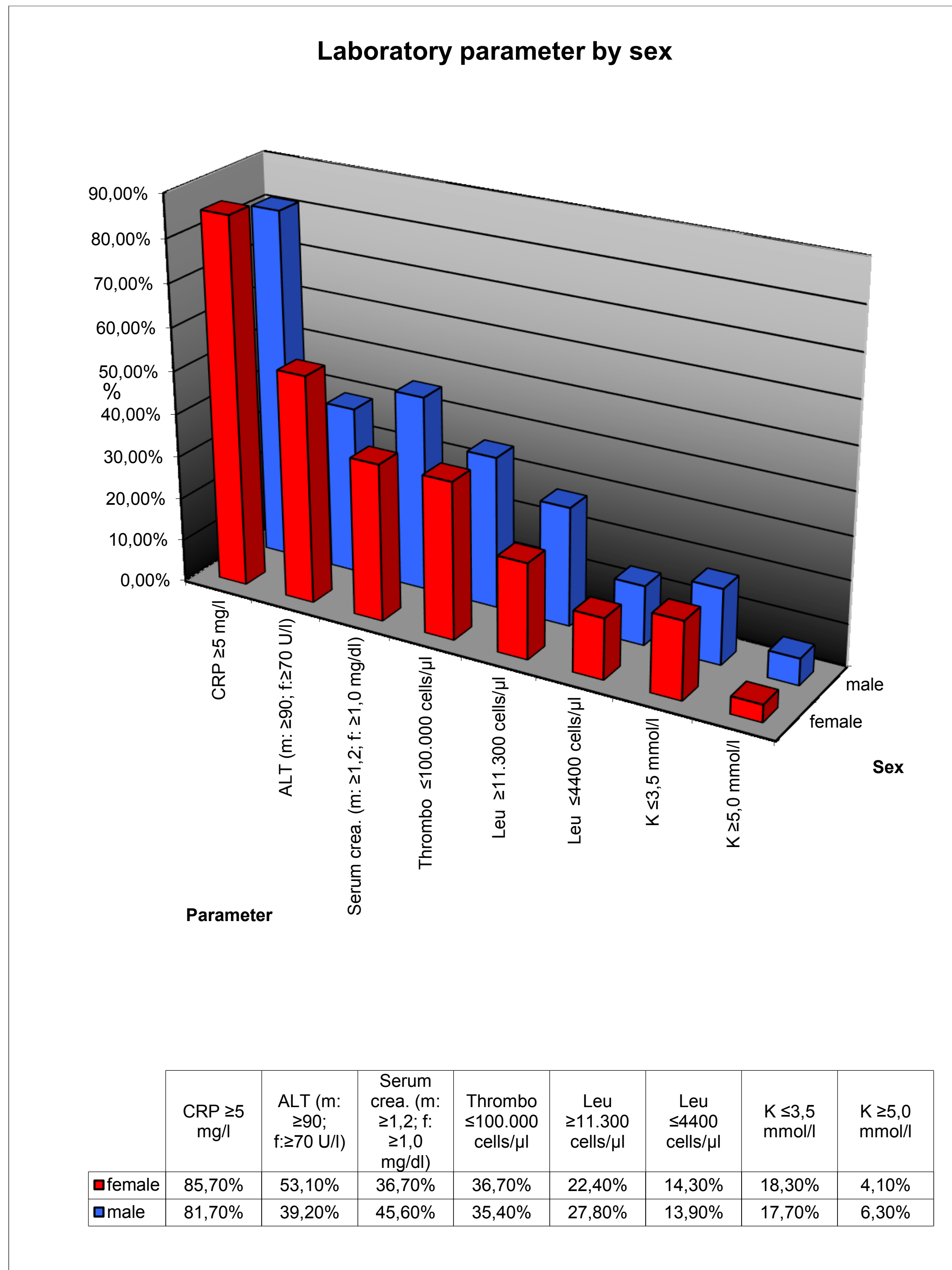
Serum creatinine

In 36 male patients (45.6%), but only 18 female patients (36.7%) lab results showed elevated serum creatinine.

Potassium

9 (18.4%) of all female patients and 14 (17.4%) of all male patients suffered from hypokalaemia, whereby only 5 male (6.3%) and 2 female (4.1%) had hyperkalaemia at time of admission.

There were no statistically significant differences in symptoms between sexes. Data are shown on the chart below.



	CRP ≥5 mg/l	ALT (m: ≥90; f:≥70 U/l)	Serum crea. (m: ≥1,2; f: ≥1,0 mg/dl)	Thrombo ≤100.000 cells/µl	Leu ≥11.300 cells/µl	Leu ≤4400 cells/µl	K ≤3,5 mmol/l	K ≥5,0 mmol/l
female	85,70%	53,10%	36,70%	36,70%	22,40%	14,30%	18,30%	4,10%
male	81,70%	39,20%	45,60%	35,40%	27,80%	13,90%	17,70%	6,30%

Figure 19: Laboratory parameters split by gender

4.7 Serovars

In cooperation with the AGES Mödling we analysed causative serovars of *Leptospira* infections. The AGES Mödling did not receive serum samples from 48/128 (37.5%) of patients. The causative *Leptospira spp.* remains therefore unknown in these 48 patients. Therefore MAT results of 80/128 patients were analysed. Out of those 21 serologies (26.3%) turned out to be negative, although patients were positive by IgM POC test.

At the end we found 59 patients with positive MAT testing. According to MAT results 30 patients (50.8%) were infected with only a single serovar, 17 patients (28.8%) with 2 serovars and 12 patients (20.3%) with 3 or more serovars.

The most frequent serovar was *L. Bratislava*, found in 18 of 59 (30.5%) patients. *L. Sejroe* occurred in 14 (23.7%) and *L. Ballum* in 13 (22%) patients, followed by *L. Australis* in 10 (16.9%) and *L. Grippotyphosa* in 8 patients (13.6%). *L. Bataviae*, *L. Canicola* and *L. Copenhageni* were each represented 6 times (10.2%). We also found *L. Hardjo* in 4 (6.8%), *L. Icterohaemorrhagiae* in 3 (5.1%) and *L. Wolfii* in 2 (3.4%) samples. Rare *Leptospira spp.* we found were *Leptospira hebdomadis* (1.6%), as well as *L. Saxköbing* and *L. Pyrogenes*. Each one was found only once in patients' blood.

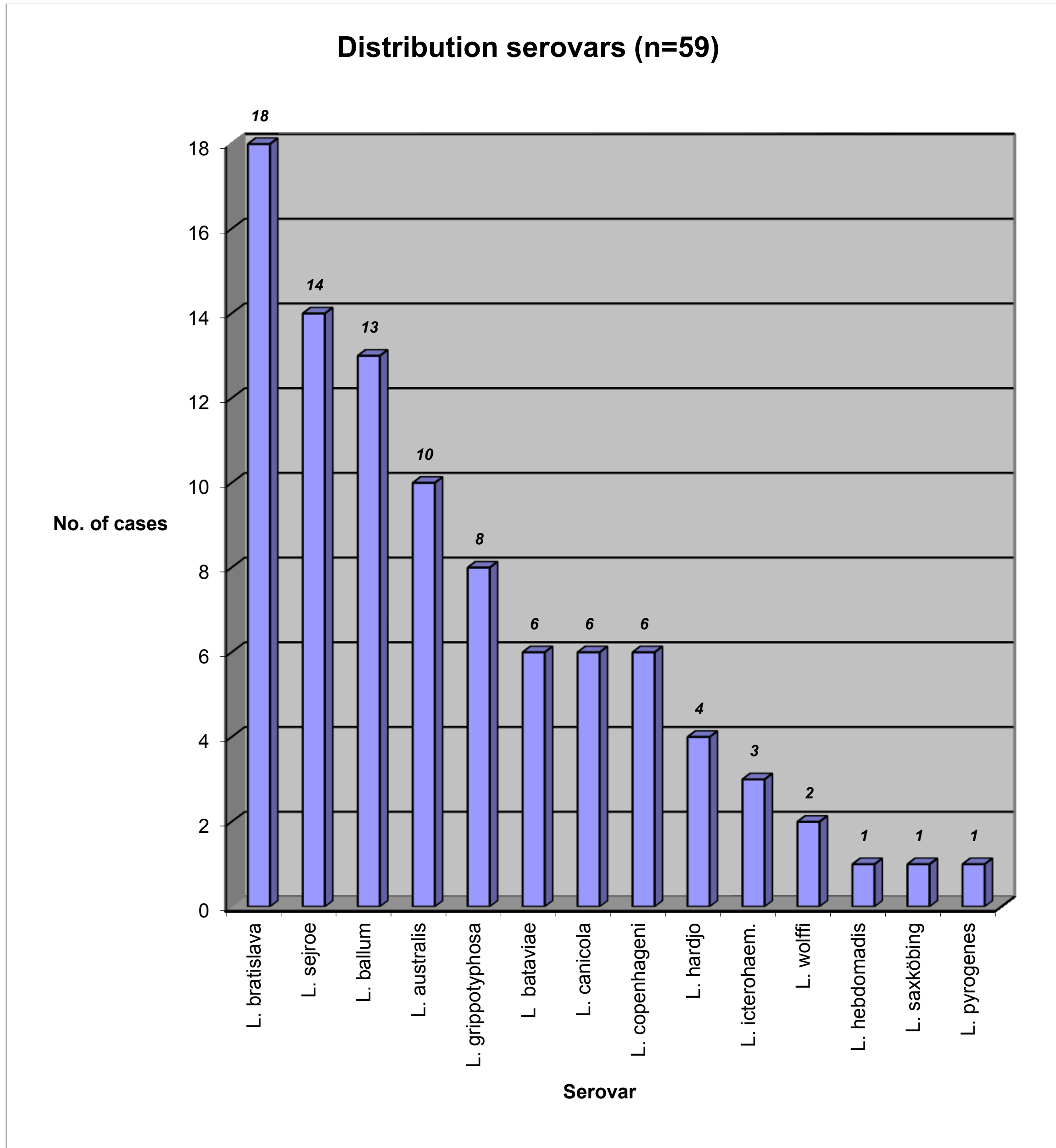


Figure 20: Distribution of Leptospira-serovars

4.8 Length of hospitalisation

The average time of hospital stay was 10.6 days and ranged from outpatient treatment (0 days at the hospital) to 66 days, median was 8 days.

Female patients averagely were hospitalised for 9.8 days, males for 11.3 days and therefore a little longer.

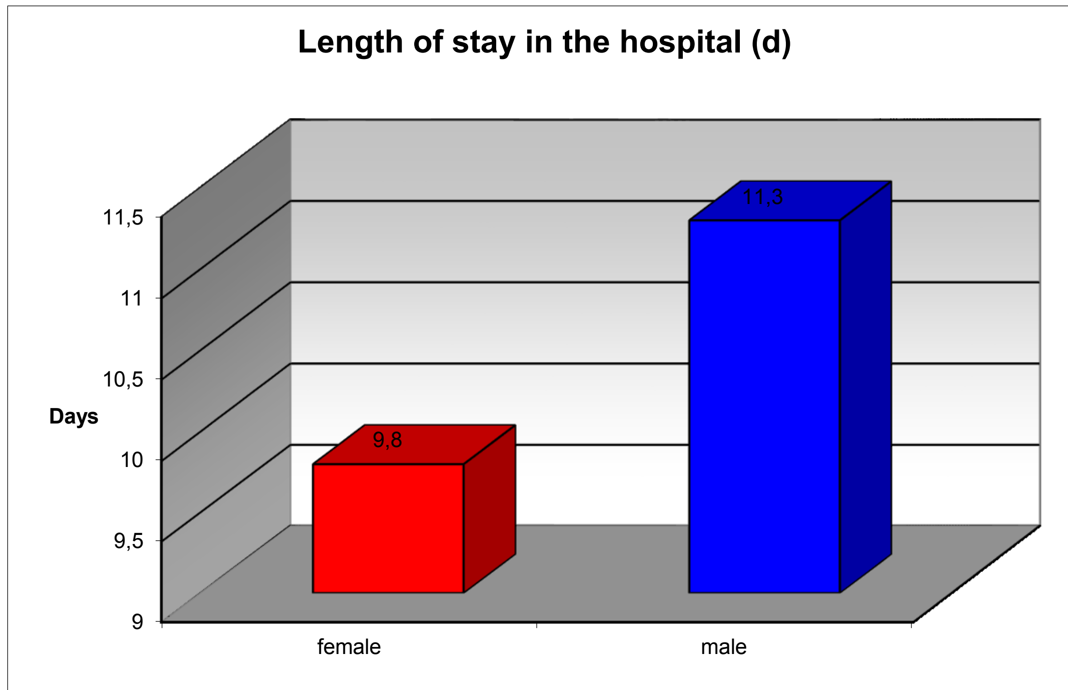


Figure 21: Average length of stay in the hospital

5 DISCUSSION

5.1 Methods

We retrospectively analysed risk factors for acquiring leptospirosis in south- east Austria. Patients in a 9- year period, from 2004 until 2012, that had been tested positive for leptospirosis (IgM POC test) at the Microbiology Laboratory, Medical University of Graz, were included. Data concerning clinical course of the disease, as well as recreational and residential risk factors and laboratory results were collected via an electronic patients database (MEDOCS) and by a self-composed questionnaire via telephone. Because the investigation starts at 2004, we could not get information of all the patients of the 9 year period. For some patients we could not find the correct telephone numbers, others could not been reached via telephone, and a few patients had died in the meantime. Particularly, patients of the earlier years were not always able to give exact information, e.g. they did not remember all their symptoms, or if they had been swimming or doing other activities which were potentially sources of infection. Mostly they remembered only activities that they were explicitly asked for during their stay at the hospital. If symptoms were not remembered they were looked up in the patients' history in MEDOCS.

Information of which type of *Leptospira spp.* patients have been infected with was gathered via MEDOCS and in direct cooperation with the AGES Mödling. All results from patients' blood samples that had been sent to Mödling between 2007 and 2012 were saved in an electronic database, results from the years before had to be looked up by hand. At the AGES Mödling serum was received from about 2/3^{rds} of patients only.

5.2 Results

All in all we collected data of 128 patients, 79 (61.7%) of them were male, 49 (38.3%) female, which is unusual, especially in temperate zones and developed countries. In a study performed in Germany and Austria from 1998-2008 achieved by Hoffmeister et al. the percentage of female infections was 14% (28). In another study from New Zealand evaluating cases between 1990-1998 the percentage of infections in females was only 9.6%. In that study a male predominance in high-risk exposure occupations was thought to be the main reason for the sex distribution. Further they detected no gender difference in the incidence of leptospirosis in meat processing and forestry- related workers, but in farmers, which was attributed to personal hygiene measures (36). Also in a study from the Netherlands by Goris et al., evaluating cases of leptospirosis from 1925-2008, the percentage of infections in females (8.9%) was very low. Almost the same results were seen in studies from Hawaii by Katz et al. between 1999-2008, and in France by Abgueguen (37, 38). In our study we detected that many female patients were frequently reporting risk factor related activities, especially gardening, or activities in woods or wet area. A study published by Jansen et al. evaluating cases in Germany from 1997-2005 concluded that male patients often show a more severe course of leptospirosis than females, so infections in females may be under recognized, although seroprevalences did not differ significantly between both sexes (39). In our study we could not find a relevant difference in severity of the disease between males and females. Another cause for the high rate of females found in our study could simply be the rising awareness of the disease at the LKH Graz.

Median age at time of infection was 43 years, and female median age was higher (46 y) than male (41 y). A peak of infections was seen at the age of 20-29, and 30-39 years, which is slightly different to other studies of leptospirosis in temperate zones that show a peak of infections at the age between 30 and 50 (13, 40). Probably we find the peak between 20-40 years in our study because of outdoor related activities like water sports, trekking and hiking that are done more often at this age, but also occupational expose to potentially contaminated water, soil or infected animals.

In females the highest peak occurred between 20 and 29, in males between 30 and 39. In males another peak between 60 and 69 years was seen, which was not found in female patients.

An explanation for this peak between 60 and 69 was not found in male patients. On the other hand in all female patients aged between 60-69 that answered the questionnaire we found the risk factor handling lots of soil during garden work.

In distribution of cases over the 9-year-period, as seen on table 5, an interesting change of incidence was found. The number of infections in the years from 2004 until 2007 was rising, a peak of 29 cases was found in 2007.

After 2008 (25 reported cases) the number is falling again, and starting from 2009 until 2012 we clearly see a decreasing trend of infections. While 82.8% of infections occurred between 2004 and 2008, only 17.2% of all 128 cases were recognized in the last 4 years. We tried to find out if there was a correlation between the climate conditions, like rainfall, snow and warm temperatures, and the appearance of infections. Therefore we looked up the weather trend in Austria, especially in Styria in the years between 2004-2012, in the Division for Meteorology and Geodynamics (ZAMG).

The peak in 2007 could be a result of an extremely warm winter 2006/2007. Warm temperatures could lead to an increase of rodent population. On the other hand there was not much rainfall in 2007. Although *Leptospira* can also survive some time in dry areas, they prefer much a moist environment. The year 2004 showed no unusual events in weather, temperature was normal, in some months slightly cooler and rainfall in Styria was also in average, 14 cases of infection were recognized. 2005 was in some parts of Styria slightly cooler than average, rainfall was also in normal range. In this year 17 infections occurred. In 2006, after a long, cold winter and lots of snow, heavy rainfalls occurred during summer, and also temperatures in the high thirties were recognized more often than usual. The number of cases increased, compared to the previous year, and 21 cases were diagnosed. The year 2008 was characterized by heavy rainfalls, and with regard to temperatures seen over the year, was one of the warmest ever recorded. The second highest peak of reported cases occurred in that year.

In 2009 storms and heavy rainfall lead to flooding in Styria and also temperatures in spring and summer were above average, however the rate of infections decreased to 6 infections.

2010 was considered as “normal”, temperatures were not above or below average, the amount of rain in Styria was also as usual.

2011 was overall dry and warm, especially in spring and fall there was hardly any rainfall. About 15% less rain and snow when compared to an average year were recorded. In each of those 2 years only 5 infections were reported.

Last year, 2012, was characterized by a very cold winter, but also unusually warm in summer, all in all 6 cases of leptospirosis occurred (41).

Overall, we could therefore not find a significant correlation between the climatic conditions in Styria and the number of infections with leptospirosis.

Although there were some years with heavy rainfall, snowfall and warm temperatures and a higher number of infections than average, there were other years with no correlation. From this data we cannot conclude with certainty that leptospirosis in Styria occurs strictly together with heavy rainfall or flooding.

A reason for that could be good sanitation and hygienic measures in Austria, which aggravates an extraordinary growth of rodent population.

The most common reported symptoms were fever, gastrointestinal manifestation (nausea, vomiting, diarrhoea, abdominal pain) and myalgia/arthralgia, followed by prostration, jaundice, and ARF. According to Hoffmeister et al. (28) these symptoms were more likely found in imported infections, whereby sepsis, ARF and jaundice were the most common symptoms in autochthonous infections. Onset of the first symptoms was between day 4 and 10 after the infection. Overall we found 3 septic patients, 1 case of ARDS, as well as 1 case of meningoencephalitis and 1 patient with SPHS, which means, only 6 (4.7%) patients showed a severe form of autochthonous leptospirosis. Only one of all imported cases showed a severe clinical course. This patient was working as GSM technician in Afghanistan and developed severe symptoms. Back in Austria he was diagnosed with Weil`s disease.

Jaundice was found in 24.2% and ARF in 11.7% of our patients. In larger case series summarized by P. N. Levett signs and symptoms of leptospirosis differ in different countries. He reported, for example, large numbers of patients that show jaundice in Brazil and Barbados, less often this sign occurs in Vietnam, Korea and Seychelles. Headache seems to be a very common symptom, 70% to 98% of patients complained about cephalea. Gastrointestinal discomfort like nausea, vomiting and diarrhoea were also frequently reported symptoms. Variations are found for vomiting (18%-69%), abdominal pain (26%-43%), nausea (29%-75%) and diarrhoea (11%-36%). Myalgia was a very common symptom in Brazil (94%), China (100%) and Puerto Rico (97%), but was less commonly found in Barbados (49%) and Korea (40%). An interesting comparison can be drawn in the symptom conjunctival suffusion. In our study we only found a very low percentage of patients suffering from this symptom, listed among "others". In contrast, 28.5% of all patients in Brazil, 42% in Vietnam, more than 50% in Barbados (54%) and Korea (58%), and even 97% in China and 99% in Puerto Rico showed conjunctival suffusion (1). The reason for that may be a different clinical course and slightly different symptoms caused by different *Leptospira* serovars.

Differences in symptoms between male and female patients were seen in gastrointestinal symptoms. Males suffered from abdominal pain/diarrhoea significantly more often than females (p=0.037). On the contrary, females reported headache more frequently than males (p=0.045). Prostration and nausea/sickness were also specified more often by females, but we did not find any statistically significance. Also in all other symptoms we did not find significant differences. Cardiovascular symptoms, e.g. myocarditis or arrhythmia, occurred only in 2 cases. Also dyspnoea, cough, involvement of the eyes (iridocyclitis, conjunctival suffusion) and exanthema only occurred on single occasions. Because of the mostly unspecific symptoms and variability of clinical presentations leptospirosis in Austria is probably under-recognized. If a patients presents with fever, arthralgia or myalgia, gastrointestinal symptoms and jaundice, leptospirosis should therefore be considered in differential diagnoses.

The main focus of the study was to find out recreational and residential risk factors for acquiring leptospirosis. This part of the study turned out to be the most time- consuming. Out of 128 patients we were able to collect data of 82 individuals.

The most commonly reported recreational risk factors were activities in woods or wet area, gardening and working or cleaning up a basement/hut/attic, followed by swimming in a lake, pond or biotope. Also camping and trekking, and fields, stall or canal work provide the possibility of acquiring the infection.

In the study by Jansen et al. the most common risk factors were, traveling, fishing, swimming and canoeing. Also gardening, which is listed among *recreational risk factors* in our study, was found out to be a risk factor (13). We also noted cases of leptospirosis acquired during a sport event like it happened in ECO- Challenge 2000 (20), in a triathlon in Langau, Austria, in July 2010. After the event 4 serologically confirmed cases of leptospirosis in athletes were reported. All 4 patients were previously healthy males, age 40-44 years. It´s noticeable that there were heavy rainfalls a few days before the triathlon (42).

With regard to residential risk factors rats and/or mice in surroundings was mentioned by almost three quarters of all patients, followed by contact to pets and working and/or living on a farm. Also growing own fruit or vegetable and water in surroundings (pond, biotope, lake, well) showed to be considerable risk factors. These factors were also listed by Jansen et al.(13)

All these risk factors may lead to contact with infected urine, soil, water or dust. Food directly from a farm or farmers market and excavation work were also mentioned by some patients. Nearly none of the patients used protective measures, like wearing gloves or a mask when working with soil or in dusty areas. Most of the patients were aware of the presence of rodents, or their excrements. When interviewing the patients, almost everybody told, that he/she did not know of the possibility of contracting any sort of infection like leptospirosis when doing everyday commodities.

Two patients also remembered a tick bite, which would be unusual to lead to transmission of leptospirosis, but may be a rare possibility.

We also found a few occupational risks in some patients. One veterinarian was examining the quality of meat in a slaughterhouse, when he got accidentally splashed his face with urine from a pig. He did not wear protective glasses and was infected by the contaminated urine. Also two canal workers acquired leptospirosis during work.

One of them did not wear a protective mask when cleaning a closed dusty sewage plant and inhaled lots of dust, a few days later he developed the first symptoms. Another one cut his finger through his protective gloves when working in the canal system. We also found 3 patients working as landscapers in a nursery and handling lots of soil every day.

The most important differences in recreational risk factors between sexes were gardening and activities in woods or wet area. While 56.3% of all interviewed females reported gardening as a risk factor, only 40% of all males did so. This may be cause of a generally higher preference of females for gardening. Activities in woods or wet area was done by 64% males, less often (46.9%) by females. The difference may be due to a male preference for walking with their dogs, or jogging in a forest rather than in urban areas.

Also in the risk factor trekking we found a difference between males (12%) and females (6.3%). In other recreational risk factors like camping, swim/snorkel/dive and caves/surfing were no significant differences. An interesting finding was that one patient probably acquired the infection when surfing on the Mur in the area of Graz, and another while he was climbing in a cave and got some abrasions.

Differences between sexes seen in residential risk factors were also found. 18.8% of all female, and only 6% of all male patients bought fruits or vegetables directly from a farmer or farmers market.

If the products are sold right after harvesting they may be still covered with soil or other possible infected agents. If not properly washed or cooked before consumption, this could be a conceivable way of acquiring the infection. Working or living on a farm also differs between genders. 11.4% of all male patients, but only 4.2% females reported this risk factor. This reflects that in Austria more males than females are farmers in general.

Nearly the same is seen in the risk factor handling of farm animals, which was answered positively by 12.7% of male, but only by 4.2% of female patients.

A slight difference is also seen in the risk factor water in surroundings. This includes ponds, biotopes, streams, wells and tarns, right next to the home or place of frequent stay, and work. 12.5% females and 22% males had water in their surroundings.

The water can be contaminated with leptospirosis, for example by mice urine, and during activities an infection may occur. The last difference was keeping domestic animals, who may act as vector for the disease. 65.6% females and 58% males held pets at the time when they acquired leptospirosis. 9 of 82 (11%) patients had two or more pets at their home. Male patients held 14 dogs and 15 cats, female patients 7 dogs and 14 cats.

We could not show statistically significant differences ($p < 0.05$) in recreational or residential risk factors between genders.

Because the incubation period can be as long as 21 days, an association between exposure to risk factors (e.g. water) and symptoms may not be realized the very first time.

The seasonal distribution of autochthonous leptospirosis shows slight differences to the comparable study by Hoffmeister et al.. While in this study the highest peak was by far in August, followed by October, we found no such significant peaks (40). In the study by Jansen et al. a peak of infection was seen in August, followed by September, October and November.

All other months showed lower numbers of cases (13). In our study the highest number of infections occurred in July (17 cases), followed by June, August and October (each month 16 cases). We did find a small peak in March, but the "*Leptospira*-season" really seems to start in May. 49.2% of all infections were recorded in the months from May to August. In September the number of cases decreases slightly before increasing again in October. A reason for the increasing appearance of infections in March could be on the one hand the beginning of spring, rising temperatures, snowmelt- and resulting wet conditions and the start of outdoor activities. On the other hand spring cleaning of holiday cabins, basements or the attic may cause the peak. All factors can lead to contact with potentially infected excrements of rodents.

In the summer months a tremendous increase of outdoor activities, and warm climate could be the reason for the high number of cases.

The small peak in October could also be the result of some warm days and augmented outdoor activities again. We also find a little more rain in general in September and October in temperate zones, and moist conditions facilitate leptospirosis.

From November until February we only find small numbers of cases, which is a result of cold temperatures, almost no rodent population, and minimized contact to possible infected sources, like water, mud and soil. In winter most outdoor activities like gardening and swimming cannot be done because of snow and frost, and there are no fruits or vegetables that are harvested freshly in Austria and can be eaten raw. So the risk of infection is also almost completely eliminated.

With regard to laboratory findings 85.2% of all patients showed elevated CRP at time of admission.

Leucocytosis (≥ 11.300 cells/µl) as an often seen sign of bacterial infection was found in 25.8% of patients, but also leukopenia (≤ 4400 cells/µl) occurred in 13.3%.

Thrombocytopenia (≤ 140.000 cells/µl) occurred in 35.9% of all patients.

Involvement of the liver (ALT m:≥ 90 U/l; f:≥70 U/l) was found in 44.5% of all patients.

Affection of the kidneys (serum creatinine m:≥1.2 mg/dl; f;≥1.0 mg/dl) was found in 42.2%.

18% of all patients suffered from hypokalaemia (≤ 3.5 mmol/l) and 5.5% from hyperkalaemia (≥ 5 mmol/l).

We also analysed differences in laboratory results between sexes.

We did not find significant differences in leukopenia, but leucocytosis was more frequently seen in males (27.8%) than in females (22.4%).

In males thrombocytopenia (35.4%) was slightly less common than in females (36.7%). We didn´t find differences in potassium or CRP values.

Another distinction was elevation of serum creatinine. 45.6% of male patients showed elevated values, but only 36.7% of female patients did so.

The most significant difference was found in elevation of ALT, which was found in 39.2% of male patients, but in 53.1% of female patients. The reasons for that are not identified yet.

Sethi et al. analysed leptospirosis cases in India between 2004- 2008. Their findings in laboratory parameters were similar with regard to thrombocytopenia (18.6%). In the study ALT was considered elevated when ≥60 U/l, and leucocytosis if ≥11.000 cells/µl. Alanine aminotransferase showed elevation in 81.4%, and 61.6% of all patients that suffered from leucocytosis. Because of the slightly different chosen range of ALT and leucocytes, the percentages of the Indian study are higher in those parameters. Nonetheless it shows that in case of an infection with leptospirosis, similar to our results, ALT level, leucocytes and thrombocytes are affected. The most common complication in the Indian study turned out to be renal failure, determined by serum creatinine ≥1,4mg/dl. 60.5% of all patients suffered from this complication (40).

In Styria we also see in about 40% an elevation of serum creatinine (≥1.2 mg/dl), but not as often as in India.

In cooperation with the AGES Mödling we found out the distribution and incidence of the various serovars of leptospirosis.

The most common turned out to be *L. Bratislava* (30.5%), followed by *L. Sejroe* (23.7%) and *L. Ballum* (22%) and *L. Australis* (16.9%), which are common serovars in Europe. *L. Grippotyphosa* was ranked on 5th place, right before *L. Bataviae*, *L. Canicola* and *L. Copenhageni*, followed by *L. Hardjo*. Surprisingly we found *L. Icterohaemorrhagiae*, causing Weil´s disease in only 3 patients (5.1%). We also found rare serovars in 3 patients, which were *L. Hebdomadis*, *L. Saxköbing* and *L. Pyrogenes*.

In contrast to our results the incidence of *L. Icterohaemorrhagiae* in France, Spain, Denmark and Portugal is by far the highest according to a study in France from 1985-2003. The vast majority of infections with leptospirosis in these countries is cause by *L. Icterohaemorragiae* (37.8%), followed by *L. Grippotyphosa* (20.7%). Less common serovars were *L. Australis* (9.3%). *L. Sejroe* (7.1%), *L. Panama* (6.9%) and *L. Canicola* (6.2%). Also in the Netherlands L. Icterohemorrhagiae and L. Grippotyphosa were the most common serovars.

Only a low number of cases were caused by *L. Bataviae* and even less by *L. Ballum*, which is very common in Austria. It´s noticeable that cases of leptospirosis, caused by in Austria dominant *L. Bratislava*, were reported much less common in France (12, 14).

Reasons for this difference may be a slight distinction in climatic conditions that also causes disparities in vector population and fauna, but maybe also a different focus on agriculture, for example in livestock breeding.

The last point we focused on was the median time of hospitalisation. Leptospirosis and its complications provoked altogether 1371 days of hospitalization in a 9-year-period. Male patients (11.3 d) averagely stayed 1.5 days longer in the hospital than female patients (9.8 d), mean was 10.6 days. This data is almost identical to the outcome of the study by Hoffmeister et al.. In this study median length of hospitalisation was 11 days (28).

6 CONCLUSION

Leptospirosis is, because of its rarity and often broad clinical spectrum and various symptoms still an under recognized problem in Austria. Compared to other countries in tropical areas we have a very low incidence of infections, but in association to our climate and density of population it´s not an orphan disease.

In the years between 2004- 2012 we diagnosed 128 cases of leptospirosis. Only 8 of them were probably imported, all others occurred autochthonously. As in many studies documented, cases of leptospirosis are also increasing in other developed countries due to an increase in outdoor activities. We found out that the most important residential risk factors for acquiring leptospirosis in Austria were rats or mice in surrounding, pets, working and/or living on a farm, as well as growing own fruit or vegetables. In recreational risk factors activities in woods or wet area ranked first, followed by gardening, tyding up an attic/basement/ hut, and swimming in a pond, lake or biotope. Because we have a relatively high percentage of infections in females, we also tried to work out differences between sexes. The percentage of males living or working on a farm was higher than in females, while the risk factor food from farm or farmers market was denoted more often by female patients. In recreational risk factors we found that male patients more often do activities in woods or wet areas, whereas females do a lot more gardening.

The most common mentioned symptoms were fever, myalgia/arthralgia, gastrointestinal disorders, prostration, headache and jaundice. In females headache and prostration were seen more often, in males gastrointestinal symptoms were mentioned more often.

Laboratory results showed elevated CRP in 65.6%, elevated ALT and serum creatinine in 42.2% and 39.1%. We also found leucocytosis in 21.9%, but also leukopenia in 13.3% and thrombocytopenia in 20.3%. Hypokalaemia occurred in 18% and hyperkalaemia was seen in 5.5%.

The most common leptospira serovars were *L. Bratislava*, *L. Sejroe* and *L. Ballum*. Those three serovars caused 75.4% of all infections, and the median stay at the hospital because of the infection was 10.6 days.

As there is no immunization for humans, prevention is the only way to avoid infection. In activities related to higher risk of infection, like working in sewage plants or canal systems, protective clothing and masks should be worn. Also when gardening or handling lots of soil protective gloves can be used for protection. If fruits or vegetables are grown in the backyard or bought directly at a farmer or at farmers market, it has to be at least washed properly.

7 References

1. Levett P. Leptospirosis. Clin Microbiol Rev. 2001;14:296 - 326.
2. T. Löscher GDB. Tropenmedizin in Klinik und Praxis mit Reise- und Migrationsmedizin. Stuttgart: Thieme; 2010. 1117 p.
3. WJ T. Historical perspectives in leptospirosis. Indian J Med Microbiol. 2006(24):316-20.
4. Library PHI. Leptospira interrogans [Multimedia files]. CDC; [updated 03/18/2005]. Available from: http://phil.cdc.gov/phil/details.asp.
5. Turner L. Leptospirosis. British Journal of Medicine. 1969 1:231-5.
6. Bharti AR, Nally JE, Ricaldi JN, Matthias MA, Diaz MM, Lovett MA, et al. Leptospirosis: a zoonotic disease of global importance. The Lancet Infectious Diseases. 2003;3(12):757-71.
7. PN L. Leptospirosis. Clinical Microbiology Reviews. 2001;14(2):296-326.
8. Leptospirosis; georaphical distribution 2011 [cited 2013 02/28]. Available from: http://www.caribvet.net/en/diseases/leptospirosis.
9. Leptospirosis 2012 [updated 08/09/2012]. Available from: http://wazzupdok.blogspot.co.at/2012/08/leptospirosis.html.
10. Center TLI. Life cycle of pathogenic leptospires: © 2004-2009 www.leptospirosis.org; 2004-2009 [updated 2009]. Available from: http://www.leptospirosis.org/topic.php?t=27.
11. Pappas G, Papadimitriou P, Siozopoulou V, Christou L, Akritidis N. The globalization of leptospirosis: worldwide incidence trends. International Journal of Infectious Diseases. 2008;12(4):351-7.
12. Baranton G, Postic D. Trends in leptospirosis epidemiology in France. Sixty-six years of passive serological surveillance from 1920 to 2003. International Journal of Infectious Diseases. 2006;10(2):162-70.
13. Jansen A, Schöneberg I, Frank C, Alpers K, Schneider T, Stark K. Leptospirosis in Germany, 1962–2003. Emerging infectious diseases. 2005;11(7):1048.

14. Goris MG, Boer KR, Duarte TA, Kliffen SJ, Hartskeerl RA. Human Leptospirosis Trends, the Netherlands, 1925–2008. Emerging infectious diseases. 2013;19(3):371.

15. Karande S KH, Kulkarni M, De A, Varaiya A. Leptospirosis in children in Mumbai slums. Indian J Pediatr 2002;69(10):855-8.

16. Watson JT, Gayer M, Connolly MA. Epidemics after natural disasters. Emerging infectious diseases. 2007;13(1):1.

17. Al-shere TA, Ujiie M, Suzuki M, Salva E, Belo MCP, Koizumi N, et al. Outbreak of leptospirosis after flood, the Philippines, 2009. Emerging infectious diseases. 2012;18(1):91.

18. A.M. Monahan ISM, J.E. Nally. Leptospirosis: risks during recreational activities. Journal of Applied Microbiology. 2009;107:707-16.

19. all JSe. Leptospirosis in “Eco-Challenge” Athletes, Malaysian Borneo, 2000. Emerg Infect Dis 2003;9(6):702-7.

20. Sejvar J, Bancroft E, Winthrop K, Bettinger J, Bajani M, Bragg S, et al. Leptospirosis in “eco-challenge” athletes, Malaysian Borneo, 2000. Emerging infectious diseases. 2003;9(6):702.

21. World Health Organization W. Leptospirosis worldwide, 1999. Wkly Epidemiol Rec. 1999;74(29):237-42.

22. Desai S vTU, Lierz M, Espelage W, Zota L, Sarbu A, Czerwinski M, Sadkowska-Todys M, Avdicová M, Reetz J, Luge E, Guerra B, Nöckler K, Jansen A. Resurgence of field fever in a temperate country: an epidemic of leptospirosis among seasonal strawberry harvesters in Germany in 2007. Clin Infect Dis 2009;48(6):691-7.

23. Sitprija Visith TT. Leptospirosis2011 April 2011.

24. Feigin C, Demmler, Kaplan. Textbook of Pediatric Infectious Diseases. 5 ed. Philadelphia, USA: Saunders; Elsevier; 2004. 3347 p.

25. JC AJB. Leptospirosis in man and animal. Edinburgh UK: E. & S. Livingstone; 1958.

26. Daher EDF, Abreu KLSd, Silva Junior GBd. Insuficiência renal aguda associada à leptospirose. Jornal Brasileiro de Nefrologia. 2010;32:408-15.

27. Lee MG, Char G, Dianzumba S, Prussia P. Cardiac involvement in severe leptospirosis. West indian medical journal. 1986;35(4):295-300.

28. Hoffmeister B, Peyerl-Hoffmann G, Pischke S, Zollner-Schwetz I, Krause R, Müller MC, et al. Differences in clinical manifestations of imported versus autochthonous leptospirosis in Austria and Germany. The American journal of tropical medicine and hygiene. 2010;83(2):326.

29. Edwards CN, Nicholson GD, Hassell TA, Everard C, Callender J. Thrombocytopenia in leptospirosis: the absence of evidence for disseminated intravascular coagulation. The American journal of tropical medicine and hygiene. 1986;35(2):352-4.

30. Andrade L, de Francesco Daher E, Seguro AC, editors. Leptospiral nephropathy. Seminars in nephrology; 2008: Elsevier.

31. al. GMe. Prospective Evaluation of Three Rapid Diagnostic Tests for Diagnosis of Human Leptospirosis. PLoS Negl Trop Dis. 2013;7(7).

32. Faine S. Guidelines for the control of leptospirosis: World Health Organization Geneva; 1982.

33. Limmathurotsakul D, Turner EL, Wuthiekanun V, Thaipadungpanit J, Suputtamongkol Y, Chierakul W, et al. Fool's gold: why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. Clinical infectious diseases. 2012;55(3):322-31.

34. ME G. Antimicrobial therapy of leptospirosis. Current Opinion in Infectious Diseases. 2006;19(6):533-7.

35. Sejvar J, Bancroft E, Winthrop K, Bettinger J, Bajani M, Bragg S, et al. Leptospirosis in "Eco-Challenge" Athletes, Malaysian Borneo, 2000. Emerg Infect Dis. 2003;9:702 - 3.

36. Thornley C, Baker M, Weinstein P, Maas E. Changing epidemiology of human leptospirosis in New Zealand. Epidemiology and Infection. 2002;128(1):29-36.

37. Katz AR, Buchholz AE, Hinson K, Park SY, Effler PV. Leptospirosis in Hawaii, USA, 1999–2008. Emerging infectious diseases. 2011;17(2):221.

38. Abgueguen P, Delbos V, Blanvillain J, Chennebault JM, Cottin J, Fanello S, et al. Clinical aspects and prognostic factors of leptospirosis in adults. Retrospective study in France. Journal of Infection. 2008;57(3):171-8.

39. Jansen A, Stark K, Schneider T, Schöneberg I. Sex differences in clinical leptospirosis in Germany: 1997–2005. Clinical infectious diseases. 2007;44(9):e69-e72.

40. Sethi S, Sharma N, Kakkar N, Taneja J, Chatterjee SS, Banga SS, et al. Increasing trends of leptospirosis in northern India: A clinico-epidemiological study. PLoS neglected tropical diseases. 2010;4(1):e579.

41. 2013. Available from: http://www.zamg.ac.at/cms/de/klima/klimauebersichten/jahrbuch.

42 Radl, Christoph, et al. "Outbreak of leptospirosis among triathlon participants in Langau, Austria, 2010." *Wiener klinische Wochenschrift* 123.23-24 (2011): 751-755.

8 Appendix

8.1 QUESTIONNAIRE

Name: Number: Telephone number:

Date of birth:

Sex: *m* *f*

Stay abroad: *Y* *N*

If YES: Where? How long before onset of the first symptoms?

Begin of symptoms:

Symptoms:

Date of admission to the hospital:

Date of diagnose:

RISK FACTORS:

Occupation:

Recreational	*NO*	*YES*	*How long before onset of the first symptoms?*
Fishing			
Swim/ Snorkel/ Dive			
Camping/ Trekking			
Caves/ Surfing			
Gardening/ Hunting			
Woods/ Wet area			
Field/ Stall/ Canal work			

Residential	*NO*	*YES*	*WHAT?*
Pets			
Farm animals			
Rats/ Mice in surrounding			
Water in surrounding			
Excavation work			
Food from farmer/ farmers market			
Animal bite			
Own fruit/ vegetable growing			

Clinical course *MILD* *SEVERE*

Weil´s disease

SPHS

ARDS

Meningitis/ Encephalitis

Others:

Laboratory parameter:

Thrombocytes (cells/µl):

Leukocytes (cells/µl):

CRP (mg/l):

Potassium (mmol/l):

Serum creatinine (mg/dl):

ALT (U/l):

L. serovars:

Length of stay at the hospital (d):