ESTONIAN UNIVERSITY OF LIFE SCIENCES
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CANINE LEPTOSPIROSIS – OWNER KNOWLEDGE IN ESTONIA

KOERTE LEPTOSPIROOS – OMANIKE TEADLIKKUS EESTIS

Final Thesis
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Leptospirosis is a zoonotic bacterial disease with a worldwide distribution infecting most mammals, including both dogs and humans. The aim of this descriptive study was to obtain an overview of the current knowledge of dog owners in Estonia about canine leptospirosis, its distribution, causative agent, clinical signs, risk factors, transmission, vaccination and zoonotic significance.

The data was collected via questionnaire in which a total of 116 dog owners visiting the small animal clinic of Estonian University of Life Sciences during a 5-month period participated. The main findings were that most owners (68%, n=79) had never heard of canine leptospirosis before and that only 14% (n=5) of the owners with prior knowledge about the disease (n=37) knew about the possibility of zoonotic transmission between dogs and humans. Most owners with prior knowledge (84%, n=31) knew about the availability of a vaccine against leptospirosis but only less than half of the people had vaccinated their dog. Majority of the owners did not know that vaccination does not fully prevent their dog getting infected.

The results indicate that dog owners in Estonia have limited knowledge on canine leptospirosis. Based on our results, there is a need to raise awareness of canine leptospirosis, particularly about its prevention and zoonotic risks it poses to public health.

Keywords: *Leptospira* spp., zoonosis, dog, vaccination

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Leptospiroos on ülemaailmse levikuga zoonootiline bakteriaalne haigus, mis nakatab enamik imetajatest, sealhulgas ka koeri ja inimesi. Selle kirjeldava uuringu eesmärk oli saada ülevaade Eesti koeraomanike praegustest teadmistest koerte leptospiroosi, selle leviku, tekitaja, kliinilste tunnuste, riskifaktorite, edasikandumise, vaktsineerimise ja zoonootilise tähtsuse kohta.

Andmed koguti küsimustiku kaudu, millest võtsid osa 116 koeraomanikku, kes külastasid Eesti Maaülikooli väikeloomakliinikut 5 kuulise ajaperioodi vältel. Peamised uurimuse tulemused olid, et enamus omanikke (68%, n=79) ei olnud leptospiroosist kunagi varem kuulnud ja ainult 14% (n=5) nendest omanikest, kellel oli haiguse kohta varasem teadmine (n=37), teadsid võimalikust zoonootilisest ülekandumisest koerte ja inimeste vahel. Enamus omanikkudest, kellel oli varasem teadmine (84%, n=31), teadsid leptospiroosi vastase vaktsiini olemasolust aga ainult vähem kui pooled nendest inimestest olid oma koeri vaktsineerinud. Suurem osa omanikest ei teadnud, et vaktsineerimine ei hoia täielikult ära nende koera nakatumist.

Tulemused viitavad, et Eesti koeraomanikel on koerte leptospiroosi kohta piiratud teadmised. Tuginedes meie uuringu tulemustele saame öelda, et koerte leptospiroosi, eelkõige selle ennetamise ja zoonotilise riski osas on vaja teadlikkust tõsta, kuna see kujutab ohtu rahva tervisele.

Märksõnad: *Leptospira* spp., zoonoos, koer, vaktsineerimine

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LIST OF ABBREVIATIONS

AKI	acute kidney injury
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUFI	acute undifferentiated febrile illness
CK	creatine kinase
CKD	chronic kidney disease
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
ELISA	enzyme linked immunosorbent assay
IgG	immunoglobulin G
IgM	immunoglobulin M
ILS	International Leptospirosis Society
L4	quadrivalent antileptospiral vaccine
LPHS	leptospiral pulmonary haemorrhage syndrome
MAT	microscopic agglutination test
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
RRT	renal replacement therapy
SIRS	systemic inflammatory response syndrome
SPHS	severe pulmonary hemorrhagic syndrome
UPC	urine protein to creatinine ratio
WHO	World Health Organization

INTRODUCTION

Canine leptospirosis is a worldwide zoonotic disease caused by pathogenic bacteria of *Leptospira* spp. (Sykes, 2014). Dogs may acquire the disease via direct contact with rodents or by indirect contact with contaminated soil or water (Levett, 2001). The clinical signs vary from asymptomatic infection to multiorgan failure (Schuller et al., 2015b). Leptospirosis is a zoonosis that can be transmitted to humans as well, resulting in similar clinical manifestation as in dogs (Major et al., 2014). Dog owners can acquire the infection both via contact with their dog's urine and by exposure to the pathogen in the same environment in which their pet became infected (Barmettler et al., 2011).

Major et al. (2014) reported that *Leptospira* spp. are more common in Europe than it has previously been thought. Moreover, they stated that increasing numbers of leptospiral infections in humans have been noted parallel to increasing numbers of canine cases in Europe. Vaccination is an integral part of prevention of leptospirosis (Sykes, 2014) and it has been associated with decreased number of dogs diagnosed with the disease. A recent study reported a rapid uptake of the quadrivalent vaccine in Switzerland, probably due to effective owner education (Francey et al., 2020). Even so, the authors pointed out that there still is a continuing need to increase awareness not only among owners, but also within veterinarians.

This final thesis provides an overview of canine leptospirosis and describes owners' current level of knowledge about the disease in Estonia. In the literature review, the main aspects related to the pathogen, distribution, transmission, clinical disease, diagnostics, treatment, prevention, and public health implications are discussed. The emphasis is on aspects relevant in clinical veterinary practice as well as in the zoonotic significance of the disease. The aims of our descriptive study were to characterize whether dog owners are aware of canine leptospirosis, what do they know about it and which areas of knowledge require improvement.

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Finally, special thanks to my family and friends for the support during these six years.

1. LITERATURE REVIEW

1.1. *Leptospira* bacteria

Leptospira spp. (*leptos* = "thin," *spira* = "coiled") are flexible, motile bacteria with unique hook or question mark-shaped ends (Evangelista & Coburn, 2010; Sykes, 2014). There are both saprophytic *Leptospira* species, which do not infect animals, and pathogenic *Leptospira* species that cause the disease in animal hosts. Even though pathogenic leptospires do not replicate outside the host and are easily inactivated in certain harsh conditions (Sykes, 2014), they do survive in moist environmental conditions such as water and wet soil for weeks to months (Trueba et al., 2004).

Currently, there are more than 20 recognized species within the genus *Leptospira* which are further classified into over 250 different serovars (Reagan & Sykes, 2019). Most of the serovars are zoonotic pathogens (Schuller et al., 2015b). These pathogenic serovars are grouped into 24 serogroups (Cerqueira & Picardeau, 2009). Generally, a certain serovar is adapted to a specific host species that acts as its carrier and reservoir in the environment. Dogs are suspected to be the host species only for serovar *Canicola.* Despite of the adaptation to certain hosts species, however, pathogenic leptospires can cause a clinical disease in several animal species. A wide range of serovars are capable of causing the disease in dogs (Schuller et al., 2015b).

1.2. Distribution

Globalization allows tropical infections to rapidly spread and among them, leptospirosis has become a significant cause of imported morbidity and mortality (Pappas et al., 2008). *Leptospira* spp. are now considered one of the most widespread zoonotic bacteria (Liimatta et al., 2017). According to Pappas et al. (2008), the most important foci of the disease are the Caribbean and Latin America, the Indian subcontinent, Southeast Asia, Oceania and to a lesser degree Eastern Europe.

Pappas et al. (2008) state that factors that are associated with leptospirosis endemicity include tropical climate, poor sanitation, standing water and flooding which are generally not present in Europe. However, they also suggest that host exposure and the presence of wild and domestic animal reservoir hosts affect geographic distribution of the disease.

Moreover, leptospirosis is widely spreading to nonendemic areas due to recreational exposures, tourism (Evangelista & Coburn, 2010) and climate change (Liimatta et al., 2017).

In Europe, the highest incidence of leptospirosis has been demonstrated in Central and Eastern countries (Pappas et al., 2008). However, Major et al. (2014) stated that leptospires are more widespread in Europe than it has commonly been thought. Pappas et al. (2008) developed a list about annual incidence of leptospirosis worldwide. European countries that were listed among the 28 countries with highest annual incidence of human leptospirosis included, in declining order, Croatia, Ukraine, Romania, Portugal, Denmark, Latvia, Slovenia and Slovakia. Russia was also listed among these countries with annual incidence of 17.2 per million population but the actual incidence of leptospirosis there was unknown. According to the zoonoses report by Estonian Agriculture and Food Board leptospirosis infected 2-6 people annually in Estonia during 2015 – 2019. In 2019, 5 human cases were registered and annual incidence was 0,4 per 100 000 population (Estonian Agriculture and Food Board, 2019).

During 2012–2017 in the Estonian Veterinary and Food Laboratory, 322 canine samples were analysed out of which 43,8% (n=141) were positive based on microscopic agglutination test (MAT). However, the diagnosis for leptospirosis in dogs can also be made by on-site SNAP® Lepto test or by sending the samples to Laboklin laboratory in Germany (Mik, 2019). Data on the total number of seropositive dogs in Estonia is therefore currently not available.

1.2.1 Prevalence of different serogroups in Europe

According to Ellis (2010), there is some variation in the prevalence of different serogroups in Europe. The most common serogroups to which dogs are exposed in Europe are *Icterohaemorrhagiae*, *Canicola*, *Grippotyphosa*, *Australis* and *Sejroe*. Serogroup *Icterohaemorrhagiae* is widespread due to its ubiquitous maintenance host, rat, and remains to be the most common recognized cause of clinical leptospirosis in European dogs (Ellis, 2010). Claus et al. (2008) stated that, contrary to the prevalence of the serogroup *Icterohaemorrhagiae*, *Canicola* seroprevalence has been decreasing in Europe. They suggested that the cause for this is widespread vaccination, which should therefore be continued to prevent the decrease in the population immunity. Serogroup *Grippotyphosa* is causing clinical leptospirosis in dogs particularly in mainland Europe (Ellis, 2010), being

the predominant serogroup associated with the disease in Germany (Geisen et al., 2007). Serogroup *Australis*, particularly serovar *Bratislava*, has been the cause of infection in several European countries (Claus et al., 2008). Seroprevalence to serogroup *Sejroe* has been reported, however there is a lack of strong evidence of widespread infection or clinical disease in Europe (Ellis, 2010).

Ellis (2010) argued that serogroup *Pomona's* epidemiology is not well-understood and a low level of exposure has been reported in various European countries. However, for example in Germany the seroprevalence was previously reported to be low (Brem et al., 1990; Geier-Dömling et al., 2003; Geisen et al., 2007) but later studies have shown an increased exposure (Mayer-Scholl et al., 2013; Knöpfler et al., 2017). In a study about serogroup distribution in a small animal clinic in Berlin, *Pomona* was among the most prevalent serogroups causing clinical leptospirosis in dogs along with *Australis* and *Grippotyphosa* (Mayer-Scholl et al., 2013). Moreover, a recent study by Habuš et al. (2020) reported *Pomona* as a causative serogroup for majority of canine leptospirosis infections in Croatia during years 2009 to 2017. Additionally, *Pomona* has been reported to be the most common serogroup in Estonia (Liimatta et al., 2017).

1.3. Transmission and pathogenesis

Leptospirosis is maintained in the environment by wild or domestic reservoir hosts (Bharti et al., 2003), of which rodents are considered the most important ones. Infection to incidental host is acquired by direct or indirect transmission as illustrated in Figure 1 (Schuller et al., 2015b). Direct transmission can occur via contact of mucous membranes or skin abrasions with the urine of an infected animal. Indirect transmission occurs via contaminated water or soil (Levett, 2001). Dogs have been reported to acquire an infection also via bite, placenta, ingestion of infected tissues, and venereal transfer (Sykes et al., 2011).

Infection in incidental hosts can lead to a fatal disease (Levett, 2001), however reservoir hosts are generally asymptomatic (Schuller et al., 2015b). Once a reservoir host becomes infected, it maintains a carrier state, harboring the bacteria in its renal tubules and spreading leptospires in its urine for long periods of time (Reagan & Sykes, 2019). The potential to produce a persistent renal carriage is a crucial part of *leptospira's* life cycle (Ko et al., 2009).

Infection with pathogenic leptospires results in a multi-systemic disease via hematogenous spread (Schuller et al., 2015b). The initial, bacteremic phase lasts up to one week (Adler &

de la Peña Moctezuma, 2010) and during this leptospires spread in the organism and evade the immune response of the host (Barbosa et al., 2009). Once the acquired immune response of the host is effective, leptospires are cleared from the blood circulation (Schuller et al., 2015b). During this second, so-called immune phase, antibodies are produced and bacteria start shedding into urine (Branger et al., 2005).

According to Schuller et al. (2015b), the main organs that leptospirosis affects are the kidneys and the liver. They pointed out that multiple other organs can be affected too, including spleen, lungs, pancreas, reproductive organs, endothelial cells, muscles, eyes and meninges. A German study reported multi-organ involvement in 98% of the cases (Knöpfler et al., 2017). The extent of damage to internal organs depends on several factors, including the virulence of the organism, the serovar and the strain as well as the host susceptibility (Goldstein, 2010).

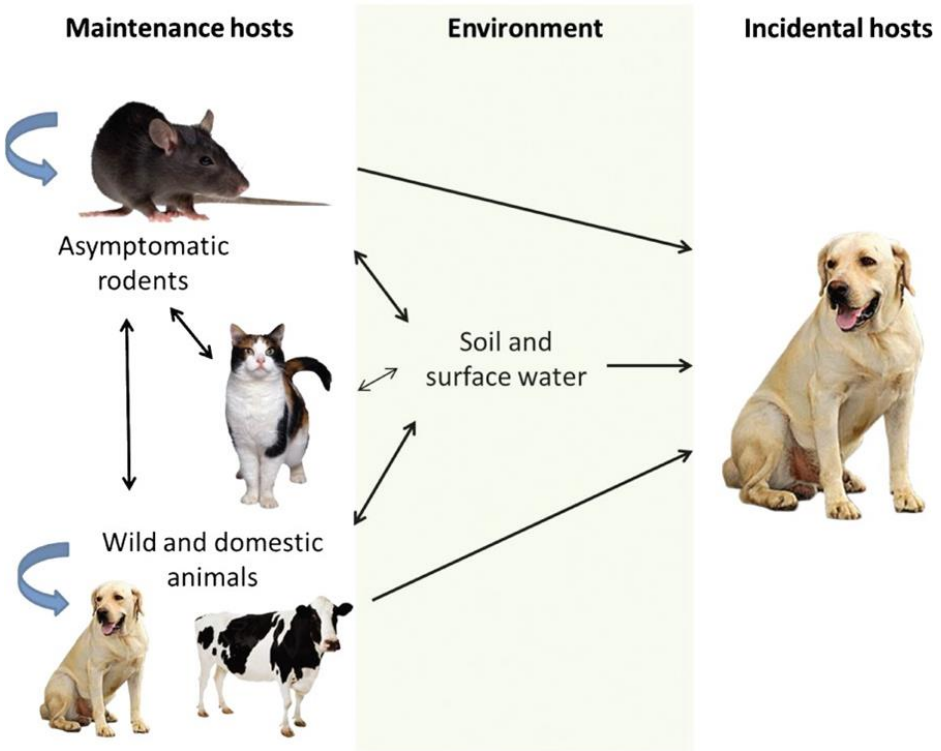

Figure 1. Transmission cycle of pathogenic *Leptospira* spp. (Schuller et al., 2015b, 161).

1.4. Clinical disease in dogs

The outcome of the infection ranges from subclinical or minimal clinical disease to severe, lethal disease (Schuller et al., 2015b). The symptoms of leptospirosis are non-specific (Liimatta et al., 2017). Generally, the main clinical signs of acute disease are associated with acute kidney injury (AKI) and liver insufficiency (Schuller et al., 2015b). Particularly in the acute phase of the disease, fever is usually present (Sykes, 2014). Other common clinical signs include lethargy, inappetence, polyuria and polydipsia, and gastrointestinal signs such as diarrhea and vomiting (Goldstein et al., 2006). Liver damage may cause icterus (Sykes, 2014).

Respiratory signs such as tachypnea, harsh lung sounds and breathing difficulties may be present in case of pulmonary involvement (Sykes, 2014). Leptospiral pulmonary haemorrhage syndrome (LPHS) is a critical form of leptospirosis described in both humans and dogs (Schuller et al., 2015b). Pathogenesis of LPHS is poorly understood but it has been suggested that autoimmunity may be involved in it (Schuller et al., 2015a). Schuller et al. (2015b) stated that the prognosis is poor as LPHS is associated with high mortality due to severe pulmonary hemorrhage which eventually leads to respiratory arrest. Between 2006 and 2010, 70% of dogs with leptospirosis that were treated at the Small Animal Clinic of the Freie Universität Berlin had clinical and radiological pulmonary changes (Kohn et al., 2010). However, pulmonary involvement does not always refer to LPHS (Schuller et al., 2015b).

Ophthalmological conditions have been recognized occasionally (Sykes, 2014). According to Gallagher (2011), leptospiral uveitis is rare in dogs but at times it can be the only symptom and thus leptospirosis should be considered a differential diagnosis in cases of uveitis. Reproductive complications, such as abortions, stillbirth and neonatal deaths occur rarely (Sykes, 2014). Infrequently, tachycardia and arrhythmias have been described, suggesting myocardial damage. Canine leptospirosis has also been associated with systemic syndromes such as systemic inflammatory response syndrome (SIRS), disseminated intravascular coagulation (DIC) and multiple organ damage (Mastrorilli et al., 2007).

Interestingly, Goldstein et al. (2006) suggested that infection with serogroup *Pomona* causes more severe renal disease and leads to a worse outcome compared to the disease caused by other serogroups. Clinical signs such as vomiting were described to be more severe in these patients. According to their study, only 50% of dogs with suspected *Pomona* infection were

discharged from the hospital alive. More clinically relevant associations with the other serogroups and clinical syndromes were not detected.

Even though leptospirosis is commonly reported as an acute disease (Goldstein et al., 2006), it is important to point out that chronic conditions have been associated with the disease as well (McCallum et al., 2018; Sant'Anna et al., 2019). A recent study by Sant'Anna et al. (2019) showed a link between leptospiral infection and chronic kidney disease (CKD). Based on their findings, CKD can develop not only after acute leptospirosis but also in asymptomatic carriers, creating a public health concern in endemic areas. McCallum et al. (2018) suggested that leptospirosis may also cause chronic hepatic disease without obvious renal involvement.

1.5. Diagnosis

1.5.1. Laboratory abnormalities

Laboratory abnormalities in dogs with leptospirosis include changes in hematology, serum biochemistry and urinalysis (Knöpfler et al., 2017). Common hematological findings are leucocytosis, thrombocytopenia and mild to moderate anaemia. Biochemical changes include azotemia, increased liver enzyme activity and a variety of electrolyte abnormalities (Schuller et al., 2015b). In a study from North-East Germany, 84% of the dogs diagnosed with leptospirosis had increased urea concentrations and 81% had increased creatinine concentrations (Knöpfler et al., 2017). Many studies have shown that dogs with severe azotaemia have a poorer prognosis compared to dogs with mild to moderate increases of creatinine concentrations (Goldstein et al., 2006; Mastrorilli et al., 2007; Knöpfler et al., 2017). What is more, increased activity of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and hyperbilirubinaemia, indicating hepatic damage, generally occur in conjunction with azotemia (Geisen et al., 2007). Hypo- and hyperkalaemia, hyper- and hypophosphataemia, hyponatraemia and hypochloraemia are commonly observed, depending on the degree of renal and gastrointestinal dysfunction (Schuller et al., 2015b). Other occasional findings in the serum biochemistry include hypoalbuminemia, increased creatine kinase (CK) activity due to myositis, increased pancreatic lipase due to pancreatitis or enteritis and increased troponin concentrations suggesting myocardial damage. Finally, there are often changes in the

hemostatic function, seen as variable increases in fibrinogen, D-dimer, and fibrinogen degradation product concentrations (Sykes, 2014).

Urinalysis from dogs with leptospirosis usually reveals isosthenuria (Knöpfler et al., 2017), occasionally hyposthenuria, and variable glucosuria, proteinuria, hematuria (Mastrorilli et al., 2007) and cylindruria. Urine protein to creatinine ratios (UPC) may be elevated (Knöpfler et al., 2017). Leptospires are not visible by routine urinary sediment examination (Schuller et al., 2015b).

1.5.2. Confirmatory testing

The early diagnosis of acute canine leptospirosis remains a challenge (Troìa et al., 2018). This is mainly due to the nonspecific clinical signs of the disease and numerous limitations of the diagnostics tests available (Lizer et al., 2018). Currently, the most useful diagnostics tools include microscopic agglutination test (MAT) and conventional polymerase chain reaction (PCR) or real - time quantitative PCR (qPCR) (Schuller et al., 2015b). The MAT detects antileptospiral serum antibodies, namely immunoglobulin M (IgM) and immunoglobulin G (IgG) (Troìa et al., 2018), whereas PCR detects leptospiral DNA (Schuller et al., 2015b).

In MAT, serial dilutions of patient serum are reacted with live antigen suspensions of leptospiral serovars after which the agglutination of the serum–antigen mixtures is examined. The MAT is a serogroup-specific analysis, meaning that reactivity to a serovar does not necessarily indicate exposure to the serovar tested but rather exposure to the corresponding serogroup (Levett, 2001). According to Schuller et al. (2015b), serogroups included in the test panel should be chosen based on the antibody prevalence data in the appropriate geographic location. They recommend the inclusion of serogroups *Australis, Autumnalis, Canicola, Grippotyphosa, Icterohaemorrhagiae, Pomona, Pyrogenes* and *Sejroe* in the test panel in Europe. Infecting serogroup, however, cannot be accurately predicted by MAT due to the marked limitations the test has (Miller et al., 2011).

The limitations of MAT include cross-reactivity, equality of high titers, and paradoxical reactions (Miller et al., 2011). False positive and false negative results occur (Schuller et al., 2015b). Especially in acute-phase samples, high level of cross-reaction between the serogroups can be expected (Levett, 2001). Moreover, infected dog can show negative results in the acute phase as serum antibodies develop with a delay (Schuller et al., 2015b).

Equally high titers occur relatively often and complicate the interpretation as the infecting serogroup cannot be definitively identified (Miller et al., 2011). In paradoxical reactions, the highest titers do not develop against the infecting serogroup but to a serogroup unrelated to it and likewise make interpretation difficult (Levett, 2001). What is more, high titers in a non-infected dog can be caused by vaccination (Schuller et al., 2015b). Additionally, changes in the MAT results overtime in individual dogs have been reported and marked interlaboratory variation in the results have been described.

Despite these limitations, however, MAT remains a valuable test for diagnosing canine leptospirosis as identification of the infecting serogroup does not influence the treatment plan of infected individuals. Knowing the responsible serogroup plays a bigger role in epidemiological surveys, control of wildlife reservoirs and vaccine planning (Miller et al., 2011).

Testing paired samples is recommended in order to detect seroconversion and thereby improve the sensitivity of the MAT (Schuller et al., 2015b). The samples should be collected one or two weeks apart (Fraune et al., 2013). Schuller et al. (2015b) suggest that in a hospital setting, this can be achieved by taking the initial sample at admission, and the convalescent sample at the time of discharge. When there is at least a fourfold rise in MAT or when the first sample is negative and the convalescent titre is at least 800, leptospirosis can be strongly suspected (Schuller et al., 2015b). In a study in Italy, a notable percentage of dogs would have been misdiagnosed without a convalescent sample as seroconversion occurred in 14.3% dogs (Troìa et al., 2018). In addition to interpretation of paired titers, Miller et al. (2011) recommend sending samples to laboratories that participate in a proficiency scheme such as the International Leptospirosis Proficiency Testing Scheme provided by the International Leptospirosis Society (ILS).

On live animals, PCR can be performed on blood and urine, and post-mortem on kidney tissue. To ensure high sensitivity, only fresh samples should be used for PCR, especially in the case of urine (Branger et al., 2005). According to Schuller et al. (2015b), leptospirosis can be strongly suspected in a dog with positive PCR on blood along with suggestive clinical symptoms but a positive PCR on urine only indicates urinary shedding. This can occur in case of an acute infection but also in a chronic carrier state. In fact, PCR on urine is used for the detection of renal carriers. PCR cannot rule out leptospirosis, because negative result may be due to transient leptospiremia, delayed or intermittent leptospiruria or antibiotic

therapy (Schuller et al., 2015b). Another limitation for routine diagnostic PCR is its inability to predict the infecting serovar (Levett, 2001).

In addition to MAT and PCR, rapid patient-side tests are becoming more popular (Schuller et al., 2015b). An enzyme linked immunosorbent assay (ELISA) detects *Leptospira*-specific antibodies IgM and/or IgG in canine sera (Lizer et al., 2018). The SNAP® Lepto test is an in-clinic ELISA from IDEXX Laboratories for the detection of *Leptospira spp.* antibodies to a major outer membrane protein, LipL32 (Curtis et al., 2015). However, Schuller et al. (2015b) state that as a serological test, it has the same limitations as MAT. These include negative results in the early stages of infection and inability to differentiate between infectious and vaccinal antibodies. The main advantage of SNAP® Lepto is that it is widely available and can be easily performed in a clinic setting, providing quick results and therefore giving guidance on whether treatment should be administered and if precautions should be taken to minimize the risk of zoonotic infection (Curtis et al., 2015).

According to Schuller et al. (2015b), definitive diagnosis of leptospirosis can be achieved by positive culture of blood, urine or tissue, however, culturing leptospires is challenging and may take several months. Therefore, it is not routinely performed and other diagnostic tests are preferred.

1.6. Treatment

Appropriate antimicrobial therapy and supportive care for the affected organs constitute an effective treatment of canine leptospirosis (Schuller et al., 2015b). Doxycycline is currently the antibiotic of choice as it eliminates leptospires from all tissues. Leptospiruria and thereafter spreading of leptospires is terminated once doxycycline clears the organism from the renal tubules (Mauro & Harkin, 2019).

Schuller et al. (2015b) recommend starting antimicrobial therapy as early as possible, even before definitive laboratory confirmation. They suggest that early treatment may prevent from severe clinical presentation and zoonotic transmission. Based on current evidence, the European Consensus Statement recommends that all dogs infected with leptospirosis should be treated with 5 mg/kg q12h or 10 mg/kg q24h doxycycline for 14 days. However, many dogs present gastrointestinal signs such as vomiting and diarrhea and may not tolerate oral doxycycline. These patients are recommended to be initially treated with an intravenous penicillin derivative such as ampicillin, penicillin G or amoxicillin, until doxycycline can be

used (Schuller et al., 2015b). After resolving of the gastrointestinal signs, a full two-week course of oral doxycycline should be administered (Sykes et al., 2011). As for dogs with renal failure, the dose of antimicrobials should be adapted (Sykes, 2014).

A recent study presented a case series of five dogs who remained PCR positive for pathogenic leptospires despite receiving the recommended treatment with either an initial beta lactam or doxycycline (Mauro & Harkin, 2019). In case the clinical signs are not resolving after correct treatment, factors to be considered include problems in oral absorption of doxycycline (Mauro & Harkin, 2019), pre-existing chronic kidney disease, systemic bacterial infection with nosocomial pathogens (Schuller et al., 2015b) or an atypical leptospirosis serovar infection with unique antimicrobial susceptibility (Mauro & Harkin, 2019). Additionally, Mauro and Harkin (2019) suggest that antimicrobial resistance in pathogenic leptospires could have a major effect on the treatment of the disease although is not well-documented. What is more, they point out that reinfection remains a possibility despite of not being documented in the literature. Based on current evidence, Mauro and Harkin (2019) recommend performing a PCR on urine after 7 days of antimicrobial therapy in order to confirm eliminated leptospiruria.

In addition to appropriate antimicrobial therapy, supportive treatment has a major role in the management of canine leptospirosis (Sykes, 2014). Depending on the severity of the disease, supportive treatment should consist of appropriate fluid therapy, treatment of systemic hypertension and gastrointestinal complications, pain management and parenteral or enteral nutritional support (Schuller et al., 2015b). Severely ill dogs should be referred to a 24-h care hospital to ensure intensive monitoring (Sykes, 2014).

Fluid therapy should be carefully selected according to patient status. Dogs with polyuria require high fluid rates, whereas oliguric or anuric patients may develop severe complications such as respiratory failure in case of overhydration. In these patients it is recommended to carefully monitor urine output by using a closed, indwelling urinary catheter and collection bag system (Sykes, 2014). In case of severe AKI and a failure of urine production after adequate hydration, renal replacement therapy (RRT) is strongly advised.

In dogs with respiratory involvement, precautionary measures should be adopted including minimization of manipulations and stress and avoidance of systemic hypertension,

overhydration or hypervolaemia (Schuller et al., 2015b). Dogs with LPHS may require oxygen therapy and, in severe cases, mechanical ventilation (Sykes, 2014).

As concurrent infection of other dogs in the same household may occur, it is recommended to treat all dogs that are living with a dog diagnosed with leptospirosis (Sykes et al., 2011). For these dogs, 5 mg/kg q12h or 10 mg/kg po q24h doxycycline treatment for two weeks should be administrated (Schuller et al., 2015b).

1.7. Prevention

Many risk factors for acute canine leptospirosis have been analyzed with conflicting results (Lee et al., 2014). According to a recent meta-analysis by Ricardo et al. (2020), potential risk factors included sex, age, breed, exposure to other dogs, exposure to rodents, exposure to environmental water sources, presence of rodents in the household, street access and living environment (urban/rural). In the meta-analysis, adult male dogs that had street access and that were in contact with environmental water were analyzed to have a higher risk for infection. However, it has been suggested that male dogs being at increased risk for infection might be due to their gender related behavior such as sniffing and licking of urine (Major et al., 2014). Regardless of the studies on the possible risk factors for canine leptospirosis, it is recommended to suspect the disease as a possible diagnosis despite of the signalment of the patient (Schuller et al., 2015b).

A preventative measure for canine leptospirosis is restricting dogs' access to wildlife, rodents and environmental water sources (Sykes, 2014). In planning of preventive actions, it is beneficial to have knowledge about the epidemiology of leptospirosis and patterns of global incidence (Pappas et al., 2008). Vaccination is recommended for dogs that are at risk of exposure (Sykes, 2014). In the European Consensus Statement, Schuller et al. (2015b) advise annual revaccination for all at-risk dogs after a basic vaccination of two applications three to four weeks apart. They point out that vaccination, however, does not protect from all serogroups that cause leptospirosis in dogs.

Bivalent vaccines against canine leptospirosis have been available for approximately 60 years, consisting traditionally of serogroups *Icterohaemorrhagiae* and *Canicola* (Jull & Heath, 1960). Currently, the use of quadrivalent vaccines is recommended in Europe (Schuller et al., 2015b). According to Klaasen et al. (2013), these should further include serogroups *Icterohaemorrhagiae* and *Canicola* and additionally *Grippotyphosa* and

Australis. The choice of these four serogroups is based on current epidemiological situation of canine leptospirosis in Europe. According to Ellis (2010), most of the information about leptospires in dogs has been acquired from seroprevalence studies performed using the MAT.

A recent research by Francey et al. (2020) examined the effects of quadrivalent antileptospiral vaccine (L4) which was introduced in Switzerland in 2013. Use of the L4 vaccine was associated with a marked decrease in the number of dogs diagnosed with leptospirosis and AKI as shown in Figure 2. This study showed the importance of including the most prevalent serogroups into the vaccine to ensure its effectiveness. Several studies in Europe have discussed about the inclusion of serogroup *Pomona* to the vaccine (Claus et al., 2008; Mayer-Scholl et al., 2013; Arent et al., 2013). Further seroepidemiological studies are needed to ensure that available vaccines will be adapted to include the most important circulating serovars (Mayer-Scholl et al., 2013).

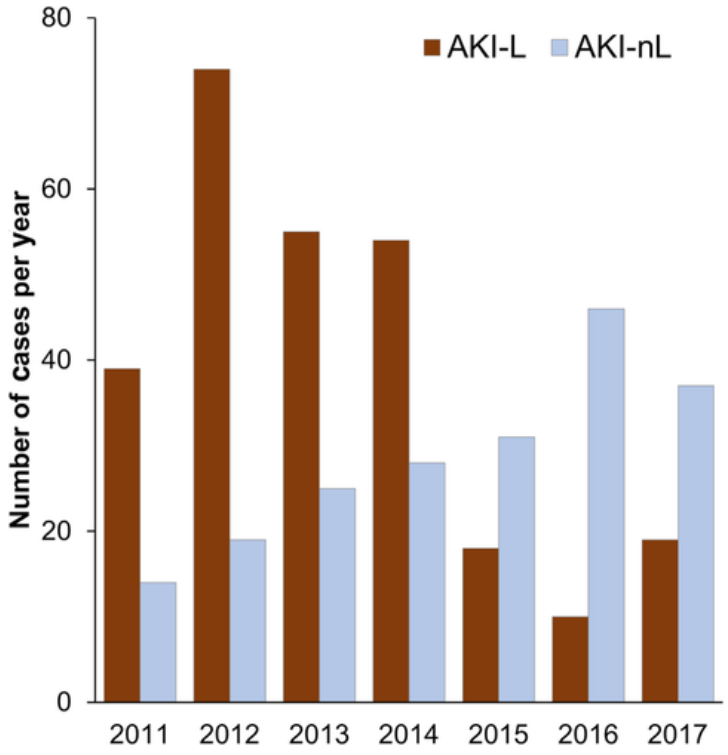

Figure 2. Diagnosed leptospirosis cases (AKI-L) and AKI cases not caused by leptospirosis (AKI-nL) in Switzerland during 7 years (Francey et al., 2020, 2408).

1.8. Zoonotic aspects

In humans, leptospirosis is most commonly either subclinical or mild influenza-like illness (Levett, 2001). Due to its nonspecific symptoms, the disease can be difficult to differentiate from other acute undifferentiated febrile illness (AUFI)-causing etiologies such as dengue, hepatitis and malaria (Karpagam & Ganesh, 2020). More severe manifestation includes multiorgan failure with impaired renal and hepatic function (Sykes, 2014). Pulmonary involvement is seen in 20–70% of patients and may lead to severe pulmonary hemorrhagic syndrome (SPHS) (Karpagam & Ganesh, 2020). A study from Switzerland states that the clinical manifestation in dogs presents similar characteristics to the human disease (Major et al., 2014). Thus, the authors propose that considering canine leptospirosis a model to human infection could be useful in further studies.

Sykes (2014) argues that leptospirosis should be suspected in all dogs with acute renal failure. She suggests that all these dogs should be managed as leptospirosis suspects and appropriate precautions should be taken in the hospital environment. This approach ensures that dogs which are diagnosed by seroconversion after the time of hospitalization are handled properly (Barmettler et al., 2011). The aim of the safety measures is to minimize zoonotic transmission (Sykes, 2014) and all workers that may have contact with patients suspected to have leptospirosis should be aware of them (Sykes et al., 2011).

Many authors have listed in-hospital precautions for dogs with diagnosed or suspected leptospirosis (Barmettler et al., 2011; Sykes, 2014; Schuller et al., 2015b). Routine precautions include warnings signs, minimizing movement, using of protective clothing such as gloves, gowns, eyewear and face shields as well as regular handwashing. Additionally, pregnant or immunocompromised staff should not be allowed to work with the patient. Moreover, indwelling urinary catheterization for monitoring urinary output is advised and if urinary catheter is not in place, dogs should be taken outside to urinate in a designated area (Sykes, 2014). All body fluids from dogs suspected to have leptospirosis should be treated as medical waste (Schuller et al., 2015b) and urine spills should be disinfected rapidly (Sykes, 2014). Collected urine can be inactivated with disinfectant solutions and must be disposed of properly. 1:1 aqueous dilution of 10% bleach solution, iodine-based disinfectants, accelerated hydrogen peroxide, and quaternary ammonium solutions are effective for disinfection (Sykes et al., 2011).

Sykes (2014) states that it is essential to educate owners about the zoonotic potential of leptospirosis. She points out that even though the risk of dogs infecting their owners is probably low, people should have adequate knowledge about the zoonotic nature of the disease as well as the precautions to be taken with infected dogs. According to Barmettler et al. (2011), owners can acquire the infection not only via contact with their dog's urine but also by exposure to the bacteria in the same environment in which their pet became infected.

The low risk of direct inoculation from pets to owners is explained by urinary shedding not occurring until 7 to 10 days after the onset of illness, and antimicrobial treatment resulting in ceasing of leptospiruria within first few days (Sykes, 2014). However, there is a demand for further study about the urinary shedding of leptospires in dogs during treatment (Schuller et al., 2015b).

The precautions owners should take at home with their dogs with known or suspected infection include avoiding contact with dog's urine and regularly washing hands after handling their dogs. Additionally, it is advised to take dogs to urinate in a place away from water, other pets and people, and to properly clean possible urinary accidents occurring indoors (Sykes, 2014). Owners should also be informed to seek medical attention if they fall ill themselves around the time their dog is diagnosed with leptospirosis. Immunocompromised owners should contact their medical practitioner for advice (Sykes et al., 2011).

2. AIMS OF THE STUDY

The aim of this descriptive study was to obtain an overview of the current knowledge of dog owners in Estonia about canine leptospirosis, its distribution, causative agent, clinical signs, risk factors, transmission, zoonotic significance and prevention, including vaccination.

The data for this study was collected through a questionnaire that was filled by dog owners visiting the small animal clinic of Estonian University of Life Sciences in Tartu during a 5-month period in 2020.

3. MATERIALS AND METHODS

3.1 Questionnaire

The questionnaire (Appendix 1 and 2) was filled by dog owners visiting the small animal clinic of Estonian University of Life Sciences in Tartu. The answers were collected from 25th May 2020 to 25th October 2020. Participation was voluntary and anonymous, and no personal information from the participants was collected. A short introduction in the beginning of the questionnaire provided information about the purpose of the study. The questionnaire was available in Estonian and in English. There were 14 questions in total of which eight were multiple choice questions and six were two- or three-choice questions with answers yes, no +/- I don't know.

Based on the participant's answer to the first question, "Have you ever heard about canine leptospirosis?", they were advised to either reply to all following questions or only to questions number 7 and 14. If the participant had heard about the disease before, they were advised to answer all questions based on their current knowledge. If the participant had not heard of canine leptospirosis before, they were advised to answer only to two more questions (7th and 14th) which were not concerning leptospirosis specifically: whether their dog has been abroad and whether they have ever received information from their veterinarian about diseases humans can get from dogs or precautions to take with dogs to reduce the risk of disease.

3.1 Data handling

Data from all 116 questionnaires were included in the study although some questionnaires were incompletely filled. In cases of incompletely filled questionnaires, missing answers were described in the statistical analysis as "no reply".

If the participant replied to all questions even though based on their answer to the first question, they should further only reply to questions 7 and 14, their answers to other questions were excluded and only answers to questions 1, 7 and 14 were included in the study. Some owners chose multiple options in questions where only one option was correct. In this case, their answer was regarded as "no reply" because it was assumed that the participants who marked more than one option did not have very good knowledge about the disease.

There were nine questions in total in the questionnaire that measured the participants' level of knowledge about leptospirosis. The questions evaluated owners' knowledge about vaccination, distribution, causative agent, clinical symptoms, risk factors, transmission and zoonotic potential of leptospirosis. Correct answers to the questions were determined based on the literature. Options without references to publications were regarded as incorrect. Each correct answer gave one point to the participant. In the two multiple-choice questions about the symptoms and risk factors of the disease, the participant could achieve one point per question by choosing at least one correct option and simultaneously not choosing any incorrect option(s). Total points were calculated to each owner by which the participants were divided into three groups: high, moderate and poor level of knowledge. The maximum number of points was nine. High level could be achieved with more than or equal to seven points, moderate level with four to six points and poor level with less than or equal to three points.

Questions that did not measure owner knowledge about leptospirosis by points included the first question about whether owners have heard of the disease, the second question about sources of knowledge, the fourth question asking whether the participant's dog is vaccinated against leptospirosis, the seventh question concerning travelling with dog and the fourteenth and final question asking whether owners have received information about zoonotic diseases dogs can transmit to humans. The question about travelling asked whether the participant's dog had been abroad. Some owners specified that their dog was brought from abroad but had not been outside of Estonia since. These answers were regarded as "no". Answer was regarded as "yes" only if the dog had been abroad during the time they had been with their current owner. Additionally, the number of owners that had or had not been travelling with their dog and had or had not heard of leptospirosis was calculated.

The questionnaire included four questions in total that concerned vaccination of dogs against leptospirosis: can dogs be vaccinated against leptospirosis, how often should they be vaccinated, can the dog get infected when it is correctly vaccinated and whether the participant's own dog is vaccinated against the disease. The last one did not evaluate owner knowledge and thus did not affect the total points.

The tenth and eleventh questions in the survey were multiple choice questions in which many options could be selected. Based on their answers, the participants were divided into four groups: the participants who chose at least one correct option and no wrong options, the

participants who chose at least one correct option and at least one wrong option, the participants who chose no correct options or chose “I don’t know”, and the participants who did not reply to the question at all.

Correct answers to the tenth question were fever, signs of kidney failure (for example dehydration and vomiting), signs of liver failure (for example icterus), respiratory signs (for example tachypnea) and ocular signs (for example conjunctivitis) (Sykes et al., 2011). Dermatological signs (for example rash and itchy skin) and neurological signs (for example balance issues and seizures) were regarded as incorrect because these options did not have references. Neurological signs and some dermatological signs (e.g. petechiae), however, could be associated with certain complications of leptospiral infection such as hepatic encephalopathy (Ashna et al., 2019) and as a consequence of for example bleeding disorders (Mastrorilli et al., 2007) but in these cases they are not considered the primary signs of leptospirosis.

Correct answers for the eleventh question were warm weather, exposure to lakes, rivers or streams and contact with wildlife (Sykes et al., 2011). Exposure to ticks, old female small breed dogs and dry and cold environment together with multi-dog household were regarded as incorrect because these options did not have references. Exposure to other dogs has been reported to be a potential risk factor for canine leptospirosis but dry and cold environment has not been associated with higher risk (Ricardo et al., 2020). As multi-dog household and dry, cold environment were grouped together as one option, this answer was regarded as incorrect.

Responses to survey questions are reported as rounded percentages and may consequently not sum up to exactly 100%.

3.2 Statistical analysis

Data from the questionnaires was entered into Microsoft Office Excel (Version 2103) where all answers were transformed into numerical codes. Each questionnaire was assigned an identification number. The data was described by using descriptive statistics. IBM SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, N.Y., USA) was used to calculate the proportions and to create the figures and tables.

4. RESULTS

The questionnaire was answered by a total of 116 dog owners. The final sample size was 116 as no participant was completely excluded from the study. The results are presented in two groups: first the data from questions number 1, 7 and 14 to which all participants were advised to reply to and secondly the data from the rest of the questions that were aimed for the participants who had heard of leptospirosis before.

4.1 All participants

The responses to the first question in the questionnaire are shown in Figure 3. Most of the owners (68%, n=79) reported that they have never heard of canine leptospirosis before.

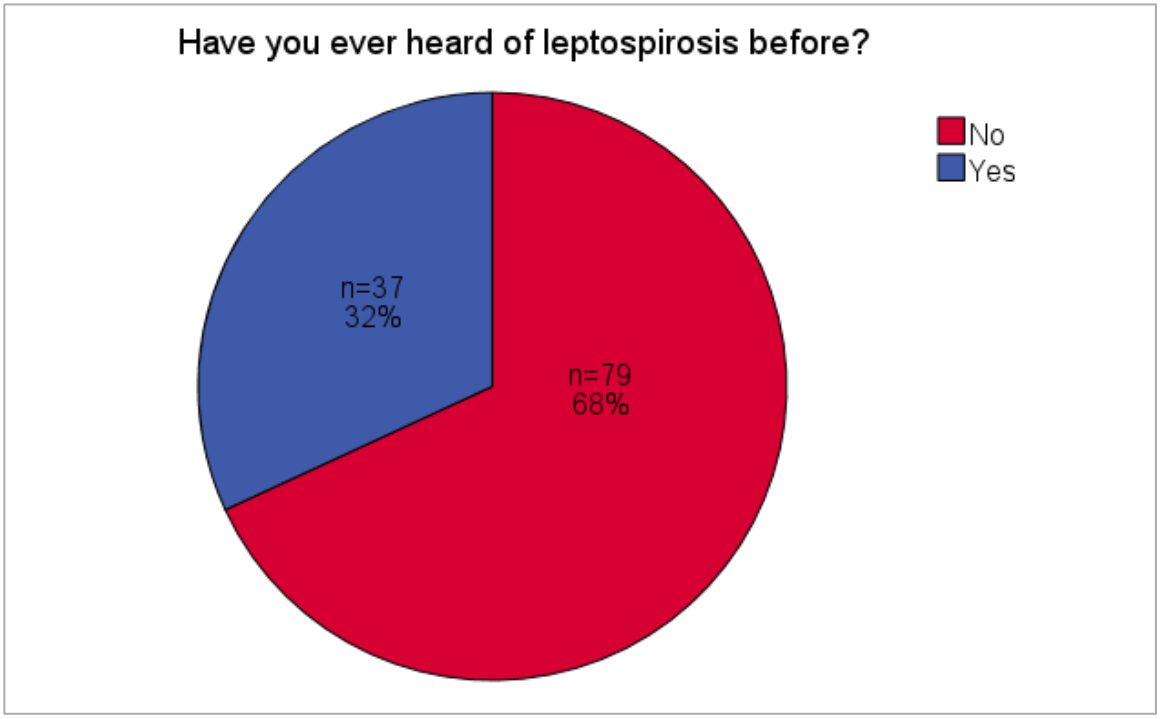

Figure 3. Proportions of participants that have or have not heard of leptospirosis before (n=116).

The seventh question was concerning dogs' travel history. Most (69%, n=80) of the participants' dogs had not been abroad during the time they had been with their current owner (Table 1). If owner stated that their dog had been abroad, they were asked to name the countries where their dog had been to. Common answers included Baltic and Nordic

countries, particularly Latvia and Finland. Out of the owners that had been travelling with their dog (n=35), 43% (n=15) had heard of leptospirosis and 57% (n=20) had not heard of the disease before. Out of the owners that had not been travelling with their dog (n=80), 26% (n=21) had heard of leptospirosis and 74% (n=59) had not.

Table 1. Responses to the 7th question "Has your dog been abroad?" (n=116). If option "Yes" was selected, the participant was requested to list the countries where their dog had been to.

	Count (n)	Percentage (%)
Yes	35	30%
No	80	69%
No reply	1	1%

The results for the 14th question are presented in Figure 4. Most of the owners (66%, n=76) had never received information about zoonotic diseases that dogs can transmit to humans. Only 23% (n=27) had received some information.

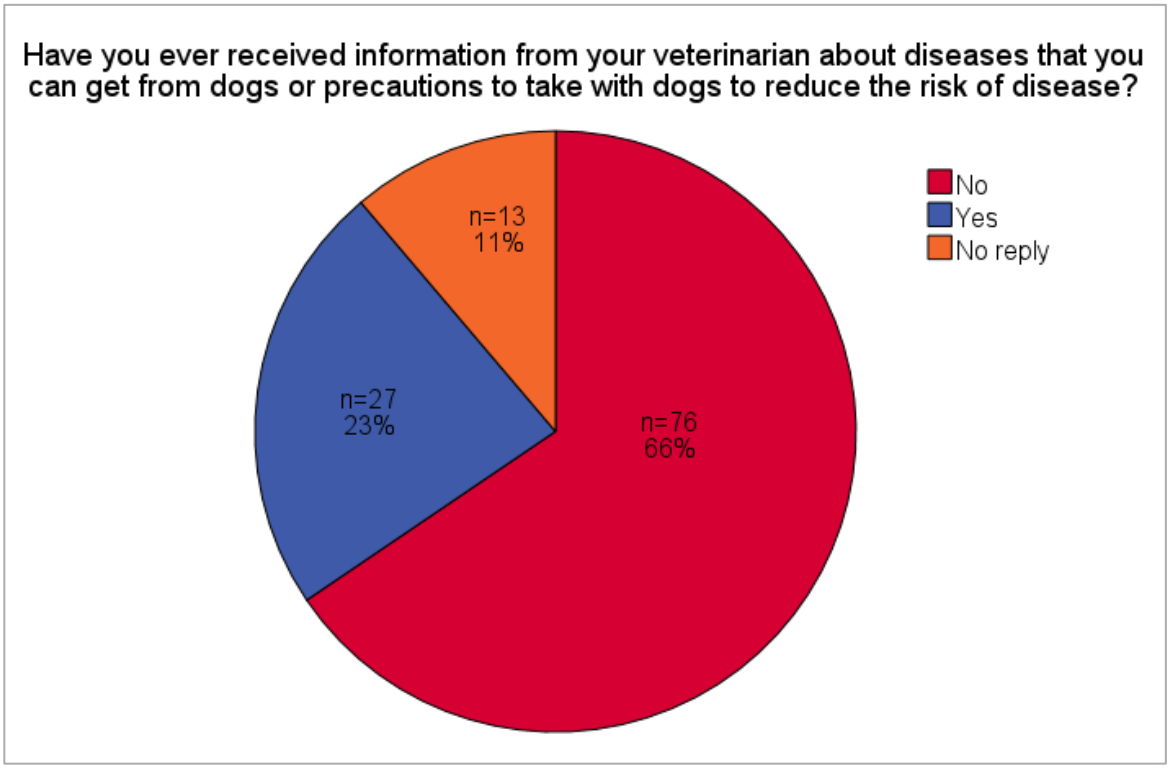

Figure 4. Proportions of participants that have or have not received information from their veterinarian about zoonotic diseases (n=116).

4.2 Participants with prior knowledge about leptospirosis

Out of all participants, 32% (n=37) had heard about leptospirosis before (Figure 3) and were expected to reply to all questions in the questionnaire based on their current knowledge. The following paragraphs will analyze the data concerning these 37 owners who had some prior knowledge about the disease.

4.2.1 Sources of knowledge

Most of the participants (59%, n=22) said that they have heard about the disease from veterinarians and/or internet sources including web pages and social media. Almost half of the owners (49%, n=18) had heard about the disease from media/press. Only 3% (n=1) had heard about leptospirosis from a medical doctor. Two participants (5%) chose option "other" and were asked to specify their answer in writing. Their answers were educational institutes. The responses are summarized in Figure 5.

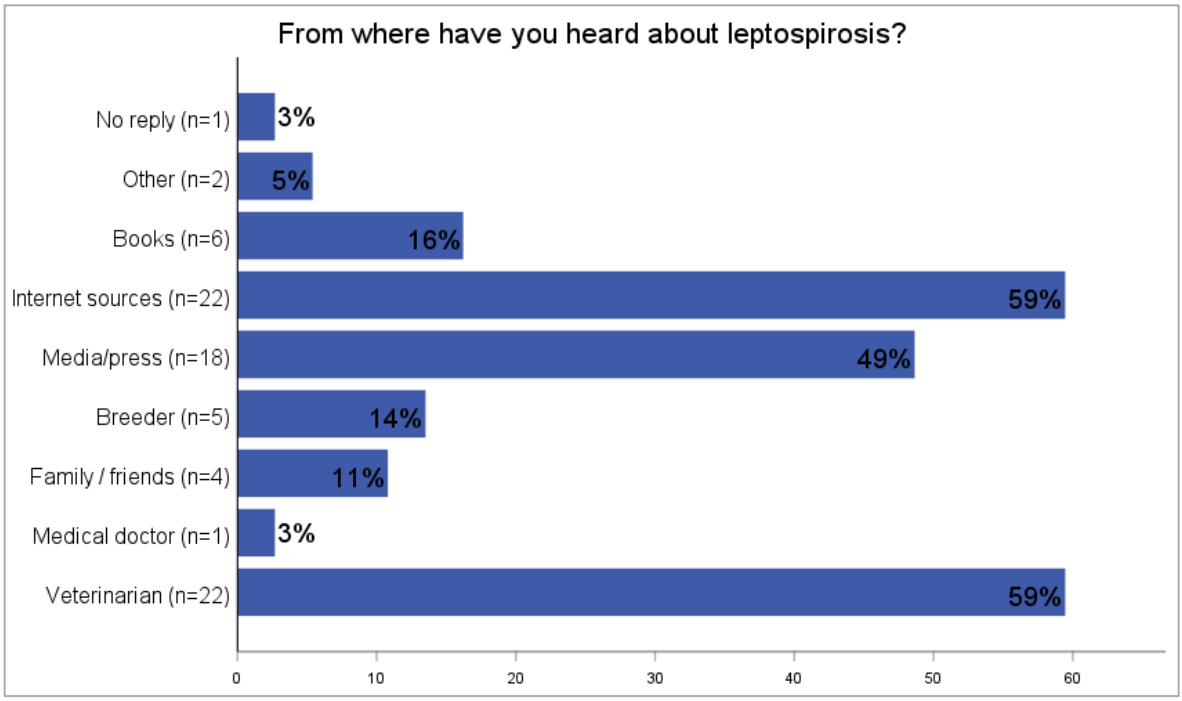

Figure 5. Sources from where dog owners have heard about canine leptospirosis (n=37). More than one option could be selected by the same participant. Total amount of options selected was n=81. If option "Other" was selected, the participant was required to specify their answer.

4.2.2 Knowledge about vaccination against canine leptospirosis

The answers to the four questions regarding vaccination are presented in Table 2. Most dog owners (84%, n=31) knew that there is a vaccine available for canine leptospirosis. Most owners (62%, n=23) also knew that dogs should be re-vaccinated against leptospirosis every year. However, only 27% (n=10) knew that the vaccine does not fully protect their dog from getting infected. Small percentage (11%, n=4) answered that once a dog is correctly vaccinated, it cannot get infected and a majority (54%, n=20) chose option “I don’t know”. Less than half (46%, n=17) of the participants answered that their dog is vaccinated against leptospirosis and 19% (n=7) of the owners did not know whether their dog is vaccinated against the disease or not.

Table 2. Number and proportion of answers to questions considering vaccination of dogs against leptospirosis (n=37).

Questions	**Answers**[a]	**Participants** Total n = 37 n (%)
Can dogs be vaccinated against leptospirosis?	**Yes**	31 (84%)
	No	0 (0%)
	I don't know	5 (14%)
	No reply	1 (3%)
How often should dogs be vaccinated against leptospirosis?	**Every year**	23 (62%)
	Every 3 years	2 (5%)
	There is no vaccine for leptospirosis in dogs	0 (0%)
	I don't know	11 (30%)
	No reply	1 (3%)
When a dog is correctly vaccinated against leptospirosis, can the dog still be infected with it?	**Yes**	10 (27%)
	No	4 (11%)
	There is no vaccine for leptospirosis in dogs	0 (0%)
	I don't know	20 (54%)
	No reply	3 (8%)
Has your dog been vaccinated against leptospirosis?	Yes	17 (46%)
	No	12 (32%)
	I don't know	7 (19%)
	No reply	1 (3%)

[a] Correct answers based on the literature (Schuller et al., 2015b) are highlighted with bold font.

4.2.3 Knowledge about distribution, causative agent, risk factors, clinical signs, transmission and zoonotic potential

The eighth question in the questionnaire asked about distribution of leptospirosis. Most (57%, n=21) of the owners knew that leptospirosis is a worldwide disease (Bharti et al., 2003). The rest of the participants either chose option "I don't know" or did not reply to the question (Figure 6). None of the participants chose any of the three other options: "everywhere except for Nordic countries", "everywhere except for Nordic and Baltic countries" or "outside of Europe".

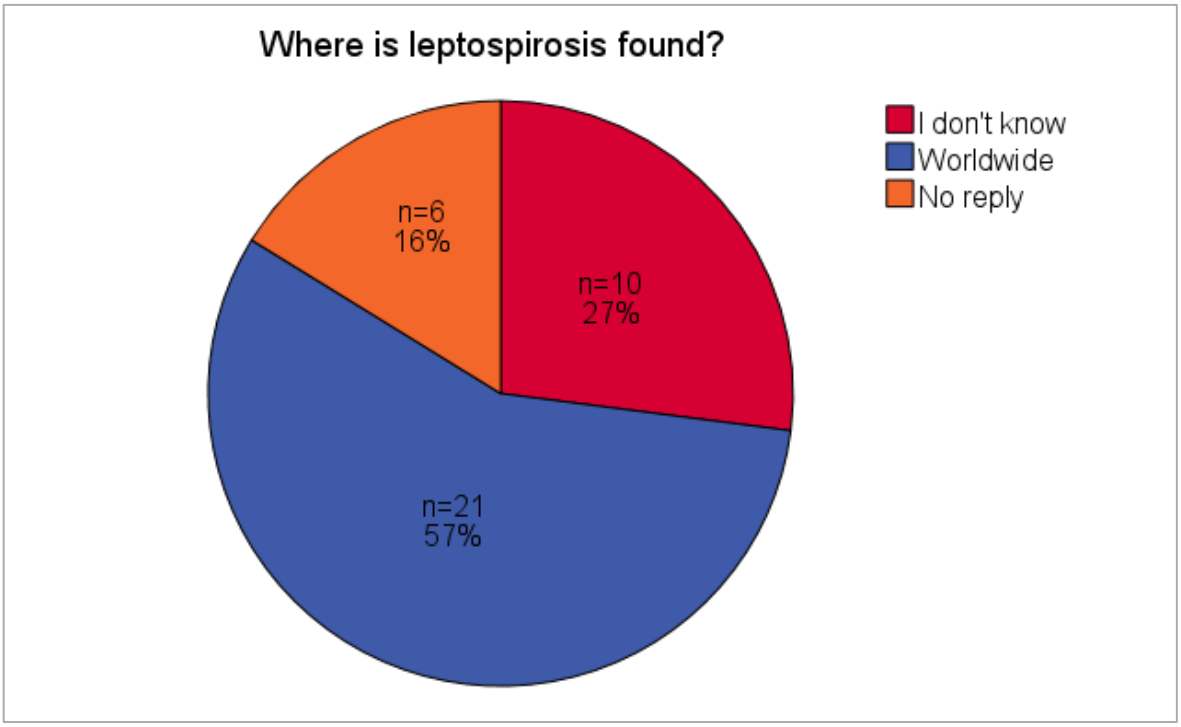

Figure 6. Answers to the eighth question about distribution of leptospirosis (n=37). Correct answer is "Worldwide" (Bharti et al., 2003).

The ninth question was concerning the pathogen causing the disease. 38% (n=14) of the owners knew that leptospirosis is caused by bacteria (Schuller et al., 2015b). No one answered fungi as causative agent. Many owners (32%, n=12) did not reply to this question at all. Results are summarized in Figure 7.

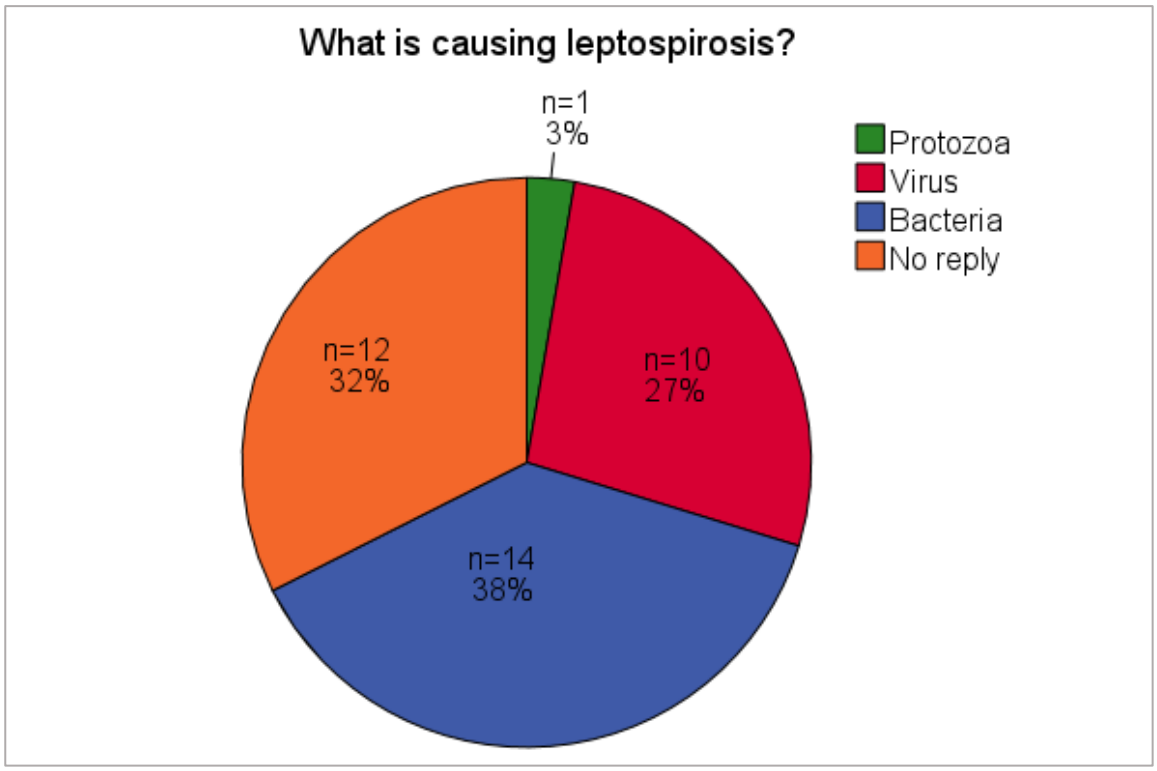

Figure 7. Answers to the ninth question about the causative agent of leptospirosis (n=37). Correct answer is "Bacteria" (Schuller et al., 2015b).

The answers to the two multiple choice questions about clinical signs and risk factors are summarized in Table 3. Almost half of the participants (46%, n=17) knew at least one possible symptom leptospirosis can cause but only 27% (n=10) chose only correct option(s). Majority of the owners (65%, n=24) knew at least one risk factor for the disease and a total of 59% (n=22) chose only correct option(s). Both questions were lacking answers from 19% (n=7) of the participants.

Table 3. Number and proportion of answers to the multiple choice questions concerning symptoms and risk factors for leptospirosis (n=37).

Questions	**Answers**[a]	**Participants** Total n = 37 n (%)
What are possible symptoms of leptospirosis in dogs?	At least one correct answer and no incorrect answers [b]	10 (27%)
	At least one correct answer and at least one incorrect answer [c]	7 (19%)
	None correct or "I don't know"	13 (35%)
	No answer	7 (19%)
Which risk factors predispose dogs to leptospirosis?	At least one correct answer and no incorrect answers [d]	22 (59%)
	At least one correct answer and at least one incorrect answer [e]	2 (5%)
	None correct or "I don't know"	6 (16%)
	No answer	7 (19%)

[a] Correct answers were determined based on the literature (Sykes et al., 2011).

[b] Correct options: fever, signs of kidney failure (for example dehydration and vomiting), signs of liver failure (for example icterus), respiratory signs (for example tachypnea) and ocular signs (for example conjunctivitis) (Sykes et al., 2011).

[c] Incorrect options: dermatological signs (for example rash and itchy skin) and neurological signs (for example balance issues and seizures).

[d] Correct options: warm weather, exposure to lakes, rivers or streams and contact with wildlife (Sykes et al., 2011).

[e] Incorrect options: exposure to ticks, old female small breed dogs and dry and cold environment with multi-dog household for example dog shelters.

The twelfth question asked about transmission of leptospirosis. Only 35% (n=13) selected the correct answer, "Via urine" (Schuller et al., 2015b). Most of the participants (54%, n=20) chose either option "I don't know" (16%, n=6) or did not reply at all (38%, n=14) (Figure 8).

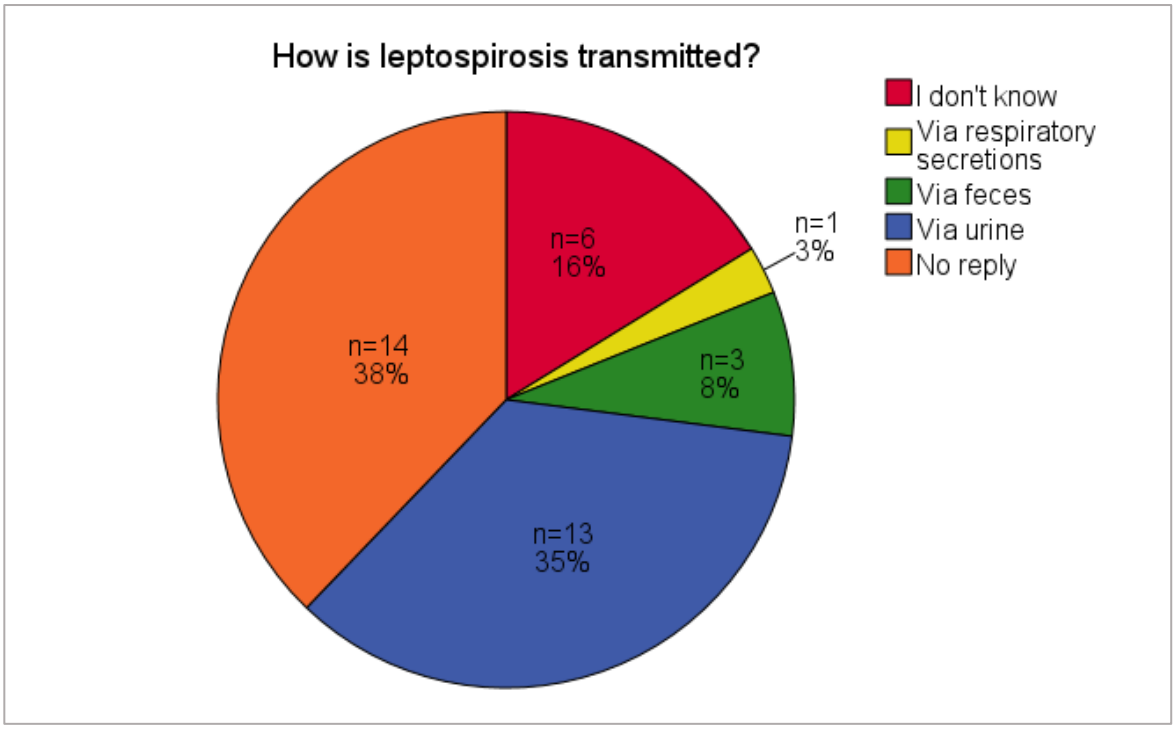

Figure 8. Answers to the twelfth question about transmission of leptospirosis (n=37). Correct answer is "Via urine" (Schuller et al., 2015b).

The thirteenth question was the last question which evaluated owner's current knowledge about leptospirosis. It was concerning the zoonotic potential of the disease, more specifically whether dogs can transmit leptospirosis to humans. Only 14% (n=5) of the owners knew that canine-to-human transmission is possible (Barmettler et al., 2011). Results are shown in Figure 9.

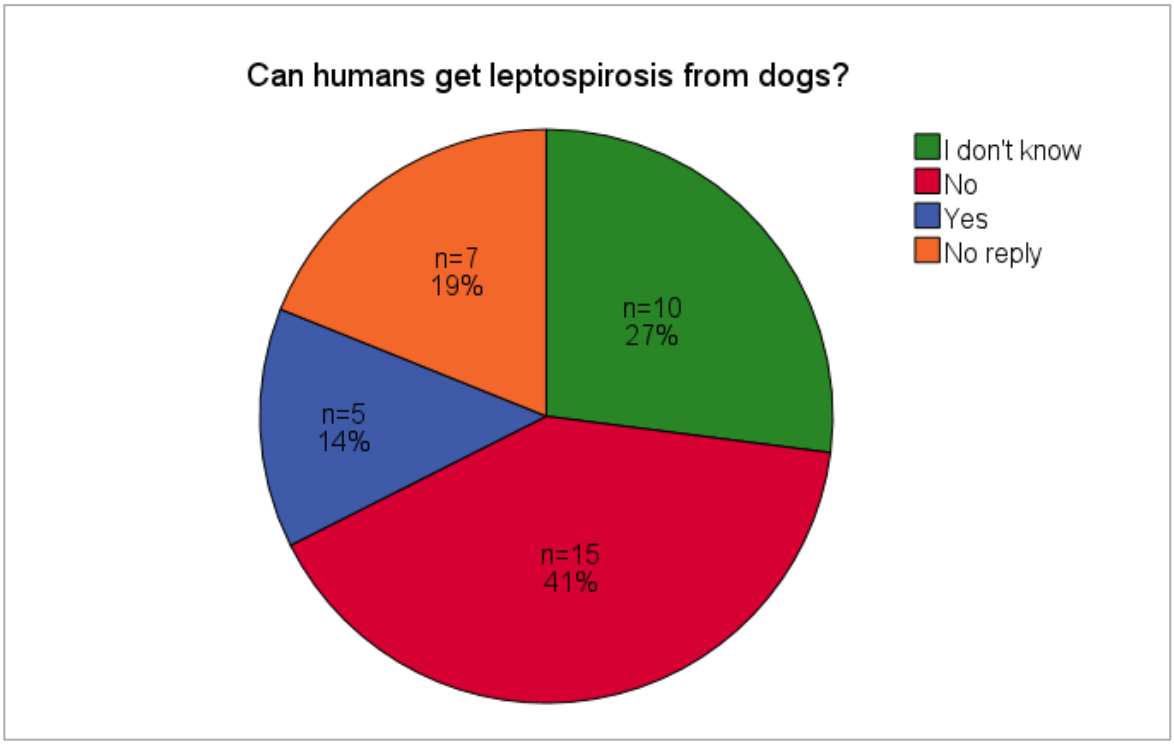

Figure 9. Answers to the 13th question about canine-to-human transmission of leptospirosis (n=37). Correct answer is "Yes" (Barmettler et al., 2011).

4.2.4 Level of knowledge

Participants' total points are illustrated in Figure 10. Most of the participants (78%, n=29) did not achieve high level of knowledge according to our scale. Nearly half of the participants (49%, n=18) achieved equal to or less than three points and thus were regarded as having poor level of knowledge. Only 22% (n=8) earned equal to or more than seven points and reached high level of knowledge.

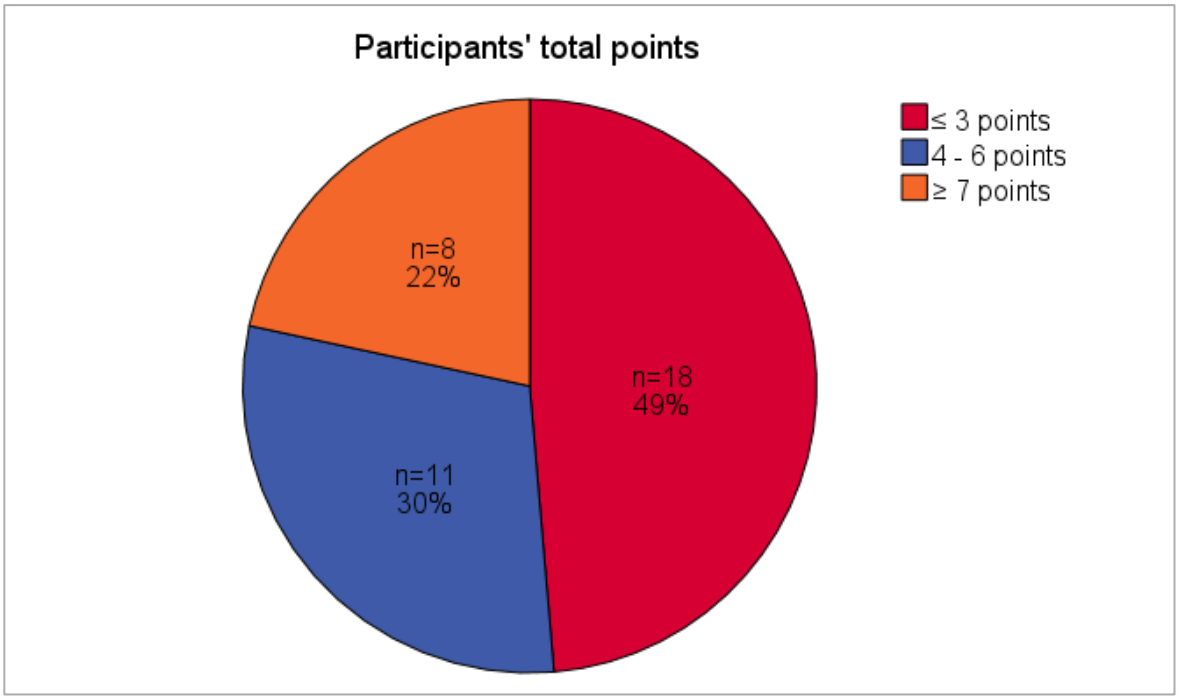

Figure 10. Level of knowledge among participants (n=37). More than or equal to 7 points out of total points of 9 was considered high level of knowledge. 4-6 points was considered moderate level of knowledge and less than or equal to 3 points was considered poor level of knowledge.

Most points were collected from question number 3, "Can dogs be vaccinated against leptospirosis?", as 84% (n=31) of the participants received a point from it. Most participants also earned a point from questions about vaccination interval, distribution of leptospirosis and risk factors: 62% (n=23), 57% (n=21) and 59% (n=22), respectively. The least points were collected from question number 14 about zoonotic transmission from which only 14% (n=5) of the owners earned a point. The number of points collected from each question are presented in Figure 11.

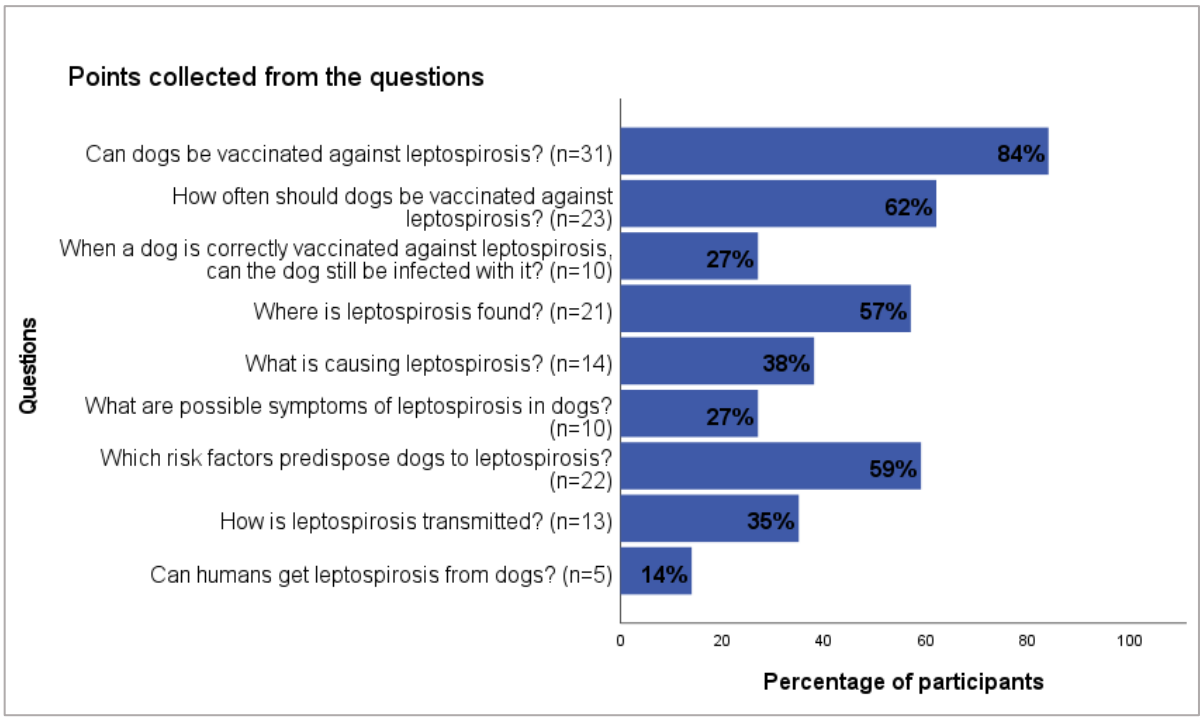

Figure 11. Number of points collected from each question in the questionnaire by the participants (n=37). Correct answers were determined based on the literature.

5. DISCUSSION

The main aim of this study was to assess dog owners' knowledge on canine leptospirosis in Estonia. Several studies have described people's knowledge and attitudes associated with animal leptospirosis (Agampodi et al., 2010; Steneroden et al., 2011; de Navegantes de Araújo et al., 2013; Ricardo et al., 2018; Said et al., 2018; Mathanamohan et al., 2020). Most of them were conducted outside of Europe, mainly in areas where leptospirosis is endemic. To our knowledge, this is the first study aimed at evaluating knowledge of dog owners on canine leptospirosis in Estonia.

The study results indicate that dog owners have limited knowledge on canine leptospirosis. A majority (68%, n=79) of the owners had never heard of the disease. This number is alarming as the World Health Organization (WHO, 2003) has stated that leptospirosis is a worldwide public health problem with an emerging nature. Our results further indicate that majority of the owners who had heard about leptospirosis had, however, insufficient level of knowledge. Only 22% (n=8) of the owners reached high level of knowledge and almost half of the participants (49%, n=18) reached only poor level. These results support the statement that it is necessary to increase awareness and knowledge of this disease (WHO, 2003). Major et al. (2014) called leptospirosis a "neglected tropical disease" and stated that awareness should be increased also in non-tropical areas. Based on our study results, it is clear, that there is a demand for owner education to increase awareness in Estonia. Through our study, we hope to create more discussion around the topic among both veterinarians and dog owners.

One of our findings was that 30% (n=35) of the owners had travelled with their dog. Out of these owners, almost two thirds (57%, n=20) had never heard of leptospirosis before. When asking the owners who had been travelling with their dog to specify their travel destination, most common countries mentioned were Finland and Latvia. This creates a concern, that owners that are not aware of the disease and yet are travelling with their dog might provide a way for leptospirosis to spread to Finland, where leptospirosis is still a rare disease (Liimatta et al., 2017) and on the same way increase the number of cases in Estonia when arriving back from countries where incidence of leptospirosis has been reported to be higher, such as from Latvia (Pappas et al., 2008). Further research is needed to study for example the vaccination status of these dogs.

Our results from the question concerning whether owners have received information about zoonoses from their veterinarians are similar to previous surveys that studied owner awareness about pet-associated zoonotic diseases (Stull et al., 2012; Steele & Mor, 2015). In a Canadian study by Stull et al. (2012), 64% of the respondents indicated that they had never received information regarding pet-associated disease risks. The percentage is close to our result which was that 66% (n=76) of the owners had never received information from their veterinarian about diseases that humans can get from dogs or precautions to take with dogs to reduce the risk of disease. An Australian study by Steele and Mor (2015) reported that less than half of the owners recalled ever receiving information about zoonoses from their veterinarian. In our study, only 23% (n=27) stated that they had received some information about dog related zoonoses as 11% (n=13) did not reply to the questions at all. This indicates that veterinarians should share their public health knowledge more actively and educate owners about leptospirosis as an important zoonosis.

Despite the gap of knowledge most owners had, it was good to notice that most owners who had heard about leptospirosis had received the information from veterinarians (59%, n=22). This result was higher as compared to a study about dog owners' awareness about zoonoses in the U.S, in which it was reported that only 40% selected their veterinarian as their primary source of information regarding zoonotic diseases (Sandhu & Singh, 2014). In the same study, none of the owners quoted their primary physician as their primary source of information for zoonoses. Our findings are similar: in this study only one participant indicated that they had heard about the disease from a medical doctor. These findings suggest that veterinarians should cooperate more closely with medical doctors and educate owners together as leptospirosis is not only a threat to dogs but also to humans (Barmettler et al., 2011). Also, only 14% (n=5) of the owners had heard about leptospirosis from a breeder. As breeders often are in contact with dog owners, it would be beneficial to increase their knowledge about the disease which they could then share with the owners. A large proportion (59%, n=22) indicated that they had received information about leptospirosis from internet sources, including web pages and social media. These means of communication should therefore be used in order to reach the owners.

According to Francey et al. (2020), vaccination of dogs with L4 vaccine was associated with a significant decrease in the number of dogs with leptospiral infection. They suggested that the uptake of L4 was quick in Switzerland due to effective marketing and continuous

education efforts. In their study groups, the percentages of dogs that were not vaccinated against leptospirosis were low: 4% and 3%. A recent German study about canine vaccination and owner compliance, on the contrary, reported different results as only half of the dogs were up-to-date on leptospiral vaccine (Eschle et al., 2020). Similarly, in our study only 46% (n=17) of the owners had vaccinated their dog against leptospirosis. What is interesting, most owners seemed to know about the availability of the vaccine, yet less than half of them had vaccinated their dog. The number of vaccinated dogs, however, can be higher as 19% (n=7) of the owners were not aware whether their dog was vaccinated or not. Our small sample size could also affect the results. Additionally, we did not collect the data on how many dogs had been vaccinated correctly according to the protocol of initial two injections at an interval of three to four weeks apart, followed by yearly boosters (Schuller et al., 2015b). Some dogs that were vaccinated might have had an inadequate vaccination status if the protocol had not been followed accordingly. However, most owners seemed to be aware of the correct vaccination interval as 62% (n=23) of the participants stated that dogs should be vaccinated against leptospirosis every year. What owners knew poorly was that despite the vaccination, dogs can get infected with leptospirosis (Schuller et al., 2015b). Only 27% (n=10) of the owners knew that this could occur. Our findings therefore suggest that marketing of vaccines, particularly the L4 vaccine, should be more effective in Estonia and owners should be educated about the matter.

What owners seemed to know relatively well was the distribution of leptospirosis and the risk factors associated with the disease. Owners' knowledge about causative agent, clinical symptoms, transmission and zoonotic potential was lower. Less than 40% of the owners knew that leptospirosis is caused by bacteria and that it is transmitted by urine. Less than half could choose at least one symptom leptospirosis can cause in dogs. While knowledge about the causative pathogen may not be so relevant for the owners, awareness about the transmission route is something that should be addressed, particularly when taking into consideration the zoonotic nature of the disease. Owners should also be informed about the variability of the clinical signs leptospirosis can cause and advised to seek veterinary guidance whenever worried about their dog's health.

An alarming finding was that only 14% (n=5) knew that humans can get leptospirosis from dogs. Almost half of the owners (41%, n=15) said that canine-to-human transmission is not possible and the rest either did not reply at all (19%, n=7) or said "I don't know" (27%,

n=10). Comparable results have been published in the United States: Sandhu and Singh (2014) studied awareness among dog owners about zoonotic diseases and based on their results, less than 10% of the owners could mention leptospirosis as a disease that can be transmitted from dogs to humans. Our findings indicate an evident need for owner education about the zoonotic aspects of leptospirosis. Even though dog-to-human transmission of leptospirosis is infrequent, dogs are also considered indicators for the disease and thus, understanding and preventing the disease has important implications for human health too (White et al., 2017). Additionally, Eschle et al. (2020) suggested that owners' poor knowledge about the zoonotic threats of leptospirosis could also be one explanation for low vaccination rates. Further study is required to evaluate the association but the concern should be addressed.

Limitations of this study arise mainly from the sample size and quality of the data. The number of participants was rather low and the data was collected only from the small animal clinic of Estonian University of Life Sciences. However, being the sole University Veterinary Clinic in Estonia, its customers can be assumed to be from all over the country and thus represent the dog owners of Estonia reasonably well. Many questionnaires were incomplete or somewhat incorrectly filled. This is because the filling of the questionnaires was not controlled and the instructions for owners could have been more thorough. Additionally, multiple-choice questions leave a possibility for the respondent to choose correct answers by chance. Open questions could have provided more accurate information.

When evaluating the level of knowledge by points, blank answers gave no points for the participant. Many respondents had not answered to the second page at all, probably due to not noticing it, and thus these owners received less points. This probably affected the accuracy of the results. However, some participants left part of the questions empty probably due to not knowing the answer, especially in the questions where "I don't know" option was not provided, and therefore it can be assumed that these owners likely did not have very good knowledge about the disease.

CONCLUSION

This study demonstrated that dog owners' knowledge about canine leptospirosis in Estonia is low. As most owners have never heard about the disease and when taking into consideration the severity, emerging nature and particularly the zoonotic risks of leptospirosis, it is evident that there is a high need for increasing awareness among the public.

Based on our results, owners need education particularly about the zoonotic risks of leptospirosis and on its mode of transmission, as well as about the variable clinical presentations of the disease. Furthermore, owners' knowledge about vaccination against canine leptospirosis requires improvement. Veterinarians should more actively take their pivotal role in educating people about the disease and collaborate with medical doctors to increase awareness about leptospirosis as a risk for both canine and human health. This could be done by distributing information via web pages and social media and by providing educational materials in the clinics, as well as by advising owners at individual level on regular veterinary visits.

Further study about this topic with a larger sample size would allow more accurate analysis. In the future, open questions could be used in the questionnaire to provide more detailed information. It would be interesting to investigate the factors that affect vaccination of dogs against leptospirosis such as owner profile (e.g. education background, age, gender) or knowledge about the zoonotic potential of the disease. Moreover, it would be beneficial to examine the vaccination status of the dogs and to inspect whether owners vaccinate their dogs according to the protocol.

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APPENDIXES

Appendix 1. Questionnaire in English

We would kindly ask you to take part in our 6th year veterinary student's final thesis questionnaire. The purpose of the survey is to find out how much dog owners in Estonia know about a disease called leptospirosis.

The survey is voluntary and by answering the questionnaire you agree to participate in the study and give your permission to use the data in anonymized form.

We thank you for your participation in the survey!

Please mark the correct answer/answers with X in the 3rd (grey) column.

1.	Have you ever heard of leptospirosis before?		Yes
			No
NB! If you responded with NO to the question 1, please answer only for questions 7 and 14.			
2.	From where have you heard about leptospirosis?		From a veterinarian
			From a medical doctor
			From family / friends
			From the breeder of your pet
			From media/press
			From internet sources/web pages/social media
			From books
			Other (please elaborate) _____ _____ _____ _____
3.	Can dogs be vaccinated against leptospirosis?		Yes
			No
			I don't know
4.	Has your dog been vaccinated against leptospirosis?		Yes
			No
			I don't know
5.	How often should dogs be vaccinated against leptospirosis?		There is no vaccine for leptospirosis in dogs
			Every year
			Every 3 years
			I don't know
6.	When a dog is correctly vaccinated against leptospirosis, can the dog still be infected with it?		There is no vaccine for leptospirosis in dogs
			Yes
			No
			I don't know

7.	Has your dog been abroad?		Yes – where? _____ _____ _____ _____
			No
8.	Where is leptospirosis found?		Everywhere except for Nordic countries
			Everywhere except for Nordic and Baltic countries
			Outside of Europe
			Worldwide
			I don't know
9.	What is causing leptospirosis?		Virus
			Bacteria
			Protozoa
			Fungi
10.	What are possible symptoms of leptospirosis in dogs? (many can be selected)		Fever
			Signs of kidney failure – for example dehydration and vomiting
			Signs of liver failure – for example icterus (jaundice)
			Dermatological signs – for example rash and itchy skin
			Respiratory signs – for example tachypnea (increased respiratory rate)
			Ocular (eye) signs – for example conjunctivitis
			Neurological signs – for example balance issues and seizures
			I don't know
11.	Which risk factors predispose dogs to leptospirosis? (many can be selected)		Exposure to ticks
			Warm weather, exposure to lakes, rivers or streams
			Old, female small breed dogs
			Dry and cold environment, multi-dog household for example dog shelters
			Contact with wildlife
			I don't know
12.	How is leptospirosis transmitted?		Via feces
			Via respiratory secretions
			Via urine
			I don't know
13.	Can humans get leptospirosis from dogs?		Yes
			No
			I don't know

14.	Have you ever received information from your veterinarian about diseases that you can get from dogs or precautions to take with dogs to reduce the risk of disease?		Yes
			No

We thank you for your participation in the survey!

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Appendix 2. Questionnaire in Estonian

Palume teil osaleda meie 6. aasta veterinaaria tudengi lõputöö küsimustikus. Küsitluse eesmärk on välja selgitada, kui palju teavad Eesti koeraomanikud leptospiroosist.

Küsitlus on vabatahtlik ja küsimustikule vastates nõustute osalema uuringus ja annate loa andmete kasutamiseks anonüümsel kujul.

Täname teid uuringus osalemise eest!

Palun märkige rist sobiva vastuse/vastuste ette keskmisse, halli tulpa.

1.	Kas olete kunagi varem leptospiroosist kuulnud?		Jah
			Ei
NB! Kui vastasite esimesele küsimusele EI, palun vastake ainult küsimustele 7 ja 14.			
2.	Kust olete kuulnud leptospiroosist?		Loomaarsti käest
			Perearstilt
			Perekonnalt/sõpradelt
			Kasvatajalt
			Meediast ja ajakirjandusest
			Veebiallikatest, sotsiaalmeediast
			Raamatutest
			Mujalt (täpsustage) _____ _____ _____ _____
3.	Kas koeri saab leptospiroosi vastu vaktsineerida?		Jah
			Ei
			Ma ei tea
4.	Kas Teie koer on leptospiroosi vastu vaktsineeritud?		Jah
			Ei
			Ma ei tea
5.	Kui sageli tuleks koeri leptospiroosi vastu vaktsineerida?		Koerte leptospiroosi vastu vaktsiini ei ole
			Iga aasta
			Iga kolme aasta tagant
			Ma ei tea
6.	Kui koer on leptospiroosi vastu nõuetekohaselt vaktsineeritud, kas koer võib ikkagi nakatuda/haigestuda?		Koerte leptospiroosi vastu vaktsiini ei ole
			Jah
			Ei
			Ma ei tea
7.	Kas Teie koer on viibinud välismaal?		Jah – kus? _____ _____ _____

			Ei
8.	Kus leptospiroos esineb?		Kõikjal, välja arvatud Põhjamaades
			Kõikjal, välja arvatud Põhja- ja Baltimaades
			Väljaspool Euroopat
			Kogu maailmas
			Ma ei tea
9.	Mis põhjustab leptospiroosi?		Viirus
			Bakterid
			Algloomad
			Seened
10.	Millised on koertel leptospiroosi sümptomid? (valida võib mitu varianti)		Palavik
			Neerupuudulikkuse nähud - näiteks veetustumine (vedeliku kadu) ja oksendamine
			Maksapuudulikkuse nähud - näiteks naha ja limaskestade kollasus
			Nahaprobleemid - näiteks lööve ja sügelev nahk
			Hingamisteede nähud - näiteks tahhüpnea (kiirenenud hingamine)
			Silma nähud - näiteks silma sidekesta ehk konjunktiivi põletik
			Neuroloogilised nähud - näiteks tasakaaluprobleemid ja krambid
			Ma ei tea
11.	Millised riskifaktorid soodustavad koerte leptospiroosi? (valida võib mitu varianti)		Kokkupuude puukidega
			Soe ilm, kokkupuude järvede, jõgede või ojadega
			Vanad, emased väike tõukoerad
			Kuiv ja külm keskkond, mitme koeraga majapidamine, näiteks koerte varjupaikad
			Kontakt elusloodusega
			Ma ei tea
12.	Kuidas leptospiroos levib?		Väljaheidete kaudu
			Hingamiseritiste kaudu
			Uriini kaudu
			Ma ei tea
13.	Kas inimesed võivad koertelt saada leptospiroosi?		Jah
			Ei
			Ma ei tea
14.	Kas olete kunagi saanud oma veterinaararstilt teavet haiguste kohta, mida võite koertelt saada, või ettevaatusabinõusid, kuidas nende levikut tõkestada?		Jah
			Ei

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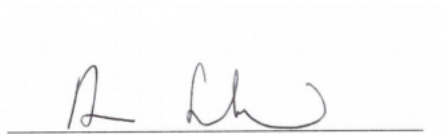
Author of the final thesis 

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