Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för biomedicin och veterinär folkhälsovetenskap

Leptospirosis in dogs in Lima, Peru

Description of changes in serology, hematology, blood chemistry and urinalysis before and after one month of treatment

Sophie Hedberg

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Leptospirosis in dogs in Lima, Peru
Description of changes in serology, hematology, blood chemistry and urinalysis before and after one month of treatment

Leptospiros hos hund i Lima, Peru
Beskrivning av förändringar i serologi, hematologi, blodkemiska tester och urinprov före och efter en månads behandling

Sophie Hedberg

Handledare:
Sofia Boqvist & Jorge Moreno-Lopez, Institutionen för biomedicin och veterinär folkhälsovetenskap
Viviana Rosa Fernández Paredes, Universidad Nacional Mayor de San Marcos, Lima, Peru

Examinator:
Richard Zuerner, Institutionen för biomedicin och veterinär folkhälsovetenskap

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ABSTRACT

This study was carried out at the Small Animal Clinic, Faculty of Veterinary Medicine, National University of San Marcos (Universidad Nacional Mayor de San Marcos, UNMSM), Lima, Peru during the period 10th of September – 2nd of November 2012. Ten dogs with a positive serologic result on the Microscopic Agglutination Test (MAT), serovars australis, bratislava, canicola, georgia, grippotyphosa, icterohaemorrhagiae and pomona included, were included in the study. All specimens were examined and treated at UNMSM or GuauGuau Wasi, a private owned small animal clinic in Lima. Serology, hematology, blood chemistry (urea, creatinine, total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALKP) and phosphate) and urinalysis were evaluated both before and after treatment. The most common clinical signs in the dogs studied were depression (n=6), vomiting (n=5), diarrhea (n=4) and polydipsia (n=4). The most common treatment was doxycycline (5 mg/kg every 12 hours, orally) although clinically unstable dogs received treatment with benzylpenicillin, amoxicillin or ampicillin prior to an oral treatment with doxycycline at home. A decrease was observed at the p=0,05 level of significance in leucocytes and neutrophiles in blood and also in proteinuria after treatment, however most of the dogs still maintained levels of neutrophiles and proteinuria above that of the reference range used by the Laboratory of Clinical Pathology at UNMSM after treatment. None of the dogs in the study were known to have anemia. None of the blood chemistry parameters showed a significant decrease (p=0,05). However all the dogs with initially increased levels of creatinine (2/10), ALT (3/8) or ALKP (4/7) had decreased values after treatment, however many still had a value above the reference range. Increased urea levels (8/10) reduced after treatment in five of eight dogs, however the other dogs' (3/8) urea levels continued to increase after treatment.

SAMMANFATTNING

Denna studie gjordes på smådjurskliniken, Veterinärmedicinska fakulteten, nationella universitetet San Marcos (Universidad Nacional Mayor de San Marcos, UNMSM), Lima, Peru under perioden 10 september – 2 november 2012. Tio hundar med ett positivt resultat på ett Microskopiskt Agglutinations Test (MAT), serovarer australis, bratislava, canicola, georgia, grippotyphosa, icterohaemorrhagiae, pomona inkluderade, inkluderades i studien. Alla hundar var undersökta och behandlade på UNMSM eller GuauGuau Wasi, en privatägd smådjursklinik i Lima. Serologi, hematologi, blodkemiska tester (urea, kreatinin, total protein, albumin, alanin aminotransferas (ALT), alkalin fosfatas (ALKP) och fosfat) och urinprov undersöktes både före och efter behandling. De vanligaste sjukdomstecknen hos de studerade hundarna var depression (n=6), kräkningar (n=5), diarré (n=4) och polydipsi (n=4). Den vanligaste behandlingen var doxycyklin (5 mg/kg var 12:e timme, oralt) även om kliniskt instabila patienter fick behandling med bensylpenicillin, amoxicillin eller ampicillin före en oral behandling med doxycyklin inleddes hemma. En sänkning observerades på signifikansnivån p=0,05 för leukocyter och neutrofiler i blodet samt proteinuri efter behandling, men många av hundarna hade dock fortfarande nivåer av blodneutrofiler och proteinuri över de normala referensvärdena som används på laboratoriet för klinisk patologi på UNMSM efter behandling. Anemi observerades inte hos någon av hundarna i studien. Ingen av blodkemiparametrarna visade en signifikant sänkning (p=0,05). Dock hade alla hundar med stegrat kreatinin (2/2), ALT (3/8) eller ALKP (4/7) minskade nivåer efter behandling, men många av dem hade fortfarande nivåer över de normala referensvärdena. Stegrade ureanivåer (8/10) sjönk efter behandling hos fem av åtta hundar, men de övriga hundarnas (3/8) ureanivåer fortsatte att stiga efter behandling.

INTRODUCTION

The bacterium *Leptospira* can be found all over the world but leptospirosis as a disease is more common in countries with a warm, humid climate that allow the bacteria to thrive (Levett, 2001: Sykes et al., 2011). Leptospira is a Gram-negative, aerobic bacterium (Holt, 1978; Zuerner, 2010), of which there are both pathogenic and saprophytic strains (Faine & Stallman, 1982; Johnson & Harris, 1967). Almost every known species of rodent, mammal or marsupial (including humans) can be a reservoir or incidental host for *Leptospira* (Babudieri, 1958; Faine et al., 1999; Levett, 2001; Picardeau, 2013). The serovars bratislava, canicola, icterohaemorrhagiae, grippotyphosa and pomona are most frequently reported in dogs. Leptospirosis can affect many organs including the blood vessels, liver and kidneys (Arent et al., 2012; Miller et al., 2011). The incubation period is approximately seven days, depending on dose, strain and host (Sykes et al., 2011). The Microscopic Agglutination Test (MAT) is the standard method for serologic diagnosis of leptospirosis (Levett, 2001).

The serological prevalence of leptospirosis in dogs in South America varies between countries. In a study from the district Chancay, Lima, Peru a seroprevalence of 27,8% (67/241) was found in the dogs (Céspedes et al., 2007). Ciceroni et al. (1997) found a seroprevalence of 14% (6/43) in Bolivia in 1992. In a Columbian seroprevalence study from 2007 at least 21,4% (182/850) of the dogs had a positive result on MAT (Romero et al., 2010). Another Colombian study from 2004 found an incidence of 41,1% (81/197) (Rodríguez et al., 2004). These results indicate that leptospirosis is a highly problematic disease for dogs in South America. Leptospirosis is considered a rare disease in Swedish dogs with 16 cases reported in 2011 and 18 in 2010 (The Swedish Board of Agriculture (SJV), 2010 & 2012).

In this study the changes in serology, hematology, blood chemistry and urinalysis are described in ten dogs, both prior to and one month after treatment. This description will hopefully give veterinarians more information about possible changes in blood and urine and the effect of treatment in dogs with leptospirosis. It is important to be able to diagnose and treat leptospirosis in dogs, both for the health of the dog (and therefore the mental wellbeing of the owner) and the health of the owners themselves, as leptospirosis is a zoonotic disease.

PART 1, LITERATURE OVERVIEW: *LEPTOSPIRA* AND LEPTOSPIROSIS

Etiology

The bacterium

Leptospira is a Gram-negative, aerobic bacterium that belongs to the order *Spirochaetales,* family *Leptospiraceae* and genus *Leptospira* (Holt, 1978; Zuerner, 2010). The bacterium, illustrated in Figure 1, has a helical appearance with a hook on each end and is as thin as a sewing thread (0,1 x 6-20 µm) (Holt, 1978). It is motile through two periplasmic flagella (Levett, 2001). The bacterium has an inner membrane and an outer membrane containing lipopolysaccharides (LPS) (Holt, 1978).

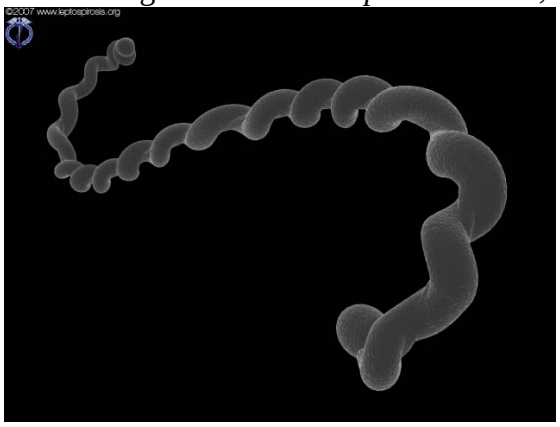

Fig 1. Artist's impression of a Leptospira bacterium.
Source: http://www.leptospirosis.org/downloads/emsim-leptospira-interrogans-02.png

Leptospira bacteria prefer a moist, warm environment, with an optimal growth temperature of 28-30^{o}C (McDonough, 2001; Zuerner, 2010). There are both saprophytic and pathogenic strains of *Leptospira* and an important difference between them is that saprophytic strains reproduce at a temperature of 13^{o}C, however the pathogenic do not (Johnson & Harris, 1967). Both strains are sensitive to dry environments and are inhibited by pH<6 and pH>8 (McDonough, 2001). In a preferable environment the leptospires can survive for a long time (Levett, 2001). According to McDonough (2001) they can survive up to 180 days in wet soil and for many months in surface water.

Taxonomy

Previously the genus *Leptospira* was divided into the two species: *L. interrogans* (pathogenic strains) and *L. biflexa* (saprophytic strains) (Faine & Stallman, 1982). By using antibodies against LPS the two species were divided into multiple serovars. Serovars that were antigenically related and cross-reacted when using serological methods formed a serogroup (Dikken & Kmety, 1978; Zuerner, 2010). In *L. biflexa* there are more than 60 serovars and in *L. interrogans* more than 200 (Levett, 2001). With modern methods, such as DNA hybridization, the leptospires can be divided into genomospecies. In one genomospecie both pathogenic and saprophytic serovars can be found (Brenner et al., 1999). At the Laboratory of Bacteriology at the National University of San Marcos (Universidad Nacional Mayor de San Marcos, UNMSM), in Lima, Peru, the serovars in Table 1 are analyzed via MAT.

Table 1. Serovars analyzed at UNMSM with their serogroups and genomospecies (after Brenner et al. 1999)

SEROVAR	Serogroup	Genomospecies
Australis	Australis	*interrogans, kirschneri, noguchii, borgpetersenii*
Bratislava	Australis	*Interrogans*
Canicola	Canicola	*interrogans, kirschneri, inadai*
Georgia	Mini	*interrogans, weilii, santarosai, borgpetersenii, meyeri, alexanderi*
Grippotyphosa	Grippotyphosa	*interrogans, kirschneri, santarosai*
Icterohaemorrhagiae	Icterohaemorrhagiae	*interrogans, borgpetersenii, noguchii, inadai, genomospecies 4*
Pomona	Pomona	*interrogans, kirschneri, noguchii, santarosai*

Epidemiology

Geography and climate

Cases of leptospirosis are more frequently seen in countries with a warm climate. Many tropical countries are low-income countries which could increase the prevalence as animals live closer to each other, sanitary problems could be present etc. (Levett, 2011; Sykes et al., 2011). In a study by Boqvist et al. (2012) a positive correlation between rainfall and seropositivity for leptospirosis in pigs was found in Sweden. Adin & Cowgill (2000) found a positive correlation between rainfall and leptospirosis in dogs in San Francisco, California, USA.

Reservoir and incidental hosts

The serovars have different reservoir hosts (in various studies called maintenance hosts) and incidental hosts (Levett, 2001). The reservoir hosts are believed to suffer from a chronic infection with *Leptospira* in their kidneys, as the leptospires colonize the surface of renal proximal tubular epithelial cells (Adler & Moctezuma, 2010; Babudieri, 1958; Picardeau, 2013). The hosts do not necessarily host all serovars of *Leptospira* but can simultaneously host multiple serovars. Whether or not an animal becomes a reservoir host depends on many factors, such as, for example, urine pH in the host, environment and possibilities of coming into contact with the bacterium. If two animals of a potential reservoir species are infected one of them can become a reservoir host while the other does not. An infected incidental host will experience more severe clinical signs than a reservoir host (Babudieri, 1958). Almost every known species of rodent, mammal or marsupial can be a reservoir or incidental host for *Leptospira* (Faine et al., 1999; Picardeau, 2013). As humans can also be infected, leptospirosis is classified as a zoonotic disease.

Transmission

Leptospirosis can be transmitted directly between host animals or indirectly via the environment (Adler & Moctezuma, 2010; Levett, 2001). Direct transmission can occur when an animal has contact with blood or other body fluids from an infected animal (incidental host) or a reservoir host. Transmission when in contact with infected urine is common (Faine et al., 1999; Forbes et al., 2012; Ngbede et al., 2012; Picardeau, 2013). The reservoir hosts are important in this aspect as they can secrete leptospires in their urine for a long time, even

throughout their entire life (Babudieri, 1958; Faine et al., 1999). Direct transmission also occurs through bites, consumption of infected viscera, inhalation of aerosols or contaminated water, sexual contact, transplacentally and transmammary. Direct, non-waterborne transmission between humans and dogs is only rarely reported and more studies are necessary to investigate this kind of transmission. Indirect transmission occurs when an animal comes into contact with water contaminated with urine or other body fluids from infected animals, for example in areas where there are infected rodents (Faine et al., 1999; Forbes et al., 2012; Levett, 2001; Ngbede et al., 2012). The leptospires can survive for a long time in diluted urine but outside the host animal they do not replicate (Babudieri, 1958).

Pathogenesis

The bacterium enters the body via intact mucous membranes (mouth, nose, eyes) or a skin with lesions and scratches (Adler & Moctezuma, 2010; Forbes et al., 2012; Langston & Heuter, 2003). Through lymphatic vessels from the infection site the leptospires enter the bloodstream (Faine et al., 1999). In the bloodstream the bacteria will multiply and spread to organs such as the kidneys, spleen, central nervous system, liver, eyes or reproductive organs (Langston & Heuter, 2003). There are three possible pathways after the systemic circulation. If the animal has a high and adequate antibody titer the body will be cleared from leptospires and no clinical signs can be seen. An animal with a moderate antibody can present with a mild or short leptospiremia followed by mild clinical signs. The leptospires are then eliminated through the kidneys and after the elimination the animal will not continue to shed leptospires. If the animal has a low or absent antibody titer there will be a multiplication of leptospires in the bloodstream. The endothelium will be damaged which can cause ischemia in different organs such as the kidneys (renal tubular necrosis), liver (hepatocellular damage) or lungs (Adler & Moctezuma, 2010; Greene et al., 2006; Langston & Heuter, 2003). Neutrophils and thrombocytes are stimulated by LPS in the outer membrane of the leptospires and this contributes to inflammation and coagulatory abnormalities (Langston & Heuter, 2003). The LPS can contribute to the renal and hepatic damage. Meningitis can develop if the leptospires enter the nervous system or cerebral spinal fluid in the acute phase of the disease. If bacteria persist despite the antibody response, then immune-complex-mediated meningitis can occur. When this phenomenon occurs in the eyes it causes uveitis (Faine et al., 1999; Greene et al., 2006).

The incubation period of leptospirosis depends on dose, infectious strain and host but is approximately seven days (Sykes et al., 2011). According to Levett (2001) antibodies become detectable 5-7 days after infection. It takes about two weeks for the leptospires to reach the proximal tubular cells and the tubular lumen in the kidneys (Greene at al., 2006). In the best case scenario, the antibodies will clear the blood and tissues from leptospires. The bacteria can also become eliminated from the kidneys and no leptospires will thus be shed in the urine. In some animals, despite an increased antibody titer, the bacteria can replicate and persist in the renal tubular cells. This may result in chronic shedding of leptospires in the urine for days to months, even years (Faine et al., 1999; Greene et al., 2006; Langston & Heuter, 2003). *Leptospira* can give permanent lesions in internal organs. An organ can be severely affected even though the animal recovers clinically (Greene et al., 2006).

Clinical findings

Clinical signs

In various studies anorexia, lethargy/depression and vomiting were the three most common clinical signs in dogs with leptospirosis. Weight loss, polyuria/polydipsia, diarrhea, abdominal or lumbar pain, musculoskeletal pain and dehydration were also common (Birnbaum et al., 1998; Goldstein et al., 2006; Greenlee et al., 2004; Prescott et al., 2002). Intestinal intussusceptions have occurred in dogs with gastrointestinal involvement. Ventricular tachyarrhythmia and eventual myocardial damage can occur in dogs where the heart is involved (Greene et al., 2006). Uveitis has also been observed. In other animals such as cattle, pigs and horses abortion is common but this is rarely the case with dogs (Picardeau, 2013). Young animals often get a more severe form of leptospirosis compared to adults (Greene et al., 2006).

It is tempting to correlate the different serovars and serogroups to specific clinical features, however this would be inappropriate as serovars that are classed in the same serogroup but are from different geographic areas can have different pathogenicity, hosts and genetic composition. Serovars within a serogroup can also be found in different genomospecies (Greene et al., 2006). Despite this, various studies have tried to correlate serovars and serogroups with different clinical signs (Goldstein et al., 2006; Greenlee et al., 2004; Greenlee et al., 2005) and according to Greene et al. (2006) serovars icterohaemorrhagiae and pomona are believed to cause hepatic disease while canicola, bratislava and grippotyphosa cause renal or hepatic disease, although pomona has also been associated with renal disease.

Hematology, blood chemistry and urinalysis

The changes in hematology, blood chemistry and urine depend on the severity of the infection and the stage that the dog is in when the samples are taken. In studies about leptospirosis in dogs leucocytosis with a left-shifted neutrophilia and anemia was seen in more than 30% of the dogs (Birnbaum et al., 1998; Goldstein et al., 2006; Prescott et al., 2002). In those studies the number of dogs included varied between 31 and 54. In two of the mentioned studies more than 30% of the dogs had thrombocytopenia (Goldstein et al., 2006; Prescott et al., 2002), the corresponding number in the third study was 14% (Birnbaum et al., 1998). When evaluating the blood chemistry a very common finding is azotemia (elevated serum urea and creatinine). In three different studies more than 81% of the dogs had azotemia, in one study it was even found in more than 93% of the dogs. Other common changes in the blood chemistry in these studies were hyperphosphatemia (in >50%), increased alkaline phosphatase (ALKP) (in >50%) and increased alanine aminotransferase (ALT) (in approximately 30%) (Birnbaum et al., 1998; Goldstein et al., 2006; Prescott et al., 2002). Increased total bilirubin varied in the studies between 17% (Birnbaum et al., 1998) and 68% (Prescott et al., 2002). The bile acids can be increased in infected dogs (Greene et al., 2006). Goldstein et al. (2006) found that 31% of the dogs had hyperglobulinemia. According to Greene et al. (2006) hyperglobulinemia is probably due to a chronic stimulation arising from the leptospiral infection. It can also be due to dehydration, secondary to, for example, a renal failure. Dogs with leptospirosis can develop hypoalbuminemia and Goldstein et al. (2006) observed that in 35% of the dogs. Different

electrolytic changes can be seen due to dysfunction in the kidneys and gastrointestinal organs. The most common electrolytic changes, apart from hyperphosphatemia, are hypo- or hyperkalemia, hyponatremia and hypochloremia (Goldstein et al., 2006; Prescott et al., 2002). Humans have additionally shown hypomagnesaemia apart from these findings, but this has not been observed in dogs. Calcium has a protein-bound fraction and because of the loss of albumin a mild hypocalcaemia can be seen in dogs with leptospirosis. If the dog suffers from a secondary pancreatitis the amylase, lipase and pancreatic lipase immunoreactivity (PLI) can be increased. Creatinine kinase (CK), C-reactive protein (CRP) and cardiac Troponin I can also be elevated in dogs with leptospirosis. The blood-pH can be lower than normal with a lower bicarbonate level (Greene et al., 2006).

Common changes in the urine is a specific gravity below 1,029, tubular or glomerular proteinuria, increased protein/creatinine ratio, hematuria, pyuria and glucosuria (Birnbaum et al., 1998; Goldstein et al., 2006). Granular casts can also be seen (Greene et al., 2006). Proteinuria can be due to a renal loss of protein or inflammation in the urinary tract (Ettinger & Feldman, 2010). An elevated protein/creatinine ratio with a low-cellularity sediment indicates that the proteinuria is caused by decreased renal function (Greene et al., 2006).

According to Greene et al. (2006) there is eventually a poorer prognosis for dogs with thrombocytopenia, hypoalbuminemia, increased cardiac Troponin I (which may indicate myocardial damage), increased C-reactive protein/haptoglobin ratio, increased urinary albumin or increased protein/creatinine ratio in urine.

Diagnosis

Direct analysis

Cultivation

Cultivation can be made from blood and urine and is a good way to know if the animal is actually infected with *Leptospira*. Unfortunately the cultivation can be time consuming, taking from a couple of days to four months, requiring weekly microscopic examination before it is possible to consider a sample as negative. This method is useful for epidemiologic studies as it gives the veterinarian a definitive diagnosis (Adler & Moctezuma, 2010). Blood can be used for cultivation up to ten days after infection. After that the concentration of leptospires is highest in the urine. Both blood and urine can be used if the time of infection is unknown (Sykes et al., 2011). The bacterium is secreted intermittently in the urine so repeated urine samples are recommended. Diuretics can be used prior to the urine collection to increase the shedding (Greene et al., 2006). If cultivation is chosen as a diagnostic method it is important to take the sample before treatment with antibiotics is initiated (Adler & Moctezuma, 2010).

Microscopy

Leptospires may be seen on microscopic evaluation of blood, urine, CSF and peritoneal or pleural exudate during the first 10 days of the infection (Faine et al., 1999; Levett, 2001). Dark field microscopy is required as the leptospires are very small (Picardeau, 2013; Zuerner,

2010), however more than 10^4 organism/ml are required to be able to see them. This method is insensitive and has a low specificity. When choosing a body fluid to analyze it is important to consider the pathogenesis and at what stage it is possible to detect the leptospires. For example, blood can only be used in the acute stage of the disease (Levett, 2001; Picardeau, 2013). Dark field microscopy must be followed by serology or cultural diagnostic methods if it is desirable to specify the serovar. The leptospires can be seen with light microscopy if using either Giemsa stain or silver impregnation on air-dried smears (Greene et al., 2006). Immunoflourescence is also possible to use (Levett, 2001).

Genetic detection

Polymerase Chain Reaction (PCR) assays have been developed but are still not commonly used even though this method is reported to have a high sensitivity (Adler & Moctezuma, 2010). Serum, urine, aqueous humor and tissues from autopsy have been used for PCR. Modern methods such as fragment length polymorphism (RFLP), pulse field gel electrophoresis (PFGE), 16S rRNA sequencing and other methods are currently being assessed (Adler & Moctezuma, 2010; Levett, 2001).

Others

There are other techniques available such as direct fluorescent antibody testing, agglutination-adsorption techniques and radioimmunoassays but they are not widely used (Greene et al., 2006; Levett, 2001).

Indirect analysis

The Microscopic Agglutination Test (MAT)

MAT is considered the standard method for the serological diagnosis of leptospirosis (Levett, 2001). To execute MAT leptospires are grown in liquid media (Figure 2). Serum is mixed with the leptospires in order to test for agglutination. Agglutination indicates that the serum contains anti-*Leptospira* antibodies (Picardeau, 2013; Sykes et al., 2011). Antibodies are detectable from 5-7 days after infection (Levett, 2001). In a study by Limmathurotsaku et al. (2012) in humans, the sensitivity of MAT was found to be around 50% and the specificity almost 100%. As the leptospires are thin and small, dark field microscopy is used to evaluate the agglutination (Figure 3). MAT is a serogroup specific diagnostic method and serovars are chosen as representatives for different serogroups (Miller et al., 2011; Picardeau, 2013). The sera can cross-react between various serovars representing the serogroups, which makes it difficult to determine the infecting serovar, especially in the acute phase of infection (Levett, 2001; World Health Organization (WHO), 2003). The ability to predict the serogroup may be as low as 40% (Levett, 2001). In general,

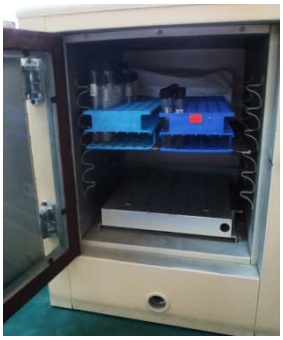

Fig 2. Incubation of leptospires.

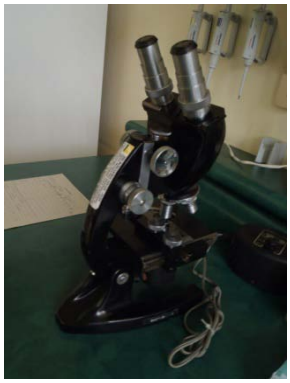

Fig 3. Dark-field microscopy used at UNMSM.

laboratories analyze serovars that are present in their local area. Normally six to eight serovars are used compared to human laboratories where they often use about 20 serovars (Greene et al., 2006).

Different dilutions are used to receive a titer. A positive sample is considered a sample with more than 50% agglutination. Analysis normally begins with the dilution 1/100 and a positive result in that dilution indicates a titer of 1/100 (Picardeau, 2003; Sykes et al., 2011; WHO, 2003). At the Laboratory of Bacteriology at UNMSM the titers 1/100, 1/200, 1/400, 1/800 and 1/1600 were used. The incubation protocol and incubation sheet used at UNMSM is shown in Figure 4. Sometimes the serum can react against many serogroups in the beginning of the infection but later shows the highest titer against the infecting strain (Levett, 2001). However this is currently debated (Sykes et al., 2011). According to Levett (2001) a four-fold increase in a paired sample or a single titer of more than 1/800 in an endemic area is required for the diagnosis of acute leptospirosis. In an area where cases of leptospirosis are rare, titers of >1/200 can be used as the cut-off point (Levett, 2001). According to Adler & Moctezuma (2010) a single titer of 1/400 or more is considered a positive sample. Levett (2001) suggests that if obvious symptoms of leptospirosis are present, 3-5 days between the samples is sufficient or 10-14 days if the patient is in the early stages of disease. Sometimes it takes longer for the antibodies to develop, up to 3-4 weeks or more (Faine et al., 1999).

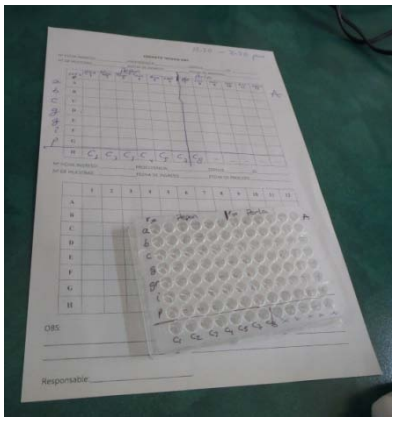

Fig 4. Incubation of samples.

Agglutinating antibodies are most frequently IgM and IgG to a lesser extent. IgM concentrations fluctuate according to the presence of the organism (Greene et al., 2006, WHO, 2003). There are not many studies done to evaluate for how long the antibodies persist. Different studies have shown results between two and 20 years (Faine et al., 1999). If an animal is successfully treated with antibiotics the titer will decrease, however sometimes a titer of 1/200 as the most can be seen until 1-4 months after treatment (Greene et al., 2006).

MAT has a high sensitivity and specificity but the difficulties are that live cultures of different serovars are necessary to carry out the method. It is also necessary that a trained person works with the samples and evaluates the results. MAT is not able to differentiate between antibodies from infection and from vaccination (Adler & Moctezuma, 2010, Picardeau, 2013).

Enzyme-Linked Immunosorbent Assay (ELISA)

There are Enzyme-Linked Immunosorbent Assays (ELISA) that measure IgM and IgG against *Leptospira*. During early infection, IgM-detection with ELISA can be a more sensitive diagnostic method than MAT (Levett, 2001; WHO, 2003). If both IgG and IgM are measured, ELISA has been found to better distinguish between natural infection and vaccine-induced antibodies. However later in the infection the serovar specificity is lower than that of MAT. In general MAT is considered a method with greater sensitivity and specificity than ELISA and

therefore ELISA should not be used as a single diagnostic test (Adler & Moctezuma, 2010; Greene et al., 2006; WHO, 2003).

Others

There are other immunoassays such as a macroscopic slide agglutination test, latex-based agglutinations tests and commercial kits that measure IgG and IgM (Adler & Moctezuma, 2010; Greene et al., 2006; Levett, 2001). When a sample is taken early in the course of disease, IgM detection is considered a more sensitive method than MAT (Levett, 2001).

Treatment of leptospirosis in dogs

The prognosis after treatment of leptospirosis can be good if the dog does not suffer from severe clinical signs and organ lesions. An early and aggressive treatment with antibiotics and fluids is required. A dog surviving a subacute infection may be considered healthy 2-3 weeks after infection. Urea and creatinine values are often normalized within 10-14 days but regeneration of kidney tissue can continue for a month (Sykes et al., 2011). The renal function can return after 2-3 weeks but if the function does not return there is a risk that the dog could have developed a chronic compensated polyuric renal failure (Greene et al., 2006). A dog that has suffered from thrombocytopenia and survives often shows an improved thrombocyte count within a week (Sykes et al., 2011).

Antibiotic treatment

According to Levett (2001) few well executed studies have been made about the treatment of leptospirosis with antibiotics. It is difficult to evaluate the results as many dogs come to the clinic in a late stage of the disease. By treating the dog with antibiotics the leptospiremia and fever can reduce. Multiplication of the leptospires with secondary pathologic changes in the kidney and liver are also avoided. The earlier in the course the antibiotics are given, the less is the risk of development of secondary chronic changes (Goldstein, 2010; Greene et al., 2006; Levett, 2001). Which antibiotic is best is actually not known. Penicillin works well against leptospiremia and is considered to diminish the renal and liver lesions caused by the infection, but it does not work well for elimination of the organism from the body (Adin & Cowgill, 2000; Green et al., 2006; Sykes et al., 2011). Doxycycline has been found to eliminate leptospires from the body (Goldstein, 2010; Sykes et al., 2011). There are studies considering penicillin as the only treatment but many are afraid that chronic shedders develop if using only penicillin (Adin & Cowgill, 2000). There are also studies saying that penicillin is non-effective (Levett, 2001). A dog that has a normal alimentation can start with oral treatment right away. To eliminate the bacteria as soon as possible doxycycline is the most common choice. In dogs that vomit, have uremia or are hepatically compromised, it is common to begin with parenteral penicillin or ampicillin and when the dog has regained a normal alimentation an oral treatment with amoxicillin or doxycycline is initiated (Goldstein, 2010). According to the Consensus Statements of the American College of Veterinary Internal Medicine (ACVIM), ampicillin should not be administered orally as it has a low absorption in the gastrointestinal tract (Sykes et al., 2011). Aminoglucosides are only used in dogs with normal renal function (indicated on a blood sample) as it is a nephrotoxic antibiotic.

Macrolides have been shown to be effective against leptospires but not as effective as penicillin and doxycycline. It has been suggested that macrolides can be used in clinically stable patients that are able to receive an oral treatment. Third-generation cephalosporins work well in cattle and humans but have not been widely used in dogs (Greene et al., 2006). Chloramphenicol and sulphonamides are ineffective whereas quinolones have an effect but are less effective than amoxicillin (Greene et al., 2006; Sykes et al., 2011). Neither are fluoroquinolones recommended as they contribute to the development of antimicrobial resistance in other bacteria (Sykes et al., 2011). If using tetracycline, high doses are necessary which may be nephrotoxic. Doxycycline is less nephrotoxic but can induce nausea and vomiting (Adin & Cowgill, 2000). Suggestions of doses for antimicrobial treatment of leptospirosis are found in Table 2. The given dose for doxycycline is also recommended by Goldstein (2010) and ACVIM, but ACVIM recommend a duration of two weeks or two weeks after the gastrointestinal signs are gone (Sykes et al., 2011). Doxycycline is mainly excreted in the feces so the dose does not have to be changed for dogs with renal failure (Greene et al., 2006). ACVIM recommends the same dose for penicillin G as Greene et al. (2006) or a dose of 20 mg/kg intravenously every six hours if using ampicillin (with dose reduction for azotemic dogs).

Table 2. Antimicrobial treatment of leptospirosis (after Greene et al., 2006)

DRUG	Dose	Administration	Interval	Duration (weeks)
Penicillin G	25000-40000 IU/kg	IM, SC, IV	12 hours	3
Ampicillin	22 mg/kg	SC, IV	6-8 hours	3
	20-40 mg/kg	PO	8 hours	3
Amoxicillin	10-20 mg/kg	PO	8-12 hours	3
Doxycycline	5 mg/kg	PO, IV	12 hours	3
Tetracycline	22 mg/kg	PO	8 hours	3
Azithromycin	20 mg/kg	PO	24 hours	3

IU = international units, IM = intramuscular, IV = intravenous, SC = subcutaneous, PO = per oral

Supportive treatment

The supportive treatment for leptospirosis depends on the dog and its needs. Patients with severe symptoms will need hospital care (Levett, 2001). If the dog is severely affected and in shock it will need fluid therapy. Fluid therapy is also necessary for dogs with fluid losses from diarrhea and vomiting. Fluids are chosen according to current electrolyte imbalances in the dog. If the dog is vomiting secondary to uremic gastritis gastric protectants such as H_2-receptor antagonists and proton-pump inhibitors can be used. It is important to consider parenteral treatment in vomiting dogs as oral treatment may be unhelpful. A dog with hemorrhages (petechial or ecchymotic) and thrombocytopenia may be in need of anticoagulation treatment. If the dog has hypoalbuminemia or if pancreatitis is suspected plasma or whole blood should be given (Birnbaum, 1998; Goldstein, 2010; Greene et al., 2006).

A dog with oliguria (under 2 ml/kg/hour) should first be treated with fluids to become rehydrated. If the decrease in renal function persists, osmotic diuretics can be added (glucose 10% or 20%, mannitol 20%) intravenously for 30-60 minutes. Dopamine or dopaminergic agents can be administered intravenously if osmotic diuretics do not work. Dopamine can be given together with furosemide to improve the effect. Peritoneal dialysis, hemodialysis or continuous renal replacement therapy can be used if oliguria cannot be treated with any of the mentioned drugs. Massive pulmonary hemorrhages are associated with high mortality in humans. To decrease the inflammation humans are treated with intravenous methylprednisolone, first with a shock-bolus dose for three days and then an anti-inflammatory dose for seven days (Greene et al., 2006). ACVIM does not consider methylprednisolone helpful for massive pulmonary hemorrhages but suggests oxygen (Sykes et al., 2011). Humans are also treated with hemofiltration, plasma exchange therapy and cyclophosphamide but to know if these treatments are possible to use in dogs or not more studies are required (Greene et al., 2006).

Prophylaxis

Immunity and vaccination

Immunity against *Leptospira* is mainly humoral although a cellular immunity may also be important. The antibodies that develop after an infection are directed against LPS in the outer membrane of the *Leptospira*. There are vaccines against leptospirosis and the ones in current use are inactivated (Faine et al., 1999; Greene et al., 2006). During clinical trials vaccines on the market have prevented disease and to a great extent shedding of leptospires in the urine (Sykes et al., 2011). Hartman et al. (1984) found that IgM-antibodies reached a maximum concentration one week after a vaccination (serovar canicola and icterohaemorrhagiae) and could be measured until seven weeks after. With a booster vaccination after 3-4 weeks IgG-titers could be measured at a maximum after one week but after six months the titers were low. After the revaccination after six months the IgG-titers were high for approximately one year.

ACVIM recommends a revaccination annually. A dog that has recovered from leptospirosis should also be vaccinated as the persistence of the antibodies is uncertain (Sykes et al., 2011). As the disease is considered rare in Sweden the recommendation for vaccination is only focused on dogs that will visit another country in Europe and spend time close to water, in woodlands, in places with many dogs or at a farm. Vaccination is not considered necessary when travelling to one of the other Nordic countries. In Sweden it is most common to use a vaccine containing the serovars icterohaemorrhagiae and canicola (The National Veterinary Institute (SVA), 2011). Vaccines that, aside from serovar canicola and icterohaemorrhagiae, also include serovar grippotyphosa and pomona are available in North America (Sykes et al., 2011) and also in Peru. The recommendation for vaccination in Sweden is two vaccinations with a 3-4 weeks interval. The second injection is done 1-3 month before travelling. The dog is then revaccinated after 6 months, thereafter annually (SVA, 2011).

Cleaning of an infected environment

When cleaning environments with suspected contamination it is important to not only use water as the leptospires can survive for a long time in infected urine diluted with water (Babudieri, 1958; Greene et al., 2006). 10% bleach solution, iodine-based disinfectants, accelerated hydrogen peroxide and quaternary ammonium solution can be used as disinfectants (Greene et al., 2006: Sykes et al., 2011). It is important to use gloves and wash hands whilst working with infected dogs and infected urine.

PART 2, FIELD STUDY

Aims

The aims of this study were as follows:

- To describe the changes in hematology, blood chemistry and urinalysis that *Leptospira* can cause in dogs before and after treatment by analyzing blood and urine samples from dogs diagnosed with leptospirosis at UNMSM
- To describe the levels of serum antibodies acting against *Leptospira* before and after treatment in dogs diagnosed with leptospirosis at UNMSM
- To describe the effect of treatment in dogs diagnosed with leptospirosis at the aforementioned clinic

Material and methods

Study population and study design

The field study was carried out at UNMSM during the period 10th of September – 2nd of November 2012 (8 weeks). The study was made as a Minor Field Study funded by SIDA (Swedish International Development Cooperation Agency) in Sweden.

Dogs that fulfilled the following criteria were included:

- Examined and treated at the Small Animal Clinic, UNMSM or the private owned clinic GuauGuau Wasi in Lima
- Received a positive serologic result (all titers) for *Leptospira* by MAT (serovars australis, bratislava, canicola, georgia, grippotyphosa, icterohaemorrhagiae, pomona) at the Laboratory of Bacteriology, UNMSM during the period 10th of August – 2nd of November (one month before the beginning of the field period till the end of the field study period, explanation below)
- Owners agreed to participate in the study

Dogs of all ages, breeds and sexes were included in the study.

Blood and urine samples

Blood and urine samples were taken when the dog came to clinic for a first consultation and again after one month of treatment. The blood samples included in the study are summarized in Table 3 with the urinalysis in Table 4.

Table 3. Blood samples included in the study

SAMPLE	Test	Instrument	Laboratory
edTA	Hematology Thrombocyte count	Abacus 5, junior vet Microscopy	
Serum	Creatinine Urea Total protein Albumin ALT (alanine aminotransferase) ALKP (alkaline phosphatase) Phosphate	Wiener laboratory test	Laboratory of Clinical Pathology, UNMSM
	Microscopic Agglutination test (MAT) Serovars: australis, bratislava, canicola, georgia, grippotyphosa, icterohaemorrhagiae, pomona	According to "Instrucciones de la técnica de MAT del Instituto Nacional de Salud" (Oficina General de Epidemiología, Instituto Nacional de Salud, 2000)	Laboratory of Bacteriology, UNMSM

Table 4. Urine samples included in the study

SAMPLE	Test	Instrument	Laboratory
Urine	pH	Urine sticks	Laboratory of Clinical Pathology
	Glucose		
	Bilirubin		
	Urobilinogen		
	Ketones		
	Hemoglobin		
	Blood		
	Nitrite		
	Protein	Sulfocalicylic acid test (SAA)	
		Nitric Acid Test	
	Leucocytes	Microscopy (Congo Red included)	
	Erythrocytes		
	Bacteria		
	Cylinders		
	Crystals		
	Cells		
	Specific gravity	Refractometer	
	Color	Manually	
	Aspect		
	Protein/creatinine ratio	EMT-168	VetDiagnóstics

Statistical analyses

Values for hematology (basophiles, eosinophiles, erythrocytes, hemoglobin, hematocrite, leucocytes, lymphocytes, monocytes, neutrophiles, thrombocytes), blood chemistry (albumin, ALKP, ALT, creatinine, phosphate, total protein, urea) and selected urinalyses (specific gravity, protein and protein/creatinine ratio) were evaluated statistically. The Shapiro Wilk Normality Test was first used to investigate if the variables were normally distributed or not (Z>0,05 = normally distributed) (Petrie & Watson, 2001). A paired T-test was used to compare analytic results before and after treatment for normally distributed, numeric variables. For the variables with Z<0,05 (not normally distributed) a histogram was made to investigate if the variables had a left- or right-tailed distribution. For data sets with a right-tailed distribution the data was normalized by taking the base 10 logarithm of each observation. The distribution of logarithmically transformed data will often be approximately normal for right-tailed variables (Petrie & Watson, 2001). The logarithmic values received were used for the paired T-test. For data sets with a left-tailed distribution (only thrombocytes) the Wilcoxon Signed-Rank Test was used. The variable proteinuria was measured qualitatively and therefore the Sign Test was used to compare the medians of the variable before and after one month of treatment. A p-value of <0,05 was considered statistically significant in all statistical analyses (Petrie & Watson, 2001). All statistical analyses were conducted using the software STATA 11.

Collection of epidemiological data

Information about the history, anamnesis, clinical signs and treatment of the dogs was collected from the paper records kept at UNMSM. To gather more information about the dogs included in the study a questionnaire was filled out by the owner (appendix 1). The questionnaire contained questions about the dog, the owner, clinical signs seen in the dogs and the owner's knowledge about leptospirosis. There is always a risk for recall bias if the owners do not remember the correct answers or avoid answering truthfully, however a questionnaire with the same questions for all the owners makes it possible to summarize their answers.

Results

Description of included dogs

All dogs that received a positive result on MAT were included, irrespective of their titer. During the field study period ten dogs received a positive result. Two owners of dogs with positive results declined to participate and two owners agreed to participate but did not come for their follow-up analyses, which resulted in six dogs participating. To include more dogs in the study, all owners of dogs that received a positive result on MAT within one month before the field study period and that fulfilled the inclusion criteria were asked to participate in the study. Four dogs entered the study this way, one owner declined. This resulted in a total sample population of ten dogs. The dogs diagnosed with leptospirosis before the field study period did not have all the necessary analyses for the project. This was considered when evaluating the results.

All the owners (n=10) with dogs included in the study filled out a questionnaire (appendix 1). Results showed that out of the ten included dogs, six were mixed breed, four were pure breed and six were male. The age varied from 7 months to 12 years, five were between 7 months and 4 years, two between 5 and 8 years and three between 8 and 12 years. Three came for their first consultation in August, four in September and three in October. The dogs included in the study are summarized in Table 5.

Table 5. Information about the included dogs

DOG	Dates for consultation[1]		Breed	Age	Sex
				Years	f/m
	B	A			
1	7-12/9	12/10	Mixed	12	M
2	10/8	19/9	Yorkshire	1	M
3	15-17/8	19/9	Schnauzer	8,5	M
4	18-21/9	20/10	Mixed	4	F[2]
5	18-21/9	20/10	Weimaraner	1	F
6	4/9	4/10	Mixed	0,6	F
7	15-20/8	25/9	Scottish terrier	6	M
8	26/9-5/10	31/10	Pekingese	7	F[2]
9	1-5/10	24/10	Mixed	1	M
10	3-5/10	30-31/10	Poodle	12	M

. = parameter not included in the history, [1] = Samples collected during that period, [2] = castrated, A = after treatment, B = before treatment, , F = female, M = male

Out of the ten dogs studied seven had received a vaccination against *Leptospira*, three had not. Of the vaccinated dogs five received their vaccination in 2011 and two received one in 2012. Five were vaccinated with Pfizer Vanguard 5L4, a vaccine currently used at UNMSM. This vaccine contains the serovars canicola, grippotyphosa, icterohaemorrhagiae and pomona. The remaining two were vaccinated with a vaccine containing the serovars canicola and icterohaemorrhagiae.

The most common clinical signs reported in the ten questionnaires were depression (n=6), vomiting (n=5), diarrhea (n=4) and polydipsia (n=4). Other less common clinical signs were, in decreasing order, darker urine than before (n=3), fever (n=3), polyuria (n=2) and hemorrhages (n=2). When asked, two of the owners thought their dog moved less than normal. No dog suffered from pain according to their owners.

Three of the dogs had received a positive titer against Toxoplasma gondii during the field study period or within one month before (No. 1, 3 & 6). 10% (1/10) suffered from myxomatous mitral valve disease (No. 10).

Blood analyses

Hematology

None of the dogs had anemia (erythrocytes<5, hemoglobin (Hb)<12, hematocrite (HCT)<37) at their first consultation nor at their follow-up after one month of treatment. Leucocytosis was found in seven and neutrophilia (segmented neutrophils) in nine of the dogs before treatment. The leucocyte count decreased in all the dogs after treatment except for one which was within normal ranges both before and after treatment but three dogs that had leucocytosis initially still had values above normal range for leucocytes. The neutrophilia decreased in six of the dogs but nine still had values above normal range for segmented neutrophils after

treatment. Both the decrease in leucocytes and neutrophiles was found to be statistically robust at the p=0,05 level. The dogs included in the study had a value of zero on basophiles, monocytes and neutrophiles (myelocytes, metamyelocytes and banded), therefore these values could not be analyzed statistically. The results from hematology including the statistical analyses are found in Table 6, 7 and 8.

Table 6. Results Hematology, part 1/2, values outside the normal reference range indicated with a bold and underlined style

DOG	Ery[a]		Hb[a]		HCT[a]		Leuc[b]		Tro[a]		Eos[a]		Baso[d]	
	x10⁶/ul		g/dl		%		x10³/ul		x10³/ul		1-5%		0-0,5%	
	5-7		12-18		37-55		8-13		200-400		80-650/ul		0-65/ul	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	7	7	15,3	16	48	49	**13,86**	6,8	400	**416**	**0**	**0**	0	0
2	7	6,79	16	14,4	46	41	10,95	10,29	394	292	2	2	0	0
3	**7,52**	5,1	14,9	13,5	49	**35**	**15,41**	12,45	397	**196**	3	3	0	0
4	6,9	**7,8**	16,4	**19**	48	**55**	11,46	10,9	289	337	2	1	0	0
5	**7,5**	**8,3**	17,7	16,8	50	54	**16,25**	**13,5**	300	285	2	**0**	0	0
6	6,8	**7,3**	14,9	16	43	48	**14,37**	9,77	**199**	**173**	1	2	0	0
7	**8,5**	**7,8**	18	17,2	53	50	**20,37**	**18,4**	**420**	397	1	**0**	0	0
8	**7,2**	6,9	15	16	38	48	**20**	8,3	**428**	**479**	**0**	2	0	0
9	6,8	**8,4**	15,4	17	44	51	8,34	9,8	**196**	282	2	**0**	0	0
10	**7,27**	6,7	16,8	16	49	50	**26,42**	**13,76**	**430**	350	**0**	4	0	0

[a] = non-significant at p = 0,05, [b] = significant at p = 0,05, [d] = not possible to receive a result, A = after treatment, B = before treatment, Baso = basophiles, Eos = eosinophiles, Ery = erythrocytes, Hb = hemoglobin, HCT = hematocrite, Leuc = leucocytes, Tro = thrombocytes

Table 7. Results Hematology, part 2/2, values outside the normal reference range indicated with a bold and underlined style

DOG	Neut[b]								Lym[a]		Mono[d]	
	Myel 0% 0/ul		Met 0% 0/ul		Band 0-3% 0-390/ul		Segm 65-75% 5200-9750/ul		15-30% 1200-3900/ul		1-4% 80-520/ul	
	B	A	B	A	B	A	B	A	B	A	B	A
1	0	0	0	0	0	0	**81**	**84**	19	16	**0**	**0**
2	0	0	0	0	0	0	**88**	**80**	**10**	18	**0**	**0**
3	0	0	0	0	0	0	**81**	**77**	16	20	**0**	**0**
4	0	0	0	0	0	0	**89**	75	**9**	24	**0**	**0**
5	0	0	0	0	0	0	**80**	**80**	18	20	**0**	**0**
6	0	0	0	0	0	0	**89**	**63**	**10**	**35**	**0**	**0**
7	0	0	0	0	0	0	**91**	**90**	**6**	**10**	2	**0**
8	0	0	0	0	0	0	**93**	**86**	**7**	12	**0**	**0**
9	0	0	0	0	0	0	74	**80**	24	20	**0**	**0**
10	0	0	0	0	0	0	**90**	**82**	**8**	**14**	2	**0**

[a] = non-significant at p = 0,05, [b] = significant at p = 0,05, [d] = not possible to receive a result, A = after treatment, B = before treatment, Band = banded, Lym = lymphocytes, Met = metamyelocytes, Mono = monocytes, Myel = myelocytes, Neut = neutrophiles, Segm = segmented

Table 8. Statistical analyses of hematology variables, software STATA11

VARIABLE	Shapiro-Wilk Z > 0,05 = normal value Z < 0,05 = not normal value logarithm (base 10)				Distribution R = right-tailed L = left-tailed		Wilcoxon p > 0,05 = non-significant p < 0,05 = significant		Paired T-test p > 0,05 = non-significant p < 0,05 = significant	
			analyzed if not normal value		*analyzed if not normal value*		*analyzed if not normal value, left-tailed distribution*		*analyzed if normal value + not normal value, right-tailed distribution*	
	B	A	B	A						
Ery	0,02	0,33	0,04	0,11	R	.	.	.	0,41	NS
Hb	0,13	0,94	.	.	.	.	.	.	0,63	NS
HCT	0,68	0,11	.	.	.	.	.	.	0,71	NS
Leuc	0,75	0,61	.	.	.	.	.	.	0,01	**S**
Thro	0,02	0,91	0,01	0,61	L	.	0,51	NS	.	.
Eos	0,14	0,71	.	.	.	.	.	.	0,57	NS
Baso	-	-	.	.	.	.	.	.	.	.
Neut (myel)	-	-	.	.	.	.	.	.	.	.
Neut (met)	-	-	.	.	.	.	.	.	.	.
Neut (band)	-	-	.	.	.	.	.	.	.	.
Neut (segm)	0,23	0,38	.	.	.	.	.	.	0,04	**S**
Lym	0,20	0,36	.	.	.	.	.	.	0,98	NS
Mono	0,01	-	.	.	.	.	.	.	.	.

. = parameter not analyzed, **-** = value 0, not possible to receive a result, A = after treatment, B = before treatment, Band = banded, Baso = basophiles, Eos = eosinophiles, Ery = erythrocytes, Hb = hemoglobin, HCT = hematocrite, Leuc = leucocytes, Lym = lymphocytes, Met = metamyelocytes, Mono = monocytes, Myel = myelocytes, Neut = neutrophiles, NS = non-significant at p=0,05, S = significant at p=0,05, Segm = segmented, Thro = thrombocytes

Blood Chemistry

Dogs with higher creatinine (n=2), urea (n=8), ALT (n=3) or ALKP (n=4) than normal before treatment were found. The values decreased in many of the dogs but were still above normal range in one out of two dogs (creatinine), six out of eight (urea), one out of three (ALT) and three out of four (ALKP). In three out of eight dogs with an increased urea level before treatment, the urea increased despite the treatment. None of the dogs had hypoalbuminemia before nor after treatment. None of the dogs had hyperphosphatemia before treatment but it was found in three after treatment. None of the changes in blood chemistry were statistically robust at the p=0,05 level. The results from blood chemistry, including the statistical analyses, are found in Table 9 and 10.

Table 9. Results Blood Chemistry, values outside the normal reference range indicated with a bold and underlined style

DOG	Crea[a] mg/dl < 1,6		Urea[a] mg/dl 10—30		TP[a] g/dl 5,5-7,5		Alb[a] g/dl 2,3-3,2		ALT[a] UI/L < 50		ALKP[a] UI/L < 190		Phos[a] mg/dl 2,4-5	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	1,5	**2,4**	**40**	**61**	**5,4**	**5,4**	2,7	2,5	**91**	**82**	**1283**	**790**	4,8	3,9
2	1,5	1,6	**39**	**55**	**5**	6,3	2,9	2,8	.	**136**	.	189	.	4,9
3	**2,1**	**1,9**	**62**	**58**	.	5,8	.	2,7	40	48	.	136	.	4
4	**1,9**	1,4	28	16	6,9	6,1	**3,9**	4	39	32	**827**	**404**	3,3	**6**
5	1,6	1,5	**32**	25	6	**7,9**	**3,9**	**4,2**	36	32	143	160	3,9	**7**
6	1,2	1,6	**33**	**32**	**8,2**	**5,1**	**3,9**	**3,3**	**69**	40	**391**	**298**	.	**1,5**
7	1,5	1,5	27	21	.	6,8	.	**3,9**	.	**140**	.	**196**	.	3,8
8	2	1,5	**54**	20	5,9	**5,3**	2,6	2,8	**61**	36	113	159	3,1	2,7
9	1,4	1,6	**39**	**34**	**5**	5,6	**3,6**	2,8	25	35	111	120	**2,1**	**5,3**
10	1,6	**2,8**	**54**	**61**	6,2	6,1	**3,7**	2,7	49	**53**	**402**	180	3,6	3,6

. = parameter not analyzed, [a] = non-significant at p = 0,05, A = after treatment, B = before treatment, Alb = albumin, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, Crea = creatinine, Phos = phosphate, TP = total protein

Table 10. Statistical analyses of biochemistry variables, software STATA11

VARIABLE	Shapiro-Wilk Z > 0,05 = normal value Z < 0,05 = not normal value logarithm (base 10) *analyzed if not normal value*				Distribution R = right-tailed L = left-tailed *analyzed if not normal value*		Paired T-test p > 0,05 = non-significant p < 0,05 = significant *analyzed if normal value + not normal value, right-tailed distribution*	
	B	A	B	A	B	A		
Crea	0,55	0,01	0,73	0,02	.	R	0,78	NS
Urea	0,22	0,25	.	.	.	.	0,31	NS
TP	0,28	0,25	.	.	.	.	0,43	NS
Alb	0,28	0,04	0,20	0,06	.	R	0,08	NS
ALT	0,59	0,00	0,97	0,03	.	R	0,17	NS
ALKP	0,08	0,00	0,31	0,08	.	R	0,09	NS
Phos	0,97	0,98	.	.	.	.	0,92	NS

. = parameter not analyzed, A = after treatment, B = before treatment, Alb = albumin, ALKP = alkaline phosphatase, ALT = alanine aminotransferase, Crea = creatinine, NS = non-significant at p=0,05, Phos = phosphate, TP = total protein

Microscopic Agglutination Test (MAT)

All dogs included in the study had at least a titer of 1/100 against one of the serovars. A titer of 1/400 or more was observed in two dogs, the remaining dogs had a lower titer. Three dogs had a titer against more than one serovar at the first consultation and six had a negative result on MAT at their follow up. The rest of the dogs received a positive titer against the same

serovar as before the treatment and eventually against other serovars too. Six dogs received a positive titer against a serovar included in the vaccine they got in 2011 or 2012. The results from MAT and information about vaccination included, are found in Table 11.

Table 11. Results on MAT

DOG	Serovar													
	Aus neg		**Bra** Neg		**Can** neg		**Geo** neg		**Gri** neg		**Ict** neg		**Pom** neg	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	.	.	.	.	**1/200**	.	.	.	.	.	.	.	.	.
2	.	.	---	.	.	.	.	.	**1/100[1]**	.	.	.	.	.
3	.	.	.	.	.	**1/200[1]**	.	.	**1/100[1]**	**1/100[1]**	.	.	.	**1/400[1]**
4	**1/100**	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	.	**1/100[1]**	.	.	.	**1/100[1]**	.	.	.	**1/200[1]**	**1/200[1]**
6	.	.	**1/200**	.	.	.	.	.	.	.	.	.	.	.
7	.	.	.	.	.	.	.	.	**1/200**	.	.	.	.	.
8	.	.	**1/100**	**1/100**	**1/800[1]**	.	.	.	**1/100**	**1/200**	.	.	**1/200**	.
9	.	.	.	.	.	.	.	.	.	.	.	.	**1/100[2]**	**1/400[2]**
10	.	.	.	.	**1/200[2]**	.	.	.	.	.	.	.	**1/400**	.
Total	**1**	**0**	**2**	**1**	**4**	**1**	**0**	**0**	**5**	**2**	**0**	**0**	**4**	**3**
Total *vaccinated*	-	-	-	-	**2 (2011) 1 (2012)**	**1 (2011)**	-	-	**3 (2011)**	**1 (2011)**	**0**	**0**	**1 (2011) 1 (2012)**	**2 (2011) 1 (2012)**

. = negative, --- = serovar not analyzed, - = no vaccine containing serovar available, [1] = vaccinated with vaccine including that serovar 2011, [2] = vaccinated with vaccine including that serovar 2012, Aus = Australis, Bra = Bratislava, Can = Canicola, Geo = Georgia, Gri = Grippotyphosa, Ict = Icterohaemorrhagiae, Pom = Pomona

Urinalysis

More than 50% of the dogs had a lower or higher specific gravity than normal before and after treatment. One dog had a higher specific gravity than normal both before and after treatment, however the other dogs did not have alternations in specific gravity that endured after treatment. In seven out of nine studied dogs proteinuria with >3+ on the sulphosalicylic test (SAA) and/or the nitric acid test (NAT) before treatment was found. According to urine stick tests four of these dogs also had hematuria (according to analysis of the urine sediment two additional dogs had few erythrocytes in their urine, bringing the total number of dogs with hematuria according to the urine sediment to six). Four of the dogs with proteinuria (n=7) before treatment still had a value above normal range after treatment. None of these dogs had hematuria at the follow-up according to urine stick tests (three dogs had few erythrocytes in the urine according to the sediment). The decrease in proteinuria after treatment was significant at the p=0,05 level. A regular amount of leucocytes in the urine before treatment was observed in four out of nine dogs. One of these four dogs had several leucocytes in the urine after treatment, the others were negative. Another dog that had few leucocytes in the urine before treatment had a regular amount after treatment. Five out of nine dogs had granular cylinders in their urine before treatment. After treatment these dogs no longer had granular cylinders in their urine, however two dogs, one that was negative for

cylinders before treatment (No. 2) and one without a urine sample before treatment (No. 3), had granular cylinders in their urine after treatment. The results of specific gravity, protein/creatinine ratio and proteins, including the statistical analyses, are found in Table 12 and 13.

Table 12. Results of specific gravity, protein/creatinine ratio and protein, values outside the normal reference range indicated with a bold and underlined style

DOG	Refractometer		EMT-168		SAA/NAT			
	Spec grav[a]		**P/C ratio**[a]		**Pro**[b]			
	1,025-1,035		< 1		neg/tr (0/1)			
	B	A	B	A	B	B (nr)	A	A (nr)
1	1,035	1,035	**2,26**	0,51	**+++**	**4**	**++**	**3**
2	**1,055**	**1,055**	.	0,78	**++**	**3**	**++**	**3**
3	.	1,032	.	0,84	.	.	**++**	**3**
4	**1,037**	**1,014**	0,41	0,36	**+++**	**4**	neg	0
5	**1,018**	1,028	0,92	0,13	Tr	1	neg	0
6	1,03	1,026	.	0,59	Tr	1	tr	1
7	1,025	**1,015**	.	**1,07**	**+++**	**4**	**++**	**3**
8	1,035	**1,018**	0,75	0,83	**+++**	**4**	neg	0
9	**1,01**	**1,042**	0,8	**1,86**	**+++**	**4**	**++**	**3**
10	**1,049**	**1,019**	**1,6**	0,57	**++**	**3**	tr	1

. = parameter not analyzed, [a] = non-significant at p = 0,05, [b] = significant at p = 0,05, A = after treatment, B = before treatment, NAT = nitric acid test, Neg = negative, Nr = number, Pro = protein, P/C ratio = protein/creatinine ratio, SAA = sulphosalicylic acid test, Spec grav = specific gravity, Tr = trace

Table 13. Statistical analyses of urine variables, software STATA11

VARIABLE	**Shapiro-Wilk** Z > 0,05 = normal value Z < 0,05 = not normal value logarithm (base 10) *analyzed if not normal value*				**Paired T-test** p > 0,05 = non-significant p < 0,05 = significant *analyzed if normal value + not normal value, right-tailed distribution*		**Sign Test** p > 0,05 = non-significant p < 0,05 = significant *analyzed is qualitative variable*	
	B	A	B	A				
Spec grav	0,97	0,40	.	.	0,24	NS	.	.
P/C ratio	0,34	0,18	.	.	0,18	NS	.	.
Pro	.	.	.	.	.	.	0,02	**S**

. = parameter not analyzed, A = after treatment, B = before treatment, NS = non-significant at p=0,05, Pro = protein, P/C ratio = protein/creatinine ratio, S = significant at p=0,05, Spec grav = specific gravity

Treatment

Antibiotics

All of the dogs (n=10) received some kind of antimicrobial treatment and nine received doxycycline. Four out of nine received the dose recommended by ACVIM, Goldstein (2010) and Greene et al. (2006) (5 mg/kg every 12 hours). Of the dogs that received doxycycline five had previously received treatment with benzylpenicillin, ampicillin or amoxicillin at the clinic prior to the oral treatment with doxycycline at home. A compilation of the antimicrobial treatment received by the dogs is found in Table 14.

Table 14. Compilation of the antimicrobial treatment received by the dogs studied

DOG	BP			Amp			Amo			Dox			Trim/sulpha			Other
	Dose IU/kg	Dur *days*	Adm	Dose mg/kg	Dur *days*	Adm	Dose mg/kg	Dur *days*	Adm	Dose mg/kg	Dur *days*	Adm	Dose mg/kg	Dur *days*	Adm	
1	.	.	.	22/12h	4	IV	.	.	.	5/12h	20+14[1]	PO	.	.	.	Cli[3]
2	.	.	.	.	.	.	.	.	.	10/12h	28	PO	.	.	.	.
3	.	.	.	.	.	.	15/24h	5	IM	5/24h	15	PO	.	.	.	Cli[3]
	.	.	.	.	.	.	.	.	.	15/24h	20	PO	.	.	.	.
4	.	.	.	.	.	.	.	.	.	5/12h	28	PO	.	.	.	.
5	.	.	.	.	.	.	.	.	.	5/12h	28	PO	.	.	.	.
	30000	6	IM	.	.	.	.	.	.	5/12h	7+7+7[2]	PO	.	.	.	.
6	.	.	.	.	.	.	.	.	.	10/12h	30	PO	.	.	.	Cli, oxy
7	.	.	.	.	.	.	.	.	.	.	.	.	24/12h	7	-	.
8	20000	4	-	.	.	.	.	.	.	4/12h	14	PO	.	.	.	Cep
9	.	.	.	.	.	.	18/-	2	IM	5/12h	14	PO	.	.	.	.
10	.	.	.	.	.	.	.	.	.	8/12h	15+5[1]	PO	.	.	.	.

. = not given, **-** = not mentioned, [1] = The treatment was prolonged, [2] = 7 days treatment, 7 days rest x 3, [3] = against toxoplasmosis, Adm = method of administration, Amp = ampicillin, Amo = amoxicillin, BP = benzylpenicillin, Cep = cephalotin/cephalexin, Cli = clindamycin, Dox = doxycycline, Dur = duration, IU = international units, IV = intravenously, IM = intramuscular, Oxy = oxytetracycline, PO = per oral, Trim/sulpha = trimethoprim/sulphonamide

Supportive treatment

Almost all the dogs (n=9) received some kind of supportive treatment (fluid therapy, antacida, antiemeticum, vitamins, silymarin or others). A compilation of the supportive treatment the dogs received is found in Table 15.

Table 15. Compilation of the supportive treatment received by the dogs studied

DOG	Fluids			Antacida			Antiemeticum	Vitamins			Silymarin	Other
	SP	RL	NaCl	Ran	Ome	Sucr	Meto	Amino	Gli	Hep		
1	+	+	+	+	+	.	+	+	.	+	.	Dex, LM, Sedo, Tram, Diet
2	.	.	+	+	.	.	+	.	.	.	.	Diet k/d
3	+	+[1]	.	.	.	.	+	.	.	+	.	.
4	.	.	.	+	.	.	.	.	.	.	+	Diet
5	.	.	.	+	+	+	+	.	.	.	+	
6	+	.	+	+	+	.	+	+	.	+	.	Meta, Dex, Pred, NSAID[2]
7	.	.	.	.	.	.	.	.	.	.	.	.
8	+	+	.	+	.	.	+	.	+	+	+	Diet
9	.	.	+	+	.	.	.	.	.	.	.	Tram, Dron, skin gel
10	.	.	+	.	.	.	.	.	.	.	+	Fur, Dig, Enal, Spi, Theo

[1] = dextrose added, [2] = local treatment in eyes, Amino = aminoplex, Dex = dexamethasone, Dig = digoxine, Dron = droncit, Enal = enalaprile, Fur = furosemide, Gli = glicopan, Hep = hepatina or hepabionta, LM = leche de magnesia (laxative), Meta = metamizole, Meto = metoclopramide, Ome = omeprazole, Pred = prednisolone, Ran = Ranitidine, RL = Ringer Lactate, Sed = sedotropine (anti-bloating), SP = suera polielectrolítica, Spi = spironolactone, Sucr = sucralphate Theo = theophylline, Tram = tramadol

Discussion

Included dogs

During the field study period at UNMSM ten dogs participated in the study. This was a small group of dogs which makes it difficult to draw firm conclusions about leptospirosis in dogs in general based on the result in the studied dogs. All dogs did not have all the analyses included in the study which made the study sample even smaller for some of the variables. A control group with healthy dogs was not included in this study. With a control group a comparison of changes in blood and urine samples could have been made between healthy and ill animals. It would also have been possible to compare serological results in clinically healthy dogs and dogs with clinical signs. All the dogs that fulfilled the inclusion criteria and received a positive titer on MAT entered the study but the dogs that received a negative result on their first consultation were not included. It is possible that some of the dogs were in an acute stage of the disease and had not yet seroconverted. A new MAT after approximately two weeks would have been appropriate in the seronegative dogs, but in general this would not have been economically possible for the study nor for the owners. In order to include as many dogs as possible no discrimination on basis of breed, age or sex was made. To investigate the prevalence and lesions in different categories of dogs, a bigger group and a longer field study period would be required.

Clinical findings

Clinical signs

Depression, vomiting, diarrhea and polydipsia were the most common clinical signs in this study. Anorexia has been found in 67-75% of dogs in other studies (Birnbaum et al., 1998,

Prescott et al., 2002; Goldstein et al., 2006). Anorexia was not included as a question in the questionnaire for the owners and only one owner wrote it in the "Other" column. This could be the reason why anorexia was not a common clinical sign in this study.

Blood analyses

In a study done by Goldstein et al. (2006) 53% (29/54) of the dogs had anemia. Anemia can be due to acute renal failure with over hydration, chronic renal failure with decreased production of erythropoietin, chronic inflammatory disease or acute blood loss due to tissue thrombosis and/or acute hemorrhaging in the gastrointestinal tract or the respiratory organs (Greene et al., 2006). In this study none of the dogs had low hematocrite either before or after treatment. This could be a true finding or could be due to a false-normal hematocrite if the dogs suffered from both anemia and dehydration (Ettinger & Feldman, 2010). In this study, the decrease in leucocytosis and neutrophilia was found to be statistically robust (p=0,05). This could be due to a decreased inflammatory response attributed to the treatment (Ettinger & Feldman, 2010). However, even if a significantly robust decrease was seen, many of the dogs still had values above normal range after treatment, especially the dogs with neutrophilia, indicating that the inflammation was still ongoing.

When describing blood chemistry changes in dogs with leptospirosis, azotemia (increased urea and creatinine) is almost always seen as a primary finding. Some of the dogs that came to the clinic before the field study, and were included later, did not have comprehensive blood chemistry results available, however all of them had at least urea and creatinine measured previously. Goldstein et al. (2006)'s finding of 93% azotemic dogs in a group with leptospirosis emphases the need to evaluate levels of urea and creatinine in dogs with this disease. The renal and liver enzymes were higher than normal in many of the dogs in the study. They decreased in some dogs after treatment, however they were still observed to be above normal range in several dogs. The urea value even increased in some dogs despite treatment. None of the changes in blood chemistry were statistically robust (p=0,05). It is therefore not possible to draw conclusions about any improvement in blood chemistry due to the treatment. In the dogs with higher values than normal after treatment, chronic lesions in the kidneys and/or liver could have been developed. Studies have shown that if urea and creatinine are increased, a minimum of 75% of the nephrons must have lost their function (Ettinger & Feldman, 2010; Greene et al., 2006). To better investigate the liver function bile acids can be evaluated (Ettinger & Feldman, 2010) but this analysis in not currently used at the Laboratory of Clinical Pathology, UNMSM. In a study by Goldstein et al. (2006) 78% of the dogs had developed hyperphosphatemia. Birnbaum et al. (1998) found hyperphosphatemia in 50% of the dogs. With those high numbers it is suggested to take a phosphate sample in dogs suspected or diagnosed with leptospirosis. The development of hyperphosphatemia could be due to development of a renal failure, but it can also be due to delayed serum separation, dietary excess, hemolysis or another cause (Ettinger & Feldman, 2010). Goldstein et al. (2006) and Prescott et al. (2002) found different electrolytic changes in dogs with leptospirosis such as hyponatremia, hypo- or hyperkalemia and hypochloremia. An evaluation of electrolytes was not included in the study as the consultation with blood and urine samples could not be too expensive for the owners participating in the study.

Four of the dogs had been diagnosed with other diseases as well, three with toxoplasmosis and one with myxomatous mitral valve disease. These diseases could have contributed to the changes in hematology, blood chemistry and urinalysis that were found in the studied dogs and the titers against *Leptospira* could have been an incidental finding. Other diseases that the dogs were not tested for could also have contributed to the changes.

Urinalysis

The specific gravity of urine is a complex parameter as the results depend on the water intake and the current status of the animals. A dog can have a changed specific gravity in one urine sample and a normal value in another. To investigate if the dog has a decreased possibility to concentrate its urine, two urine samples from different occasions should be analyzed. If the dog has decreased specific gravity in both of them a concentration disability could be suspected (Ettinger & Feldman, 2010). The urine specific gravity in the dogs in the study deviated both above and below normal values but none of them had a lower specific gravity both before and after treatment. However 40% (4/10) of the dogs had a lower specific gravity than normal after treatment and it is recommended that these dogs are reexamined in the future, in order to see if they still have a low specific gravity, a finding that could indicate a decreased renal or hepatic function.

According to the sulphosalicylic acid test (SAA) and the nitric acid test half of the included dogs had proteinuria after treatment. Only two of these dogs had an increased protein/creatinine ratio. In a study by Lyon et al. (2010) the protein/creatinine was found to be a very specific method so the ability to correctly identify negative samples was good (specificity of 99,7% in dogs) but it had low sensitivity (28,7%). The SAA had moderate specificity and a poor positive predictive value (Lyon et al., 2010). The decrease in proteinuria after treatment was found to be statistically robust (p=0,05). It is important to consider that although there was a significant decrease in proteinuria several dogs still had values above the normal range. Proteinuria can be due to a renal loss of protein or inflammation in the urinary tract (Ettinger & Feldman, 2010). Proteinuria with a low cellularity-sediment is something that can indicate that proteins are being lost through the kidneys. Therefore, before taking renal disease into consideration, it is important to examine the urine sediment and see if there are many cells there (Greene et al., 2006). It is also important to compare these results with other parameters, such as urine specific gravity, presence of granular casts in urine, albumin, creatinine and urea values in blood etc.

Diagnosis with MAT

A problem with MAT is that it cannot differentiate between antibodies due to vaccination and those from infection (Adler & Moctezuma, 2010). Many of the dogs were vaccinated during 2011 or 2012 and the majority of them received low MAT-titers. According to recommendations for vaccination the dogs should be vaccinated annually to maintain the protection (Sykes et al., 2011). However in studies involving humans, detectable antibodies have been found after up to 20 years (Faine et al., 1999) so it could be possible that the dogs still have antibodies circulating in their blood. Therefore it is very important to consider

whether the dog has received a vaccination against *Leptospira* when evaluating the MAT results and compare the results with other findings and clinical signs. This is also why a paired test with at least a four-fold increase or a titer of at least 1/400 (Adler & Moctezuma, 2001) or 1/800 (Levett, 2001) is recommended.

Only two dogs received a titer above 1/400 in this study. As mentioned above, antibodies have shown to persist for a long time and a seropositive test, above all low titers, do not equal an acute infection. However all the studied dogs received antimicrobial treatment. It is important to have in mind that the veterinarians who treat these dogs are dealing with a zoonotic disease and that the dogs are often in a very bad shape due to one or many diseases. Therefore, if the dog is ill, has clinical signs and clinical findings in accordance with leptospirosis and also has a positive result on MAT, even though it is a low titer, a treatment is often initiated.

If the dog is successfully treated with antibiotics the titer will decrease. Agglutinating antibodies that react in MAT are mainly IgM and IgG to a lesser extent. IgM increases and decreases according to the presence of the organism (Greene et al., 2006; WHO, 2003). This could probably explain why the titers on MAT would be decreased after a successful treatment. Sometimes a titer of 1/200 at the most can be seen during 1-4 months after treatment and this can be due to a long-term persistence or replication of the bacteria (Greene et al., 2006). This could have been the case in the dogs with titers against one or many serovars after treatment. The initial titer and the change in titer do not seem to correlate with the severity of the disease (Greene et al., 2006).

An important consideration with MAT is that cultivation and survival of the leptospires are important to be able to execute the test. The results are evaluated manually and are therefore subjective. A dog can also suffer from a serovar that is not included in MAT at that laboratory or there could be a weak reaction against the serovar chosen as representative for the serogroup (Greene et al., 2006).

Treatment

The most common choice of antibiotic treatment in this study and earlier studies was doxycycline, probably because the veterinarians want to use antibiotics that can eliminate the bacterium from the body. Doxycycline is also mainly excreted in the feces so the dose does not have to be changed for dogs with renal failure (Greene et al., 2006). However development of antimicrobial resistance also has to be considered before starting the treatment. According to the guidelines for the clinical use of antibiotics in the treatment of dogs and cats, formed by the Swedish Veterinary Association (SVS) (original 2002, revised 2009) there has to be evidence of infection before starting a treatment with antibiotics. More studies with clearly defined groups are needed to evaluate the best choice of antibiotics. The most common choice at UNMSM is doxycycline at a dose of 5 mg/kg/12 hours, a dose recommended by trustworthy sources (Goldstein, 2010; Greene et al., 2006; Sykes et al., 2011). However Goldstein (2010) and Greene et al. (2006) consider three weeks an appropriate duration while ACVIM recommend two weeks. The duration of the treatment in

the studied dogs varied between 14 and 28 days. The duration of treatment probably has to be corrected for the individual patient. Some may need a longer treatment and others shorter, but a minimum of two weeks seems to be appropriate.

Conclusions

For dogs with leptospirosis according to this study:

- The most common changes in hematology were leucocytosis and neutrophilia (anemia and thrombocytopenia were not found in this study but are important findings in other studies)
- The most common changes in blood chemistry were increased urea and creatinine, increased ALT, increased ALKP and hyperphosphatemia (hypoalbuminemia was not found in this study but is an important finding in other studies)
- The most common changes in urinalysis were proteinuria, pyuria, hematuria and presence of granular casts (persistent low specific gravity was not found in this study but is an important finding in other studies)
- The treatment significantly decreased the leucocytosis, neutrophilia and proteinuria ($p=0,05$) but not always to a level within the normal range

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REFERENCES

Adin, C.A. & Cowgill, L.D. (2000) Treatment and outcome of dogs with leptospirosis: 36 cases (1990–1998). *Journal of the American Veterinary Medical Association*, 216(3), 371-375.

Adler, B. & Moctezuma, A.P. (2010) Leptospira and Leptospirosis. *Veterinary Microbiology*, 140, 287-296.

Arent, Z.J., Andrews, S., Adamama-Moraitou, K., Gilmore, C., Pardali, D. & Ellis, W.A. (2012) Emergence of novel Leptospira serovars: a need for adjusting vaccination policies for dogs?. *Epidemiology and Infection*, 24, 1-6.

Babudieri, B. (1958) Animal Reservoirs of Leptospires. *Annals of the New York Academy of Sciences*, 70, 393-413.

Birnbaum, N., Barr, S.C., Centert, S.A., Schermerhorn, T., Randolph, J.F. & Simpson, K.W. (1998) Naturally acquired leptospirosis in 36 dogs: serological and clinicopathological features. *Journal of Small Animal Practice*, 39, 231-236.

Boqvist, S., Eliasson-Sellin, L., Bergström, K. & Magnusson, U. (2012) The association between rainfall and seropositivity to Leptospira in outdoor reared pigs. *The Veterinary Journal*, 1, 135-139.

Brenner, D.J, Kaufmann, A.F., Sulzer, K.R, Steigerwalt, A.G., Rogers, F.C. & Weyant, R. (1999) Further determination of DNA relatedness between serogroups and serovars in the family Leptospiraceae with a proposal for Leptospira alexanderi sp. nov. and four new Leptospira genomospecies. *International Journal of Systematic Bacteriology*, 49, 839-858.

Céspedes, M., Chun, M., Cano, E., Huaranca, I., Atoche, H., Ortiz, H., Valentin, M., Balda, L. & Huamán, T. (2007) Prevalencia de anticuerpos contra Leptospira en personas asintomáticas y en perros de Chancay, Lima 2001. *Revista Peruana de Medicina Experimental y Salud Pública*, 24(4), 343-349.

Ciceroni, L., Bartoloni, A., Pinto, A., Guglielmetti, P., Valdez, V. C, Gamboa, B.H., Roselli, M., Giannico, F. & Paradisi, F. (1997) Serological survey of leptospiral infections in sheep, goats and dogs in Cordillera province, Bolivia. *New Microbiology*, 20(1), 77-81.

Dikken, H. & Kmety, E. (1978) Serological typing methods of leptospires. In Bergan, T. & Norris, J. R. (editors) *Methods in Microbiology*. 11, 259–307. Academic Press, London.

Ettinger, S.J. & Feldman, E.C. (2010) *Textbook of Veterinary Internal Medicine*, 7. ed. St Louis, Missouri, USA, Saunders Elsevier.

Faine, S., Adler, B., Bolin, C. & Perolat, P. (1999) *Leptospira and Leptospirosis*. 2. ed. Melbourne, Australia, MediSci.

Faine, S. & Stallman, N.D. (1982) Amended Descriptions of the Genus Leptospira Noguchi 1917 and the Species L. interrogans (Stimson 1907) Wenyon 1926 and L. biflexa (Wolbach and Binger 1914) Noguchi 1918. *International Journal of Systemic Bacteriology*, 32(4), 461-463.

Forbes, A.E., Zochowski, W.J., Dubrey, S.W. & Sivaprakasam, V. (2012) Leptospirosis and Weil's disease in the UK. *Quarterly Journal of Medicine*, 105, 1151–1162.

Goldstein, R.E. (2010) Canine Leptospirosis. *Veterinary Clinics of North America: Small Animal Practice*, 40 (6), 1091‑1101.

Goldstein, R.E., Lin, R.C., Langston, C.E., Scrivani P.V., Erb, H.N. & Barr, S.C. (2006) Influence of Infecting Serogroup on Clinical Features of Leptospirosis in Dogs. *Journal of Veterinary Internal Medicine*, 20, 489-494.

Greene, C.E., Sykes, J.E., Moore, G.E., Goldstein, R.E. & Schultz, R.D. (2006) Chapter 42: Leptospirosis. In Greene, C.E. (editor) *Infectious Diseases of the dog and cat*. 4. ed. 431-447. St Louis, Saunders Elsevier.

Greenlee, J.J., Alt, D.P., Bolin, C.A., Zuerner, R.L. & Andreasen, C.B. (2005) Experimental canine leptospirosis caused by Leptospira interrogans serovars pomona and Bratislava. *American Journal of Veterinary Research*, 66(10), 1816-1822.

Greenlee, J.J., Bolin, C.A:, Alt, D.P., Cheville, N.F. & Andreasen, C.B. (2004) Clinical and pathologic comparison of acute leptospirosis in dogs caused by two strains of Leptospira kirschneri serovar grippotyphosa. *American Journal of Veterinary Research*, 65(8), 1100-1107.

Hartman, E.G., van Houten, M., Frik, J.F, & van der Donk, J.A. (1984) Humoral immune response of dogs after vaccination against leptospirosis measured by an IgM- and IgG-specific ELISA. *Veterinary Immunology and Immunopathology*, 7(3-4), 245-254.

Holt, S.C. (1978) Anatomy and Chemistry of Spirochetes. *Microbiological reviews*, 42(1), 114-160.

Johnson, R.C. & Harris, V.G. (1967) Differentiation of Pathogenic and Saprophytic Leptospires. I. Growth at Low Temperatures. *Journal of Bacteriology*, 94(1), 27-31.

Langston, C.E. & Heuter, K.J. (2003) Leptospirosis: A re-emerging zoonotic disease. *Veterinary Clinics of North America: Small Animal Practice*, 33 (4), 791-807

Levett, P.N. (2001) Leptospirosis. *Clinical Microbiology Reviews*, 14(2), 296-326.

Limmathurotsakul, D., Turner, E.L., Wuthiekanun, V., Thaipadungpanit, J., Suputtamongkol, Y., Chierakul, W., Smythe, L.D., Day, N.P.J., Cooper, B. & Peacock, S.J. (2012) 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*, 55, 322-331.

Lyon, S.D., Sanderson, M.W., Vaden, S.L., Lappin, M.R., Jensen, A.W. & Grauer, G.F. (2010) Comparison of urine dipstick, sulfosalicylic acid, urine protein-to-creatinine ratio, and species-specific ELISA methods for detection of albumin in urine samples of cats and dogs. *Journal of the American Veterinary Medical Association*, 236(8), 874-879.

McDonough, P.L. Leptospirosis in Dogs – Current Status. [online] (2001-07-19). Available from: http://www.ivis.org/advances/infect_dis_carmichael/mcdonough/ivis.pdf [2013-01-02]

Miller, M.D., Annis, K.M., Lappin, M.R. & Lunn, K.F. (2011) Variability in Results of the Microscopic Agglutination Test in Dogs with Clinical Leptospirosis and Dogs Vaccinated against Leptospirosis. *Journal of Veterinary Internal Medicine*, 25, 426-432.

Nelson, R.W. & Couto, C.G. (2009) *Small Animal Internal Medicine*. 4. ed. St Louis, Missouri, USA, Mosby Elsevier.

Ngbede, E.O., Radi, M.A., Kwanashie, C.N., Okolocha, E.C., Gugong, V.T. & Hambolu, S.E. (2012) Serological prevalence of leptospirosis in cattle slaughtered in the Zango abattoir in Zaria, Kaduna State, Nigeria. *Veterinaria Italiana*, 48 (2), 179-184.

Oficina General de Epidemiología, Instituto Nacional de Salud. Leptospirosis, Módulos Técnicos . Lima. (Serie Documentos Monográficos N°2) [online] (2000). Available from:

http://www.ins.gob.pe/insvirtual/images/otrpubs/pdf/m%C3%B3dulo%20t%C3%A9cnico%202%20leptospirosis.pdf [2013-01-01]

Petrie, A. & Watson, P. (2001). *Statistics for Veterinary and Animal Sciences*. 2. ed. MA, USA, Blackwell Science Ltd.

Picardeau, M. Diagnosis and epidemiology of leptospirosis [online] (2013-01-18). Available from: Médecine et Maladies Infectieuses, http://dx.doi.org/10.1016/j.medmal.2012.11.005 [2013-01-28]

Prescott, J.F., McEwen, B., Taylor, J., Woods, J.P., Abrams-Ogg, A. & Wilcock, B. (2002) Resurgence of leptospirosis in dogs in Ontario: recent findings. *The Canadian Veterinary Journal*, 43, 955-961.

Rodríguez, A.L., Ferro, B.E., Varona, M.X. & Santafé, M. (2004) Exposure to Leptospira in stray dogs in the city of Cali (Spanish). *Biomedica*, 24(3), 291-295.

Romero, M.H., Sánchez, J.A. & Hayek, L.C. (2010) The prevalence of antibodies against Leptospira in urban human and canine populations from the Tolima Department (Spanish). Revista *de Salud Pública (Bogotá)*, 12(2), 268-275.

Sykes, J.E., Hartmann, K., Lunn, K.F., Moore, G.E., Stoddard, R.A. & Goldstein, R.E. (2011) 2010 ACVIM Small Animal Consensus Statement on Leptospirosis: Diagnosis, Epidemiology, Treatment, and Prevention. *Journal of Veterinary Internal Medicine*, 25, 1-13.

The National Veterinary Institute. Leptospiros hos hund. [online] (2011-09-18). Available from: http://sva.se/sv/Djurhalsa1/Hund/Infektionssjukdomar/Leptospiros/ [2013-01-01]

The Swedish Board of Agriculture. Statistics of notifyable diseases 2011. [online] (2012-10-16). Available from: http://www.jordbruksverket.se/download/18.54ee169213610a1e0a68000438/Notifiable+diseases+2011.pdf [2013-01-10]

The Swedish Board of Agriculture. Årsstatistik 2010. [online] (2010). Available from: http://www.jordbruksverket.se/download/18.6160f287138226df0f18000929/Sjukdomsstatistik+2010.pdf [2013-01-10]

The Swedish Veterinary Association. Guidelines for the clinical use of antibiotics in the treatment of dogs and Cats. [online] (original 2002, revised 2009). Available from: http://svf.se/Documents/S%c3%a4llskapet/Sm%c3%a5djurssektionen/Policy%20ab%20english%2010b.pdf [2013-01-02]

World Health Organization (2003) Human leptospirosis: guidance for diagnosis, surveillance and control. [online] (2013). Available from: http://whqlibdoc.who.int/hq/2003/WHO_CDS_CSR_EPH_2002.23.pdf [2013-01-28]

Zuerner, R. L. (2010). Genus Leptospira. In Krieg, N.R., Staley, J. T., Brown, D. R., Hedlund, B., Paster, B. J., Ward, N., Ludwig, W. & Whitman, W. B. (editors) *Bergey's Manual of Systematic Bacteriology*. 2. ed. Vol 4. 232-242. New York, NY, USA, Springer-Verlag.

APPENDIX

1. Questionnaire for owners (originally in Spanish)

Project about leptospirosis in dog in Lima, Peru

You have received a questionnaire with questions about You and Your dog. The questionnaire form part of a thesis about leptospirosis in dogs in Lima, Peru, that Sophie Hedberg, veterinary student, 6th year, Sweden, is currently working with.

Leptospirosis is a bacterial disease that can affect different animals, for example dogs and also humans. The bacterium enters the body via the skin or mucous membranes. *Leptospira* is transmitted to dogs and humans via, for example, urine from infected animals. Humans can also be infected when drinking contaminated water. The clinical manifestation of the disease is fever, signs of renal and hepatic disease (vomits, diarrhea etc) and mucous membrane bleeding.

Your dog has symptoms that could be caused by an infection with *Leptospira*. The diagnosis is not yet confirmed. If your dog is diagnosed with *Leptospira* (analyses in blood and urine) You can participate in the project.

The aims of the project:

- Analyze the presence of *Leptospira* in urine in dogs
- Analyze the antibody level against *Leptospira* in blood in dogs
- Evaluate the renal and hepatic lesions that *Leptospira* can cause (hematology, blood chemistry, urine)
- Analyze samples of blood and urine after one month of treatment to be able to compare the renal and hepatic lesions and the level of antibodies in blood before and after one month of treatment

Advantages for You if Your dog participates in the project:

- One analysis **gratuitous** at the first consultation
- Follow-up consultation with the veterinarian after one month of treatment **free of charge**, in addition to all the analyses **gratuitous**

The first consultation is paid by You as the owner/responsible of the dog and the project pays for one additional analysis. The follow-up consultation after one month is very important for us to be able to make a comparison of the renal and hepatic lesions before and after the treatment. Besides, we'll establish if Your dog needs a special diet and/or continue its treatment. If You'd like to participate in the project it's very important that You and the dog come for a follow-up consultation after one month.

If You'd like to participate in the project, You'll receive information about the recovery of Your dog and that way understand the effect of the treatment. At the same time You contribute to the investigation about the bacterium *Leptospira* in dogs in Lima, Peru. All the final information about the dogs and owners is confidential.

Yes, I'd like to participate in the project about leptospirosis in dogs in Lima, Peru

Yes O No O

Date: _____

Key: _____

Owner/responsible

Signature _____

First and last name _____

DNI: _____

Questions for You

Key: _____

1. The owner

a. Residence in Lima, Peru

i. Name of district _____

ii. Name of urbanization _____

iii. Name of neighborhood _____

b. Type of living

i. House (one family) O

ii. House (more than one family) O

iii. Apartment O

iv. Other O 1. What? _____

c. More dogs at home?

i. Yes O → 1. Number: _____ 2. Age/ages: _____

ii. No O

d. Other types of animals at home?

i. Yes O → 1. What kind of animal? _____

ii. No O *(production animals, domestic animals as cats etc)*

2. The dog

a. Breed

i. Pure breed O → 1. What breed? _____

ii. Mixed O → 1. What breeds (if You know?) _____

b. Age _____

c. In what space does the dog spend most of its time?

i. Inside O → 1. Flat roof O

2. Garden O

3. Rooms/Other O

ii. Outside (street) O

iii. Other O 1. Where? _____

d. Who takes care of the dog at home?

i. The man O (possibility to choose more than one alternative)

ii. The woman O

iii. The kids O

iv. Other person O 1. Who? _____

e. To whom did it occur having a dog?

i. The man O (possibility to choose more than one alternative)

ii. The woman O

iii. The kids O

iv. Other person O 1. Who? _____

f. From where did You acquire the dog? _____

g. When did You acquire the dog?

i. <6 month O

ii. >6 month O

h. What diet does the dog get?

i. Balanced O

ii. Homemade O

iii. Mixed (bal/ho) O

iv. Other O 1. What? _____

3. More questions

a. Why did You come to the clinic with your dog today?

b. Which symptoms has Your dog shown?

			a. Times/day?	b. Since when?
i. Vomiting	1. Yes O → 2. No O			
ii. Diarrhea	1. Yes O → 2. No O			
iii. Urinates more frequently	1. Yes O → 2. No O			
iv. Urine darker than before	1. Yes O → 2. No O		----	
v. Drinks more water than before	1. Yes O → 2. No O		----	
vi. Hemorrhages	1. Yes O → 2. No O		----	
i. Where did you notice them? *1. Mouth* O *2. Skin* O *3. Feces* O		*4. Urine* O *5. Other* _____		
vii. Other	1. Pain O		---	

		2. Depressed O	---	
		3. Moves little O	---	
		4. Fever O	---	
		i. How much?		
		5. Other		
c. Do You know home many times a day Your dog urinates when it's healthy? a) < 2 times/day b) 3-4 times/day c) > 5 times/day				
d. Have You contacted another veterinarian concerning this disease (leptospirosis) the last 5 days? If the answer is "Yes", what treatment did the veterinarian prescript for the dog?	i. Yes O ii. No O		i. Times/day?	ii. Since when?
	1. Antibiotics	a. Yes O →		
		b. No O		
	2. Fluids	a. Yes O →		
		b. No O		
	3. Other			
e. Did You know anything about Leptospirosis before You came to the clinic today? If the answer is "Yes", what did You know about the disease?				
f. Has the dog been ill owing to this disease before? If the answer is "Yes", when?				
g. Do You know if there are other dogs in the neighborhood that have fallen in owing to Leptospirosis?				
h. Is the dog vaccinated against Leptospira? If the				

<table>
<tr><td>answer is “Yes”, when did it get the last vaccination? Please, show us the vaccination certificate.</td><td colspan="5"></td></tr>
<tr><td rowspan="6">i. Do You know if Your dog suffers from other illnesses? If the answer is “Yes”, which? And what treatment does it get for this?</td><td colspan="5">i. Yes O ii. No O</td></tr>
<tr><td></td><td>a. ¿Which ?</td><td>b. Medicine</td><td>c. Times/day?</td><td>d. Since when?</td></tr>
<tr><td>1. Ill 1</td><td></td><td></td><td></td><td></td></tr>
<tr><td>2. Ill 2</td><td></td><td></td><td></td><td></td></tr>
<tr><td>3. Ill 3</td><td></td><td></td><td></td><td></td></tr>
<tr><td>4. Ill 4</td><td></td><td></td><td></td><td></td></tr>
<tr><td>j. Has the dog had other illnesses such as Distemper or Parvovirus previously? Confirmed or suspected?</td><td colspan="5"></td></tr>
<tr><td>k. Does the dog use to bring dead animals at home such as for example rodents, birds etc? If the answer is “Yes”, what kind of animals?</td><td colspan="5"></td></tr>
</table>

Thank you very much for participating in the project and for filling out the questionnaire!

Sophie Hedberg

Veterinary student, 6th year, Uppsala, Sweden