UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

CLINICAL MANAGEMENT OF CANINE LEISHMANIOSIS IN PORTUGAL: THE VETERINARY COMMUNITY PERSPECTIVE

MARTA NUNES EUSÉBIO MENESES MONTEIRO

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ABORDAGEM CLÍNICA DA LEISHMANIOSE CANINA EM PORTUGAL: A PERSPETIVA DA COMUNIDADE MÉDICO-VETERINÁRIA

Resumo

A Leishmaniose canina (LCan) é uma doença endémica em mais de 70 países, incluindo Portugal. A investigação científica tem permitido desenvolvimentos relativamente à abordagem da doença, incluindo a publicação de linhas orientadoras internacionais, como as do grupo LeishVet. Apesar disso, esta abordagem tem-se revelado variável entre países e há escassa informação sobre o tratamento da doença renal associada, frequentemente presente em estádios mais avançados e considerada a principal causa de morte devido a LCan.

Este estudo teve por objetivo investigar o paradigma atual da abordagem clínica da LCan em Portugal, incluindo o tratamento da doença renal nestes pacientes.

Foi desenvolvido um questionário, incluindo 24 a 64 questões, dependendo do encadeamento de respostas de cada inquirido. O conteúdo dividiu-se em três partes: características gerais, abordagem médica (diagnóstico, tratamento e prevenção) perante diferentes cenários hipotéticos de LCan e outras questões. Esta última parte focou-se no conhecimento das linhas orientadoras existentes, nas ferramentas de diagnóstico habitualmente aplicadas e no uso de imunossupressores no tratamento de glomerulonefrite secundária a LCan. Após validação interna, o questionário foi disponibilizado online, através de uma plataforma eletrónica, e divulgado através das redes sociais, durante 2 meses, em grupos destinados à comunidade médico-veterinária.

O estudo incluiu 86 respostas e revelou que a maior parte das medidas aplicadas pelos médicos veterinários são concordantes com as recomendações internacionais. Mais ainda, o tratamento com miltefosina e alopurinol foi priorizado em relação ao uso de antimoniato de meglumina (AM) com alopurinol (selecionados por 48,8% e 23,3%, respetivamente) no estádio IV da doença, provavelmente devido à nefrotoxicidade associada ao AM, segundo alguns estudos. Para além disso, a eutanásia foi apenas considerada para o cenário do estádio IV. O protocolo preferido para o tratamento de proteinúria em cães com leishmaniose consistiu no uso de inibidores da enzima de conversão da angiotensina (IECA). O uso de imunossupressores foi considerado por 44,2% dos veterinários, com uma preferência evidente por prednisolona (94,7%) e, numa proporção muito menor, por micofenolato de mofetil (5,3%).

Estes resultados contribuíram para salientar a escassez de dados sobre o uso de imunossupressores específicos para o tratamento de cães com doença glomerular imunomediada, especialmente quando associada a uma causa infeciosa, e a preferência dada à miltefosina, em relação ao AM, no estádio mais avançado da doença.

Palavras-chave: leishmaniose canina; doença renal; questionário; abordagem clínica

CLINICAL MANAGEMENT OF CANINE LEISHMANIOSIS IN PORTUGAL: THE VETERINARY COMMUNITY PERSPECTIVE

Abstract

Canine leishmaniosis (CanL) is an endemic disease in more than 70 countries, including Portugal. Scientific research has allowed developments concerning the management of the disease, including the publication of international guidelines, such as those published by the LeishVet group. However, this management has shown to be variable among countries and there is scarce information concerning the treatment of the associated renal disease, which is often present in more advanced stages and is the main cause of death due to CanL.

This study aimed to investigate the current paradigm of the clinical management of CanL in Portugal, including the treatment of renal disease in these patients.

An online questionnaire was developed, including 24 to 64 questions, depending on the answering pathway of each respondent. The content was divided in three parts: general characteristics, medical approach (diagnosis, treatment and prevention) in face of different hypothetical scenarios of CanL and other questions. This last part focused on the knowledge regarding the existing guidelines, the diagnostic tools routinely applied and the use of immunosuppressants for treatment of glomerulonephritis secondary to CanL. After internal validation, the questionnaire was uploaded using an electronic platform and diffused online, over 2 months, via Portuguese social network veterinary groups.

The survey included 86 answers and revealed that most measures applied by the enquired veterinary clinicians are in accordance with the international recommendations. Furthermore, the treatment with miltefosine and allopurinol was prioritized to that of meglumine antimoniate (MA) with allopurinol (selected by 48.8% and 23.3%, respectively) in stage IV of CanL, probably due to the nephrotoxicity associated with MA, according to some studies. Furthermore, euthanasia was only considered for the stage IV scenario. The preferred protocol for treatment of proteinuria in dogs with leishmaniosis consisted in the use of angiotensin-converting enzyme inhibitors (ACEI). The use of immunosuppressants was considered by 44.2% of the veterinary surgeons, showing an evident preference for prednisolone (94.7%) and, in a much lesser proportion, for mycophenolate mofetil (5.3%).

These findings contributed to emphasise the lack of evidence regarding the use of specific immunosuppressive drugs for the treatment of immune-mediated glomerular disease, especially when associated with an infectious cause, and the preference given to miltefosine rather than MA in the most advanced stage of disease.

Keywords: canine leishmaniosis; renal disease; questionnaire; clinical approach

Table of contents

List of figures

List of graphics

List of tables

List of abbreviations

ACEI/IECA – angiotensin-converting enzyme inhibitors
AHCC – active hexose correlated compounds
ARB/BRA – angiotensin receptor blockers
BM – bone marrow
CanL/LCan – canine leishmaniosis
CBC – complete blood count
CCB/BCC – calcium channel blockers
CKD – chronic kidney disease
CLWG – Canine Leishmaniosis Working Group
CT – computerized tomography
DVM – Doctor in Veterinary Medicine
EEG – general examination

E.g. – *exempli gratia* (Latin) (for example)
ECVIM – European College of Veterinary Internal Medicine
ELISA – enzyme-linked immunosorbent assay
ESCCAP – European Scientific Counsel Companion Animal Parasites
FMV - Faculdade de Medicina Veterinária
FNA – fine needle aspiration
GFR – glomerular filtration rate
HEV - Hospital Escolar Veterinário
ICT – immunochromatography tests
i.e. – *id est* (Latin) (this is)
IFAT – indirect fluorescent antibody technique
IDIU – Infectious Diseases Isolation Unit
IRIS - International Renal Interest Society
LN – lymph node(s)
MA/AM – meglumine antimoniate
MSc – Master of Science
NA – not available / no answer
OMV – Ordem dos Médicos Veterinários
PCR – polymerase chain reaction
PhD – Doctor of Philosophy
SBP – systolic blood pressure
SDMA - symmetric dimethylarginine
Th1 – T-helper 1
Th2 – T-helper 2
ULisboa - Universidade de Lisboa
UPC - urinary protein to creatinine ratio
US – ultrasound
USG – urine specific gravity
VBD – vector-borne disease
WHO – World Health Organization
ZVL – zoonotic visceral leishmaniosis

List of symbols

+ - plus/with
< - lower than
> - higher than
= - equal to
% - percentage
n – size of the sample
® - registered trademark

Section 1 – Traineeship report

The final curricular traineeship was performed at Hospital Escolar Veterinário (HEV) – Faculdade de Medicina Veterinária (FMV) da Universidade de Lisboa (ULisboa), from 2 September 2019 to 31 January 2020, under supervision of Professor Doctor Rodolfo Leal and co-supervision of Professor Doctor Isabel Fonseca. Thereafter, an extra-curricular externship was conducted at the Scholar Hospital of the Faculty of Veterinary Medicine at Ghent University, from 7 February to 13 March 2020. The externship had been planned to last three months, but it had to be shortened due to the Covid-19 pandemic.

Part of this study was also presented in the Online Congress of the European College of Veterinary Internal Medicine (ECVIM) – Companion Animals (2nd-5th September 2020) (Annexe 1).

1. Internship at HEV – FMV/ULisboa

The internship included the participation in different services namely surgery, ultrasound (US), radiology, internal medicine, ophthalmology, oncology, dermatology, infectious diseases and first opinion consultations/emergencies in rotating shifts, generally of 8 hours, with slight variations among the different services. Furthermore, activities in the hospitalization unit were performed in 12h shifts, from 8 a.m. to 8 p.m. or vice-versa. Globally, the trainee was able to witness and participate in first, second opinion and referral consultations, where it was given the opportunity to put into practice overall notions of various daily, in-practice procedures, as well as developing soft skills correlated with the interpersonal relationships with the hospital staff and clients. In total, approximately 1053 hours of internship were performed.

Surgery

Approximately 117 hours were completed in this service. The activities held consisted in receiving, weighing and bringing the patients to the pre-surgical preparation room. Then, different pre-surgical procedures were conducted, including the administration of pre-operative medication, catheterization, intubation, trichotomy, cleaning and disinfection of the surgical area. In the theatre, the trainee had the opportunity to participate in various surgeries, always under supervision of a veterinary surgeon. Finally, the post-surgical monitoring of the patients was performed, as well as their delivery to the owners in that same day, when possible. Different procedures were attended, including soft tissue, orthopaedic or dental surgeries.

Ultrasound

Around 40 hours were completed in this service. During this period, the student observed various US procedures and helped holding the patients during the exam. Besides the imaging technique, other procedures were often performed during the US examination, such as the collection of body fluids through cystocentesis, abdominocentesis, pericardiocentesis, among other procedures, such as splenic or hepatic biopsies. Frequently, sedation of the animal was required.

Radiology

Approximately 80 hours were completed in this service, which included radiography and computerized tomography (CT). During the radiography examinations, the trainee participated in the positioning of the patient, sedation or general anaesthesia when required, as well as the observation and analysis of the x-ray images. In CT, the student was involved in the reception of the patients in the morning and in the technical procedures involved in the whole exam, such as catheterization, preparation and administration of anaesthetic medication and endovenous contrast media, monitoring during anaesthesia and recovery. Furthermore, it was possible to assist the writing of reports about the imaging findings.

Oncology

Approximately 64 hours were performed in this rotation. The trainee engaged in various activities, including consultations approaching the diagnosis, treatment, adverse effects, prognosis and follow-up of oncologic patients. Furthermore, it was possible to help with the chemotherapy procedures, which included the reception and weighting of the animal, catheterization, blood withdrawal for complete blood count (CBC), preparation and administration of chemotherapy drugs, monitoring and returning of the animal to the owner. All procedures were supervised by the veterinarian or nurse in service. The preparation of chemotherapy drugs was performed only by the veterinarian or nurse, given the risk that the manipulation of such compounds implies, and the safety measures required, which the student had the opportunity to perceive, such as the use of personnel protective equipment and laminar flow chambers.

Ophthalmology

An estimate of 40 hours was performed in the Ophthalmology service. The activities included first opinion, second opinion, referral and follow-up consultations, in which the trainee had the opportunity to collect the clinical history, perform physical examination and specific ophthalmologic examination, including evaluation of palpebral, threat and pupillary reflexes,

realization of Schirmer and fluorescein tests, tonometry, and fundoscopic exam, under supervision.

Internal Medicine

Approximately 304 hours were accomplished in the internal medicine service. The trainee had the opportunity to play an active role on technical procedures, consultations and discussion of clinical cases (approach, differential diagnosis, diagnostic tools, treatment, follow-up and prognosis) under supervision of the clinician in service, generally Professor Doctor Rodolfo Leal or, once a week, Dr. Joana Dias, Dr. Telmo Casimiro or Dr. Sara Prata. Most consultations consisted in referral, second opinion or follow-up of clinical cases and a high proportion of the clinical scenarios included endocrine, gastrointestinal and respiratory diseases. Furthermore, several endoscopy procedures were observed, including upper gastrointestinal endoscopy, colonoscopy, rhinoscopy, tracheobroncoscopy with bronchoalveolar lavage, and cystoscopy, some including biopsy procedures and removal of foreign bodies. It was also possible to participate in the writing of clinical reports provided to the owners, under supervision of Professor Doctor Rodolfo Leal.

Infectious Diseases Isolation Unit (IDIU)

Approximately 64 hours were completed in this area, located apart from the other services, for safety purposes. The student was familiarized with the specific approach required for the manipulation of animals affected by infectious diseases, including the appropriate use of personnel protective equipment, as well as the cleaning and disinfection procedures. The activities included the cleaning and disinfection of all divisions, preparation and administration of medication, monitorization of the patients, assistance and participation in further procedures, such as blood withdrawal or imaging, this last one conducted in the US service. Finally, it was possible to participate in visit or discharge consultations as well.

Dermatology

Approximately 40 hours were conducted in the Dermatology service. The trainee assisted and participated in first opinion, second opinion, follow-up and referral consultations, which included the realization of anamnesis, physical examination, complementary diagnostic tests, as well as the discussion of the differential diagnosis, treatment and prognosis. The complementary diagnostic tests performed were: cytology – including the sampling by apposition, swabs, adhesive tape or aspiration, and followed by coloration (generally Diff-Quick) and microscopic observation; skin scraping; trichogram, observation with the Wood lamp, fungal culture, and biopsy.

General Consultations

General consultations were attended for approximately 160 hours. In this area, it was possible to put into practice several theoretical and practical insights required for the daily routine. It was given the opportunity to introduce the consultations, including performing anamnesis and physical examination (observation, palpation, auscultation, measurement of temperature), which were thereafter verified by the clinician. Moreover, other technical procedures were practiced, including the restraint of the animal, realization of complementary diagnostic exams requiring blood withdrawal, collection of urine (through cystocentesis or urethral catheterization) or other body samples through fine needle aspiration (FNA), as well as imaging exams (radiography, US). Furthermore, the trainee participated in the preparation and administration of medication, catheterization, administration of fluid therapy, the script of requisitions for analysis in external laboratories, as well as the writing of medical prescriptions. The aetiology of the visits highly varied and included first opinion, second opinion and referral consultations. Regular vaccination and/or deworming, as well as check-up consultations were held. Apart from preventive medicine, clinical cases included gastrointestinal, urinary, cardiovascular, neurology, muscular-skeletal, respiratory or dermatology first-opinion consultations. The trainee could assist and participate in emergency consultations as well, involving patients in life threatening conditions, such as traumatic lesions, convulsions, dyspnoea, urinary obstructions, gastric dilation and/or volvulus, cardiopulmonary arrest, among others. These circumstances demanded a rapid and efficient approach by the clinician/nurse in service, including catheterization, oxigenotherapy, intubation, cardiopulmonary resuscitation, preparation and administration of medication, in which the student took part, whenever possible.

Hospitalization

Approximately 144 hours were attained in this unit. The main activities included two rounds, in the beginning and end of the shift, where the medical approach regarding each clinical case was discussed and reviewed. A general physical examination of all animals was held, at least twice a day, consisting in the observation of their behaviour (*e.g.* alert, calm, lethargic) and performing a clinical examination. Moreover, the management of the patients included preparing and administering medication and fluid therapy, giving food, water, cleaning the boxes and, depending on the ambulatory status, walking the patients as well. The animals in this unit frequently required other proceedings such as blood/urine collection, measurement of blood pressure, urinary catheterization and realization of further imaging exams or surgeries.

2. Extra-curricular externship at the Scholar Hospital of the Faculty of Veterinary Medicine – Ghent University

During the externship in Ghent, the trainee attended the services of Hospitalization, Pathology, Surgery, Internal Medicine and Clinical Nutrition, each rotation for one week. This externship gave the trainee the opportunity to engage in several activities, which included starting the consultation and performing the physical examination, followed by a discussion with the specialist about the anamnesis, differential diagnosis list, adequate complementary diagnostic exams, most probable diagnosis, treatment, prognosis and follow-up. Then, the students accompanied the clinician during the consultation and registered all important information regarding the clinical case, which was thereafter confirmed. This was the general approach, although with some variations among the different rotations. As with the activities held in HEV–FMV/ULisboa, all activities performed were supervised by the specialists responsible for each service.

In Hospitalization service – also named as Hospi 7, since this service is conducted through all 7 days of the week – the activities held consisted in the care of hospitalized patients, similarly to the ones performed in the hospitalization unit of HEV–FMV/ULisboa. However, one or more patients were attributed to each student, which was responsible for transmitting the clinical information about them during the rounds.

In Pathology, the student performed various necropsies, either required by the owners (“official necropsies”) or exclusively for an academic purpose (“non-official necropsies”) and participated in the writing of the respective reports.

In Surgery, the activities performed included the observation and participation in consultations and surgeries, from the reception of the animal until its discharge, as well as the discussion about the clinical cases presented every day.

In the Internal Medicine department, it was possible to conduct the consultations, to perform a physical examination and to discuss the diagnostic and treatment approach with the clinician in service. At the end of the week, the student took part in a group presentation, approaching a clinical case seen during that period.

In Clinical Nutrition, the trainee acquired overall notions about the nutritional management of both healthy and diseased patients. In the end of the week, group presentations were conducted, each group approaching the most adequate diet for a specific theoretical or real scenario, suggested by the specialist.

Section 2 – Literature review

1. Canine Leishmaniosis

1.1. *Leishmania* spp. – life cycle, epidemiology and transmission

Leishmaniosis is one of the world's most important emerging diseases (World Health Organization [WHO] 2010). The causative agent is a parasite of the genus *Leishmania* (Ross, 1903), characterized by a digenetic life cycle which affects the mononuclear phagocyte system of several mammalian hosts, in the form of amastigote, and is mainly transmitted through the hematophagous activity of female phlebotomine sand fly vectors – *Phlebotomus* spp. in the Old World – where it replicates as promastigote (Killick-Kendrick 1990, 1999).

Leishmania infantum (and the synonym *L. chagasi* species in Latin America) is a zoonotic species responsible for canine leishmaniosis (CanL) and zoonotic leishmaniosis. Canine leishmaniosis is endemic in more than 70 countries worldwide (Solano-Gallego et al. 2011), affecting millions of dogs (*Canis lupus familiaris*) in Europe – mostly in Southern Europe, although it is spreading northwards (Maia and Cardoso 2015) – Asia, North Africa and South America, and is an emergent disease in North America as well (Pereira 2008; Dantas-Torres et al. 2012). Portugal is an endemic country for CanL (Oliveira et al. 2010; Cardoso et al. 2012; Cortes et al. 2012; Maia et al. 2015a, 2016), with an estimated nation-wide average seroprevalence of 6.3% (Cortes et al. 2012). Some studies reported increasing seroprevalences in some areas (Cortes et al. 2007; Pereira 2008), reaching 56% in east-central Portugal regions (Pires et al. 2019). A molecular screening survey conducted in 2011-2014 in southern Portugal revealed 60.4% polymerase chain reaction (PCR) positive dogs for *L. infantum* and a high prevalence of dogs without clinical signs (Maia et al. 2016).

The dog is considered the main reservoir for human infection (Gramiccia and Gradoni 2005), such that controlling the emergence of this disease became a One Health problem (Miró and López-Velez 2018). Besides dogs, the role of other mammals as reservoirs of infection by *L. infantum* has been studied, including wild lagomorphs (hares and rabbits) (Arce et al. 2013; García et al. 2014), felids (Maia et al. 2015b, Leal et al. 2018), rodents (Helhazar et al. 2013) and wild canids (Campino and Maia 2018). Other non-vectorial forms of dissemination have been investigated, such as venereal (Turchetti et al. 2014), vertical (Svobodova et al. 2017), through blood transfusion (de Freitas et al. 2006) or even through direct dog-to-dog transmission (through bites or wounds) (Duprey et al. 2006). Although minimal compared to the vectorial route, non-vectorial transmission may have an important role in the dissemination and maintenance of disease, especially in non-endemic areas and in the absence of the biological vector (Svobodova et al. 2017).

1.2. Pathophysiology

The evolution course of *Leishmania* spp. infection and the highly variable manifestations of disease result of a complex interaction among the parasite, vector, and host (Solano-Gallego et al. 2009). The host immune response against *L. infantum* includes both innate and adaptive immune responses, the second being the most intensively investigated (Hosein et al. 2017; Santos et al. 2019, 2020; Toepp et al. 2020).

In general, disease outcome is correlated with the cell-mediated immune response (Hosein et al. 2017; Santos et al. 2019, 2020; Toepp et al. 2020). Thus, resistance is associated with a protective T-helper 1 (Th1) immune response, marked by the production of pro-inflammatory cytokines, which stimulate macrophage activation and subsequent killing of the parasites. On the other hand, susceptible dogs generally display an immune response dominated by T-helper 2 (Th2) cells, with an increased production of anti-inflammatory cytokines, high parasite burden and disease exacerbation (Hosein et al. 2017; Santos et al. 2019, 2020). The Th2 response is characterized by an exaggerated humoral activity, causing hypergammaglobulinemia, autoantibody production and formation of immune complexes (Toepp et al. 2020). The circulation and subsequent deposition of immune complexes in various tissues results in inflammatory reactions such as glomerulonephritis, vasculitis, uveitis, myositis, meningitis, and polyarthritis, which are generally correlated with disease progression (Hosein et al. 2017; Parody et al. 2019).

Furthermore, regulatory T cells may play an important role of immune regulation, being recruited to the sites of infection, downregulating the Th1 response, thus enabling parasite growth and disease progression (Toepp et al. 2020). However, according to other studies (Santos et al. 2019), anti-inflammatory and regulatory cytokines apparently do not play an important role in CanL or during treatment. Research findings have found that different tissues affected by *Leishmania* parasites may present Th1, Th2 or mixed Th1/Th2 immune responses (Barbosa et al. 2011; Rodríguez-Cortés et al. 2016; Santos et al. 2019, 2020).

1.3. Clinical signs and laboratory findings

The outcome of infection and clinical presentation of CanL by *L. infantum* is widely variable. The dog may either control the infection and remain clinically healthy (subclinical infection); or develop a large variability and severity of clinical signs and/or laboratory abnormalities (clinical disease) (Solano-Gallego et al. 2009). The incubation period may vary from few months to several years after infection, and manifestations are frequently chronic, non-specific and are often misdiagnosed in early stages of disease (Foglia Manzillo et al. 2013; Koutinas AF and Koutinas CK 2014; Lombardo et al. 2014).

The most common clinical signs and laboratory abnormalities are summarized in Table 1 (Paltrinieri et al. 2016; Maia and Campino 2018; LeishVet 2018).

Renal disease is a frequent finding in CanL (Cortadellas et al. 2006; Koutinas AF and Koutinas CK 2014). Laboratory evidence of chronic kidney disease (CKD) was identified in approximately 50% of dogs with leishmaniosis (Cortadellas et al. 2006), and another study found renal histopathologic signs of nephropathy in all CanL patients (Costa et al. 2003). Renal alterations can remain as subclinical proteinuria, or progress to excretory dysfunction and systemic hypertension, culminating as fatal end-stage renal failure (Koutinas AF and Koutinas CK 2014).

Table 1. Common findings in canine leishmaniosis.

Clinical signs	Laboratory findings
General signs - Weight loss - Generalized lymphadenomegaly - Decreased or increased appetite - Mucous membranes pallor - Lethargy - Polyuria and polydipsia - Hepatosplenomegaly - Fever - Vomiting - Diarrhoea **Cutaneous lesions** - Non-pruritic exfoliative dermatitis (with or without alopecia, localized or disseminated) - Erosive-ulcerative dermatitis - Nodular, papular or pustular dermatitis - Onychogryphosis **Ocular lesions** - Blepharitis and conjunctivitis - Keratoconjunctivitis - Anterior uveitis - Endophtalmitis	**CBC and haemostasis** - Mild to moderate non-regenerative anaemia - Leukocytosis or leukopenia: neutrophilia, neutropenia, lymphopenia - Thrombocytopathy - Thrombocytopenia - Impaired secondary hemostasis and fibrinolysis **Serum protein electrophoresis** - Hyperglobulinemia (beta and/or gammaglobulinemia) **Biochemical parameters** - Hyperproteinemia - Hypoalbuminemia - Decreased albumin/globulin ratio - Increased liver enzyme activities - Increased positive acute phase proteins (*e.g.* C-reactive protein, ferritin, adiponectin, serum amyloid A, haptoglobin, ceruplasmin) - Decreased negative APP (*e.g.* albumin, paraoxanase 1, transferrin)

Other signs - Mucocutaneous and mucosal ulcerative/nodular lesions (oral, genital and nasal) - Epistaxis - Lameness (polyarthritis, osteomyelitis and polymyositis) - Muscle atrophy (atrophic masticatory myositis) - Vascular disorders (systemic vasculitis and arterial thromboembolism) - Neurological disorders	- Renal azotaemia **Urinalysis** - Proteinuria - Decreased urine specific gravity

Adapted from Paltrinieri et al. (2016), Maia and Campino (2018) and LeishVet (2018).

1.4. Diagnosis

1.4.1. Identification of *L. infantum*

Diagnosis of *L. infantum* infection is important for various reasons: to confirm disease, to monitor the response to treatment, and as an epidemiological tool, to screen infection in clinically healthy dogs and thus preventing them from travelling to non-endemic areas, from being vaccinated and from being used as blood donors or breeding dogs (Solano-Gallego et al. 2011, 2017).

The broad spectrum of various and non-specific findings results in a complex diagnostic list, for which integrated and complementary clinical exams are required. This investigation should include information about the clinical history, clinical signs and/or laboratory abnormalities compatible with disease and further confirmation of parasite infection (Solano-Gallego et al. 2011; Paltrinieri et al. 2016; European Scientific Counsel Companion Animal Parasites [ESCCAP] 2019). The laboratory tests performed should include a CBC, renal and hepatic biochemical profiles, serum protein electrophoresis, and urinalysis (Miró and López-Vélez 2018; ESCCAP 2019). Other diagnostic tools such as abdominal US may give further information about the clinical status of the patients and help monitoring clinical progression following treatment (Torrent et al. 2018; Montoya et al. 2020).

In dogs with compatible clinical signs, a cause-effect relationship between the abnormalities and the presence of the parasite should be verified. The tests to identify *Leishmania* parasites include direct techniques, which confirm the presence of the parasite or its components and include parasitological and molecular assays; and/or indirect techniques, which evaluate the host's immune response to the parasite and consist of serological methods (Solano-Gallego et al. 2011; Paltrinieri et al. 2016; ESCCAP 2019).

Serological diagnosis comprises the detection of specific serum anti-*Leishmania* antibodies (IgG), through qualitative or quantitative techniques (Solano-Gallego et al. 2011; Paltrinieri et al. 2016; ESCCAP 2019).

Qualitative techniques generally consist of rapid immunochromatography tests (ICT) (Solano-Gallego et al. 2011; Paltrinieri et al. 2016), which are easy-to-use and provide rapid qualitative in-clinic results, with good specificity (generally over 90%). However, they only provide a qualitative result (presence/absence of specific reactive spots/bands) and present variable sensitivities, generally lower in asymptomatic dogs (Paltrinieri et al. 2016; Travi et al. 2018). A positive result requires further quantitative serology to obtain a titre for follow-up monitoring; and a negative result in a suspected dog should be followed by a quantitative test as well (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

Quantitative serology includes immunofluorescence antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA) and is preferred to qualitative rapid tests. The main advantages rely on their higher sensitivity and specificity, and the possibility of quantifying the antibody levels, which is helpful for diagnosis and monitoring the response to treatment (Solano-Gallego et al. 2011; Proverbio et al. 2014; Ribeiro 2014; Paltrinieri et al. 2016; ESCCAP 2019). High titres of anti-*Leishmania* antibodies confirm CanL in dogs with clinical signs and clinicopathological abnormalities. However, the presence of a low antibody level is not necessarily indicative of disease, as it may reflect exposure to the parasite, but not necessarily active infection, and further work-up is advised through parasitological or molecular methods (Solano-Gallego et al. 2011). High titres are those which are 3-4 times higher than an established cut-off value of the laboratory (Paltrinieri et al. 2016; Solano-Gallego et al. 2017). Other disadvantages include the poor standardization of techniques among laboratories, the fact that serological titres do not always correlate with severity of disease, and the possibility of cross reactions with other agents such as trypanosomes (Solano-Gallego et al. 2011; Paltrinieri et al. 2016). The most recent problematic has arisen along with the advent of vaccination, since some vaccines may elicit the long-standing production of antibodies which are detected by standard serological techniques, hampering the differentiation between infected and vaccinated dogs (Moreno et al. 2014; Oliva et al. 2014; ESCCAP 2019). Nevertheless, recent advances have allowed the commercialization of vaccines which overcome this problem (Solano-Gallego et al. 2017; Cotrina et al. 2018), and that are also apparently safer, leading to less (or absent) adverse effects (Fonseca 2020).

Parasitological diagnosis usually relies on direct observation of amastigotes through cytology, histology, immunohistochemistry, xenodiagnosis and culture from various samples obtained by FNA or biopsy. Parasitology allows confirmation of infection, but these techniques frequently lack sensitivity, especially in asymptomatic dogs. Parasitological assays may be performed not only in typical collectable lesions, but also in tissues that are generally rich in

monocyte-macrophage system cells, namely bone marrow (BM), lymph nodes (LN), spleen and liver (Solano-Gallego et al. 2011; Paltrinieri et al. 2016). If cytological exam is inconclusive, histological/immunohistochemical or PCR techniques may be performed (Solano-Gallego et al. 2009; Paltrinieri et al. 2016).

Molecular techniques consist in the detection of *Leishmania* spp. DNA, which is extracted from various types of samples and tissues. The most widely used technique is PCR. Despite presenting good sensitivity and specificity, its performance depends on the protocols (including different techniques and samples) used. The most sensitive samples (and, therefore, the most recommended) are BM, LN, spleen, skin and conjunctival swabs (Solano-Gallego et al. 2009; Lombardo et al. 2012; Solano-Gallego et al. 2017; LeishVet 2018). Among conventional, nested or real-time PCR, the most recommended technique is real-time PCR, and kinetoplast DNA minicircles are the most sensitive DNA sequences for PCR detection (Paltrinieri et al. 2016; Solano-Gallego et al. 2017). Although PCR results confirm the presence of the parasite, an active infection and disease cannot be evaluated with molecular techniques. Thus, PCR should always be analysed together with clinicopathological and serological data (Solano-Gallego et al. 2011). Other diagnostic methods have been applied mostly in research settings, once they require higher expertise, special equipment, and are time consuming and laborious, thus being unpractical in routine practice. Those include parasite culture, xenodiagnosis and tests to assess *Leishmania*-specific cell-mediated immune response, including the *in-vivo* Montenegro or Leishmanin skin test, which induces a delayed-type hypersensitivity response in dogs, or *ex-vivo* tests such as lymphocyte proliferation assay and measurement of interferon-gamma in circulating lymphocytes (Maia and Campino 2018).

According to the international guidelines, the diagnostic approach of dogs with clinical signs and clinicopathological abnormalities should prioritize quantitative serology and, if possible, concomitant cytological/histological exams of cutaneous lesions, lymph node or other body samples, depending on the clinical presentation. Additionally, PCR is recommended when serology, cytology and histology are not conclusive (Figure 1) (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

For screening clinically healthy dogs, the LeishVet group recommends using quantitative serology, alone or combined with PCR (especially in future blood donors) (Solano-Gallego et al. 2011; LeishVet 2018). However, the single use of PCR should be avoided, as this method confirms infection but not necessarily the development of clinical disease (Solano-Gallego et al. 2011). In endemic areas, a single sign compatible with CanL should require further confirmation of infection (Baneth et al 2008) and any dog living in a non-endemic location that has travelled to an endemic area should perform quantitative serology six months after exposure (LeishVet 2018). Although discussable, considering the seasonal activity of sandflies (between April/May and October/November, approximately), the most recommended

period to perform serological quantitative screening in endemic areas for CanL is between February and April, thus providing enough time after the previous sand fly season and being close to the beginning of the following season (Paltrinieri et al. 2016).

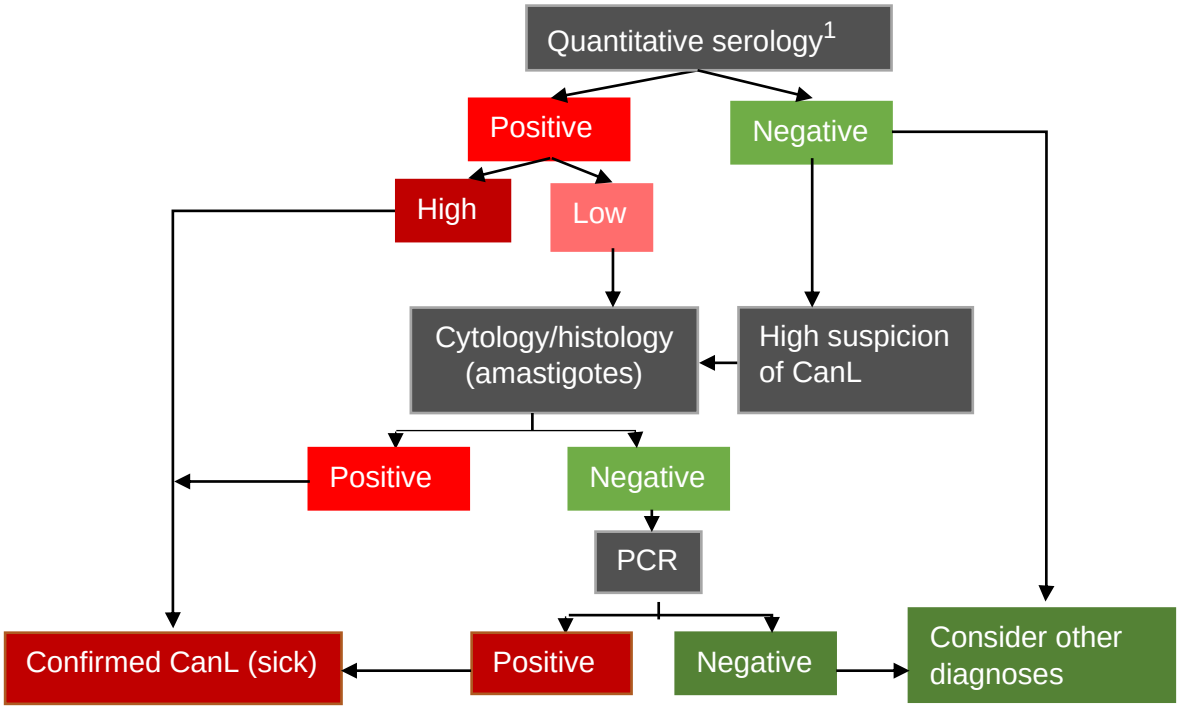


Figure 1. Diagnostic approach recommended for dogs with clinical signs and/or clinicopathological abnormalities compatible with CanL.

[1]Cytology may be conducted simultaneously in any lesional tissue or biological fluid. Adapted from LeishVet (2018).

1.4.2. Assessment of renal function in CanL

Assessment of renal function in dogs with leishmaniosis should include the measurement of serum/plasma creatinine concentration and urinalysis – including urine specific gravity (USG), dipstick, urine sediment examination and urinary protein to creatinine ratio (UPC) – as well as the measurement of systolic blood pressure (SBP). The dog should thereafter be classified in accordance with the International Renal Interest Society (IRIS) staging system (Annexes 2, 3 and 4) (Paltrinieri et al. 2016; IRIS 2019a; Roura et al. 2020). The two most important parameters used to stage renal disease in CanL are proteinuria – through UPC – and creatinine serum concentration (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

Proteinuria is frequently recognised at the time of diagnosis and often present in the absence of azotaemia. Thus, it may be helpful for the establishment of an early diagnosis, as well as for prognosis, disease monitoring and follow-up during treatment (Martínez-Subiela et al. 2002; Bonfanti et al. 2004; Cortadellas et al. 2009). Persistent renal proteinuria has been

strongly correlated with the presence of CKD (Jacob et al. 2005; Lees et al. 2005) and was considered the most important negative prognostic factor in dogs with CanL, by Geisweid et al. (2012).

The Consensus Statement on Assessment and Management of Proteinuria in Dogs and Cats, by the American College of Veterinary Internal Medicine (ACVIM) (Lees et al. 2005) provided variable recommendations regarding the management of proteinuria, including detection, evaluation, monitoring, and treatment.

Assessment of proteinuria includes the investigation of “localization” (prerenal, renal or postrenal), “persistence” (persistent proteinuria generally occurs for two or more weeks) and “magnitude”. The term “persistent renal proteinuria” is considered when proteinuria is detected for two or more weeks, and after excluding an active sediment, a prerenal, postrenal or functional renal causes of proteinuria. Then, UPC between 0.5 and 2.0 may be due to both glomerular or tubular disease, while UPC≥2.0 is more likely correlated to glomerular disease (Lees et al. 2005; Roura et al. 2020).

Renal azotaemia is a less common finding than proteinuria, associated with a lower sensitivity to detect the earliest stages of renal compromise (Zatelli et al. 2003; Paltrinieri et al. 2016; Meléndez-Lazo et al. 2018). Thus, it is generally recognised in later stages of kidney disease, often in combination with systemic hypertension (Solano-Gallego et al. 2009; Paltrinieri et al. 2016). Both proteinuria and azotaemia may improve significantly with appropriate treatment, thus being useful to monitor the response to treatment (Solano-Gallego et al. 2009).

The measurement of serum symmetric dimethylarginine (SDMA) could be a more sensitive marker, given that it is less impacted by loss of lean body mass (IRIS 2019b). However, recent research reported that SDMA did not reveal advantages as an early marker for detection of CKD in dogs with CanL, in comparison with UPC measurement (Torrent et al. 2018; Giapitzoglou et al. 2020).

Taking into account the high prevalence of systemic hypertension in dogs with leishmaniosis – ranging from 29% (Braga et al. 2015) to 62% (Cortadellas et al. 2006) – Roura et al. (2020) recommend the measurement of SBP in all CanL cases, in accordance with the international recommendations (Acierno et al. 2018; IRIS 2019a).

1.5. Clinical staging systems

In order to describe not only the wide variety of clinical manifestations and degrees of severity of CanL, but also to help determining the best treatment protocols, monitoring and prognosis, two clinical staging systems have been proposed: one by the LeishVet (Annexe 5) (Solano-Gallego et al. 2011), and other by the Canine Leishmaniosis Working Group (CLWG) (Annexe 6) (Paltrinieri et al. 2010; Roura et al. 2013, 2020). Both systems use clinical signs,

clinicopathological findings and serological titres for disease staging, including evaluation of renal function according to the IRIS recommendations (IRIS 2019a).

The LeishVet System (Solano-Gallego et al. 2011) classifies the disease into four stages of evolution: mild disease (stage I), moderate disease (stage II), severe disease (stage III) and very severe disease (stage IV). Stage II is further divided in substages A or B: substage A is characterised by a normal renal profile (creatinine<1.4 mg/dL and UPC<0.5); while substage B presents normal creatinine levels (<1.4 mg/dL), but mild proteinuria (UPC=0.5-1). This classification system attributes especial importance to the serological titres for disease staging.

The CLWG System (Paltrinieri et al. 2010; Roura et al. 2013, 2020) considers five stages: exposed dogs (stage A), infected dogs (stage B), sick dogs (stage C), severely sick dogs or those with important concomitant conditions (neoplastic, endocrine or metabolic diseases) (stage D), and dogs that are unresponsive to treatment or presenting early relapse (stage E). Contrarily to the LeishVet perspective, serology is not that much important for staging. Direct diagnostic methods, accompanied by clinical and/or laboratory abnormalities, can be sufficient to stage the disease, regardless of antibody titres. Nevertheless, quantitative serology is highly recommended as well and high serological titres are conclusive of disease. Another difference consists in the inclusion of clinically healthy patients in this staging system, classified as stages A and B (Paltrinieri et al. 2010; Roura et al. 2013, 2020).

Proverbio (2016) identified some fragilities in the LeishVet system, correlated with the classification of cases with discrepancies between the antibody levels and clinical manifestations or clinicopathological abnormalities (*e.g.* high antibody levels, but few/absent clinical signs or vice-versa); or when clinical abnormalities due to immunocomplex deposition were various and/or uncommon (*e.g.* affecting eyes or joints, but not kidneys), thwarting the staging of these cases. The CLWG uses less detailed criteria for staging, thus facilitating the classification of sick dogs, as long as significant clinical or laboratory abnormalities are present, regardless of serology. However, dogs with high serological levels and absent clinical or laboratory abnormalities (or vice-versa) may raise some doubts in staging with this system, as well (Proverbio 2016).

In CanL, severity of renal disease is a major factor influencing staging and prognosis (Solano-Gallego et al. 2011). Both systems consider proteinuria and renal condition for classification. However, while the LeishVet distinguishes different levels of proteinuria and serum creatinine concentration for each stage (or substage) (LeishVet 2018), the CLWG identifies a cut-off value for the normal values only to establish a prognosis, and does not distinguish among different levels above it (Paltrinieri et al. 2010; Roura et al. 2013). The absence or presence of severe renal disease will determine, respectively, either a “favourable

to guarded", or a "guarded to poor" prognosis, which may serve as an objective tool for the practitioner to communicate with the owner (Meléndez-Lazo et al. 2018).

Despite the differences, both systems have shown to be simple, practical and useful methods for classification of CanL in daily practice, showing a good agreement between them (Proverbio 2016).

1.6. Treatment

1.6.1. Subclinical infection

Infected but clinically healthy dogs usually present low positive or negative serological results. These patients do not require immediate antileishmanial treatment, because it could potentially break their immunological equilibrium against infection (Miró and López-Vélez 2018). However, routine monitoring is recommended to early assess seroconversion (for the seronegative and PCR-positive cases) and progression of infection towards disease (Solano-Gallego et al. 2011).

The use of domperidone as a prophylactic or immunotherapeutic drug has been increasingly investigated and recommended in veterinary practice (LeishVet 2018), showing promising results in preventing the development of clinical disease (Gómez-Ochoa et al. 2009; Sabaté et al. 2014; Miró et al. 2017).

1.6.2. Clinical disease

1.6.2.1. Antileishmanial treatment

Contrarily to healthy infected dogs, sick patients should be treated as soon as possible, accordingly with the severity of disease (Oliva et al. 2010; Solano-Gallego et al. 2011).

The most recommended regimens are the combination of allopurinol with pentavalent antimonials such as meglumine antimoniate (MA), or with miltefosine (Oliva et al. 2010; Solano-Gallego et al. 2011; LeishVet 2018; ESCCAP 2019). Both therapies have shown good efficacy and safety, leading to clinical, parasitological and serological improvement within 3 months of treatment (Manna et al. 2015; Santos et al. 2019, 2020). The first long-term follow-up study comparing both combinations of MA or miltefosine with allopurinol demonstrated that treatment with allopurinol and MA allowed faster clinical improvement and lower incidence of clinical relapse than treatment with allopurinol and miltefosine (Manna et al. 2015). Santos et al. (2020) reported a faster recovery with the protocol of allopurinol/MA compared to that with allopurinol/miltefosine. Nevertheless, miltefosine has shown promising results as well, requires an easy, oral administration, and apparently exerts a lower impact on liver and kidney function than MA (Miró et al. 2009; Proverbio et al. 2016).

In spite of the good results obtained with these two protocols, it is not absolutely proven that treatment leads to parasitological cure, and clinical relapses have been reported, following cessation of therapy (João et al. 2006; Ikeda-Garcia et al. 2007b; Manna et al. 2008, 2009, 2015). Given the low parasiticidal efficacy in this species and the risk of inducing parasite resistance, WHO discourages the administration of drugs used in human disease (such as pentavalent antimonials and miltefosine) (WHO 2017). Nevertheless, the several benefits on their use – including the reduction of parasite load and infectivity of treated dogs (Miró et al. 2009, 2011; Manna et al. 2015; dos Santos Nogueira et al. 2019) – have justified that both protocols remain the ones recommended by the veterinary experts as first-line treatments for CanL (Miró et al. 2017; LeishVet 2018; Roura et al. 2020). More information concerning treatment recommendations, including dosage and main side effects of each compound, are summarized in Annexe 7.

Allopurinol monotherapy may be used in mild cases (Koutinas et al. 2001; Miró et al. 2011; LeishVet 2018), but the use of combined therapies has shown higher clinical efficacy and less clinical relapses (Manna et al. 2009; Miró et al. 2011; da Silva et al. 2012).

In addition, a therapeutic approach including the combination of parasiticidal/parasitostatic compounds and immunomodulators may improve the result of treatment, through stimulating dogs' immune response and allowing to reduce the dose of antileishmanial drugs and consequent side effects (Lanaro 2011; Miró et al. 2017). Domperidone is an immune-stimulatory compound which stimulates the cell-mediated immune response and has been increasingly used as prophylactic agent, reducing the risk of developing an active infection in seronegative healthy dogs (Sabaté et al. 2014; Miró et al. 2017; LeishVet 2018). Furthermore, it may be applied as immunotherapeutic agent in patients with mild clinical disease, promoting the reduction of clinical signs and serological titres (Gómez-Ochoa et al. 2009; Lanaro 2011; Miró et al. 2017). Despite its advantages, an evaluation of the risk of certain side effects, such as gastrointestinal upset or potential cardiotoxicity, should be previously pondered, including evaluation of breed, age, cardiac fitness, comorbidities and drug interactions (Travi and Miró 2018). In addition, the impact of its use in dogs with a high parasite load, that may have a limited or exhausted T cell response, should be considered as well (Miró et al. 2017).

More recently, the use of dietary nucleotides and active hexose correlated compounds (AHCC) has been considered safe and capable of reducing disease progression. Moreover, the combination of these compounds with MA revealed similar efficacy to allopurinol-MA therapy and, therefore, may be a good alternative when adverse effects of allopurinol appear (Segarra et al. 2017). However, further studies are required to better understand their mechanism of action and evaluate their clinical importance in CanL treatment (Baxarias et al. 2019). Other less common treatments for CanL have been studied, namely amphotericin B,

pentamidine, aminosidine, metronidazole with spiramycin or with enrofloxacin, marbofloxacin, ketoconazole and oleylphosphocholine (Oliva et al. 2010). Marbofloxacin, for example, has been investigated as an alternative drug when first line treatments are unsuccessful and when there is renal dysfunction (Pineda et al. 2017). However, there is lack of compelling evidence supporting safety and/or efficacy of these compounds, discouraging their use in first-line therapeutic protocols (Poli et al. 1997; Oliva et al. 1998; Vexenat et al. 1998; Hernández et al. 2015; Oliva et al. 2010; Solano-Gallego et al. 2011).

1.6.2.2. Treatment of renal disease associated with CanL

1.6.2.2.1. Nephrotoxicity of antileishmanial drugs

Bearing in mind the relevance of renal disease in dogs with CanL, it is important to find an effective drug with reduced nephrotoxicity. The use of antileishmanial compounds that are potentially nephrotoxic (*e.g.* amphotericin B) should be avoided or used with caution, accompanied by close monitoring of the patient (IRIS-CGDSG et al. 2013b).

Allopurinol is well-tolerated and seems to reduce proteinuria and progression of kidney disease in these patients (Plevraki et al. 2006; Pierantozzi et al. 2013). However, the development of *L. infantum* resistance to this drug, associated with clinical relapse (Yasur-Landau et al. 2016) and occurrence of xanthinuria and urolithiasis (Torres et al. 2016) have been reported. When these adverse urinary effects occur, it is recommended to reduce the dose of allopurinol (Manna et al. 2015) or change for nucleotide analogues (Segarra et al. 2017), increase water consumption and start a low-purine diet (Osborne et al. 2010; Roura et al. 2020).

Meglumine antimoniate is mainly eliminated by the kidneys (Tassi et al. 1994; Belloli et al. 1995). The use of this drug in dogs with CanL and concomitant renal impairment has originated some controversy concerning its safety and effectiveness.

On one hand, renal failure may increase the half-life of antimonials (as shown in hamsters). Thus, dogs with CanL and reduced GFR may have an increasing risk of toxicity by this drug (Zaghloul and Al-Jasser 2004). Nephrotoxicity due to MA has been reported in human patients with leishmaniosis as well (Laguna et al. 1999; Rodrigues et. 1999). Furthermore, a study conducted on eight dogs reported that this compound failed to treat dogs with severe renal dysfunction and was correlated with transient hepatic biochemical alterations (Ikeda-Garcia et al. 2007a). Additionally, a comparative study conducted in eight healthy dogs, treated either with MA or miltefosine, reported histological kidney abnormalities (severe tubular damage) in all dogs treated with MA, but not with miltefosine, although there were no clinical signs of kidney disease in either group (Bianciardi et al. 2009).

On the other hand, other studies have reported that the treatment of MA combined with allopurinol had no negative impacts on kidney function and led to significant reductions in UPC

(Pierantozzi et al. 2013; Pardo-Marín et al. 2017; Paltrinieri et al. 2018; Daza González et al. 2019). Thus, an increase of azotaemia or proteinuria in some dogs treated with MA is more likely caused by the formation and deposition of immunocomplexes in the kidney than to nephrotoxicity of that drug (Koutinas AF and Koutinas CK 2014; Kasabalis et al. 2019).

Miltefosine has been generally accepted as a safe drug concerning hepatic or renal side effects and a good alternative to MA (Bianciardi et al. 2009; Mateo et al. 2009; Miró et al. 2009). The combination of miltefosine with allopurinol has led to a significant decrease in proteinuria after treatment, suggesting the use of this protocol particularly in dogs with CanL accompanied by impaired renal function (Proverbio et al. 2016).

Marbofloxacin, a third-generation fluoroquinolone, has shown *in vitro* leishmanicidal activity (Vouldoukis et al. 2006) and good tolerance in both non-azotemic (Rougier et al. 2008, 2012) and CKD (Pineda et al. 2017) dogs. However, clinical efficacies of only 70% (Rougier et al. 2012; Pineda et al. 2017) and clinical relapses in over 50% of treated dogs (Rougier et al. 2012) have discouraged its use. Furthermore, an *in vitro* study revealed that miltefosine was significantly more efficacious than marbofloxacin, either alone or combined with allopurinol, and that combined therapies with allopurinol were significantly more effective than monotherapies with either drug (Farca et al. 2012).

The combined therapies of allopurinol with MA or miltefosine are, therefore, the ones recommended to treat dogs with CanL and kidney disease (Roura et al. 2020). Given that both of these treatments promoted the reduction of proteinuria after four to eight weeks of therapy (Pierantozzi et al. 2013; Proverbio et al. 2016), Paltrinieri et al. (2016) suggested to add antiproteinuric treatment only after that period of antileishmanial treatment. Roura et al. (2020) recommended that when UPC>3.0, antiproteinuric therapy may be started from the beginning with the antileishmanial protocol. However, when UPC≤3.0, only an antileishmanial protocol should be started, followed by a re-evaluation of its efficacy and measurement of renal parameters (creatinine and UPC), four weeks after starting the treatment. If proteinuria persists (UPC>0.5), an antiproteinuric protocol (IRIS 2019b) should be added to allopurinol, followed by a re-evaluation and restaging of renal disease, four weeks later (Paltrinieri et al. 2016; Roura et al. 2020).

1.6.2.2.2. Antiproteinuric treatment

Any dog diagnosed with kidney disease should be classified and treated in accordance with the IRIS recommendations (IRIS 2019a, 2019b). The recommended treatment of proteinuria has shown strong agreement among the several published guidelines, which include not only those specifically focused on proteinuria (Lees et al. 2005), but also those approaching glomerular disease (IRIS-CGDSG et al. 2013a, 2013b, 2013c, 2013d), CKD

(IRIS 2019b), and the most recently published by Roura et al. (2020), detailing the management of renal disease secondary to CanL.

In fact, all of the above prioritize the inhibition of the renin-angiotensin-aldosterone system as the main approach to treat proteinuria. The most recent guidelines (Roura et al. 2020) recommend that if proteinuria is not controlled after four weeks of leishmanicidal/leishmaniostatic treatment, a low-phosphorus renal therapeutic diet should be added to allopurinol, combined or not with an ACEI (*e.g.* benazepril or enalapril). If proteinuria remains uncontrolled after four weeks of this treatment, the dose should be increased (maximum 2mg/kg orally once daily). Angiotensin receptor blockers (ARB) (*e.g.* losartan or telmisartan) or polyunsaturated (especially omega-3) fatty acids can be added as well (IRIS 2019; Roura et al. 2020). Finally, dogs with severe hypoalbuminaemia (albumin<2.0 mg/dL) should be given antithrombotic therapy, such as low-dose acetylsalicylic acid or clopidogrel. This protocol should be performed individually and may vary accordingly with the clinical evolution of the patient. Severely sick patients, for example, may require treatment with several antiproteinuric drugs at once (IRIS 2019; Roura et al. 2020).

Aldosterone-receptor blockers (*e.g.* spironolactone) are only recommended in dogs with glomerular disease that have increased serum aldosterone concentrations and have failed or not tolerated ACEIs and/or ARBs. The monitoring of response to treatment and progression of disease are based on serum creatinine concentration and UPC. When there is a stabilization of creatinine and a decreasing in UPC, the response to treatment is considered as good. The main goal of treatment is a reduction in the UPC to <0.5, or a reduction in UPC of 50% or more (Lees et al. 2005; IRIS-CGDSG et al. 2013a; IRIS 2019b).

1.6.2.2.3. Antihypertensive treatment

Patients presenting persistent SBP exceeding 160 mmHg or diastolic blood pressure higher than 100 mmHg should start antihypertensive treatment (IRIS-CGDSG et al. 2013a; IRIS 2019b). The main recommendations for treating hypertension due to glomerular disease include the administration of ACEI as first-line treatment (IRIS-CGDSG et al. 2013a; Acierno et al. 2018; IRIS 2019b). In case of ineffective treatment or if hypertension is severe, the dosage should be increased (doubled). If hypertension persists, it is recommended to combine ACEI with other antihypertensive drugs, such as calcium channel blockers (CCB) or ARB (IRIS-CGDSG et al. 2013a, 2013b; Acierno et al. 2018; IRIS 2019b; Roura et al. 2020).

The antihypertensive treatment aims to minimize the risk of extra-renal target organ damage (a frequent consequence of glomerular injury and systemic hypertension) by reducing SBP to <140 mmHg, or, at least, to decrease SBP to <160 mmHg, as well as reducing proteinuria (UPC<0.5 or reduced by 50%) (Acierno et al. 2018).

1.6.2.2.4. General considerations on the use of ACEI and ARB

The use of ACEI and ARB is correlated to some side effects, such as hyperkalaemia, hypotension and reduction of GFR, with consequent increases in serum creatinine concentration (Roura et al. 2020). Treatment with these drugs is contraindicated in dehydrated or hypovolemic animals, to avoid a precipitous decrease in the GFR of these patients. In those cases, previous rehydration, and an individual benefit/risk analysis of combining ACEI and ARB should be held, followed by a close monitoring of the patient (IRIS 2019b).

1.6.2.2.5. Immunosuppressive/anti-inflammatory treatment

In case of severe, persistent, or progressive renal disease, a renal biopsy should be considered to confirm the existence of an active immune-mediated origin (IRIS-CGDSG et al. 2013b; 2013d). In case of confirmation and absent contraindications for immunosuppressive/anti-inflammatory treatment (*e.g.* presence of systemic or urinary tract infections, among other drug-specific contraindications), this treatment can be added to the antileishmanial protocol. In the absence of a renal biopsy, empirical immunosuppressive treatment may be started as well, especially when there is persistent/progressive azotaemia (creatinine>3.0mg/dL) or severe hypoalbuminemia (*i.e,* <2.0g/dL) despite standard therapy (IRIS-CGDSG et al. 2013b, 2013c, 2013d).

There is an important lack of evidence supporting the use of specific immunosuppressive drugs in dogs with immune-mediated glomerular disease (IRIS-CGDSG et al. 2013d). An even higher therapeutic dilemma arises when there is an underlying infectious cause, as immunosuppressive treatment may compromise its control (IRIS-CGDSG et al. 2013d). However, the use of immunosuppressants has been increasingly accepted, since some dogs may die from consequences of the glomerular disease rather than nonrenal causes (IRIS-CGDSG et al. 2013b). Still, their administration should be individually discussed with the owners, assessing the benefits and risks involved, and followed by close monitoring of the patient (IRIS -CGDSG et al. 2013d; Roura et al. 2020).

The immunosuppressive drugs should be adequate to disease severity and progression. For peracute or rapidly progressive conditions, the use of mycophenolate mofetil is recommended as first-choice, alone or combined with prednisolone (IRIS-CGDSG et al. 2013d). To avoid the adverse effects of glucocorticoids, these are not recommended as a sole treatment and when applied with mycophenolate mofetil, should be tapered to the minimally effective dose as quickly as possible. For stable or slowly progressive diseases, the Group recommends mycophenolate mofetil, or chlorambucil – this last one may be administered alone or in combination with azathioprine, on alternating days – or cyclosporine. Therapeutic effectiveness should be assessed serially by monitoring proteinuria, renal function and serum albumin concentration. Treatment should be conducted for at least eight weeks for rapidly

acting nonsteroidal drugs, and for eight to twelve weeks for slowly acting drugs, before altering or stopping an immunosuppressive trial and in the absence of side effects (IRIS-CGDSG et al. 2013d).

Based on clinical experience, Roura et al. (2020) supported the use of prednisone/prednisolone at anti-inflammatory dosage (0.7 mg/kg orally once a day over 3-10 days) to reduce immune-mediated renal inflammation rather than decreasing the formation and circulation of immune complexes.

1.7. Prognosis

Most dogs with CanL show a good response to antileishmanial drugs and do not need a lifelong treatment (Solano-Gallego et al. 2011; Roura et al. 2013).

The prognosis mainly depends on the severity of the clinical signs and clinicopathological abnormalities presented at the time of diagnosis – especially those associated with renal dysfunction – and the individual response to treatment (Solano-Gallego et al. 2011). Most treated dogs show clinical improvement within the first month of treatment, but some may require longer protocols (Miró and López-Vélez 2018).

Survival time may range from less than one year to more than five (Bourdeau et al. 2014), with a mean of approximately 4 years (Geisweid et al. 2012). Survival time has been increasing due to significant developments in the management of leishmaniosis and its associated renal disease (Geisweid et al. 2012; Bourdeau et al. 2014). Indeed, kidney disease has been highly correlated with disease progression and worse prognosis in sick dogs (Miró et al. 2009; Solano-Gallego et al. 2011; Geisweid et al. 2012; Roura et al. 2013; Pereira et al. 2020). In fact, chronic renal disease is considered the main cause of mortality due to CanL (Costa et al. 2003; Zatelli et al. 2003). Furthermore, proteinuria was named the most important parameter associated with worse prognosis, followed by hypoalbuminemia and lymphopenia (Geisweid et al. 2012), as well as signs associated with immunocomplex deposition (Ikeda-Garcia et al. 2007a; Torres et al. 2011; Bourdeau et al. 2014). According to Pereira et al. (2020), survival time was correlated with the severity of anaemia, presence of concomitant infectious diseases at the time of diagnosis and the anti-*Leishmania* treatment applied. In line with previous research results, the severity of renal dysfunction, staged according with the IRIS guidelines (IRIS 2019a), highly influenced the survival time of the patients, which was of over 4 years for patients with CKD in stage 1, approximately of 2.5 years in CKD stage 2, and only of one month in dogs classified as IRIS 3-4. The IRIS staging worsened in one fourth of the dogs and CKD was the main cause of death or euthanasia in over one third (Pereira et al. 2020). The decision of euthanasia depends on the severity of the disease, with studies in France and Portugal indicating that dogs with leishmaniosis have a 10-25% probability of being euthanised, regardless of having received treatment or not (Mattin et al. 2013). Early diagnosis,

adequate treatment and monitoring are highly correlated with a more favourable prognosis (Solano-Gallego et al. 2011; Roura et al. 2020), being highly important to perform the close monitoring of infected clinically healthy animals to evaluate seroconversion and disease development (Baneth et al. 2008; Solano-Gallego et al. 2011).

1.8. Prevention and control

Prevention and control of infection are extremely important to avoid spreading of disease and improve clinical response (Miró et al. 2017). Recommendations include vector control, early diagnosis, and treatment of diseased dogs. Vaccines have been another promising preventive strategy. An optimal approach should integrate two goals: 1) to reduce the risk of infection and 2) to control infection and prevent development of disease in infected animals (Miró et al. 2017).

Concerning the prevention of infection, the most effective measure is the application of topical repellent insecticides, especially in dogs living in, or travelling to endemic areas (Miró et al. 2017). Repellents with proven efficacy consist in permethrin/imidacloprid spot-ons or sprays (Otranto et al. 2007) and deltamethrin (Maroli et al. 2001) or imidacloprid/flumethrin (Otranto et al. 2013) collars (Wylie et al. 2014; Miró et al. 2017). Other strategies to reduce the risk of infection include keeping the dogs indoors from dusk to dawn throughout the high risk season (higher sand fly activity) – generally from April/May to October/November, in southern Europe –, using mesh with 0.3 to 0.4mm^2, reducing microhabitats favourable to the vector or performing indoor insecticide treatment (Afonso and Alves-Pires 2008; Miró et al. 2017).

For prevention of disease development upon infection, three main areas have been explored: chemotherapy, immunotherapy and immunoprophylaxis (Miró et al. 2017).

Chemotherapy includes the antileishmanial drugs previously mentioned, which aim to reduce the parasite load and infectiousness of treated dogs, frequently achieving clinical cure (Miró et al. 2011; Miró et al. 2017).

Immunotherapy has been considered as adjunctive to conventional treatment in mild disease, as well as preventive measure. Domperidone has shown good results in the prevention of clinical disease (Gomez-Ochoa et al. 2009, 2012; Sabaté et al. 2014), being recommended as part of the preventive strategy in healthy dogs, along with insecticides and vaccination (Miró et al. 2017; LeishVet 2018). However, further compelling research would help clarifying its efficacy and safety, as previously mentioned (Miró et al. 2017; Travi et al. 2018).

Immunoprophylaxis aims to improve the host's immune response against the parasite upon infection, through vaccination. Various vaccines against *L. infantum* have been developed: inactivated, purified antigens, recombinant antigens, or DNA vaccines. Those based on recombinant antigens or excretion/secretion antigen products have shown the

highest efficacy (Moreno et al. 2014; Oliva et al. 2014). Currently, there are three licensed anti-*Leishmania* vaccines: one in Brazil, composed by a recombinant single-protein antigen (Leish-Tec®); and two in Europe, consisting of excreted/secreted antigens (Canileish®) and a recombinant polypeptide antigen (Letifend®) (Miró and al. 2017). However, besides not avoiding infection, their efficacy in preventing clinical disease is only of approximately 70% (Oliva et al. 2014; Fernández Cotrina et al. 2018). Therefore, vaccination should be combined with insecticides/repellents to maximize the preventive effect (Wylie et al. 2014; Miró et al. 2017).

The use of vaccination as immunotherapy has been more recently investigated, showing some conflicting results, and further research must be conducted to investigate the therapeutic effect of vaccines in diseased dogs (Solano-Gallego et al. 2017).

In the context of One Health, dog culling policies have been applied in some countries, but a recent consensus statement has considered this strategy as unethical and unjustifiable from a scientific perspective, advocating that preventive methods should be prioritized to that practice (Dantas-Torres et al. 2019).

Also on behalf of the One Health concept, the WHO reports on the control of the Leishmaniasis in Europe (WHO 2010, 2017) have suggested some strategies concerning the canine species. Those reports recognised culling as a controverse, unethical and likely ineffective measure and supported the performance of prevalence assessments through mass screening by quantitative serology (IFI, ELISA), accompanied by physical examination. Furthermore, WHO recommended the use topical insecticides and an early treatment of infected dogs following the veterinary guidelines (WHO 2017). Finally, in some countries in Europe such as Portugal, Greece and Italy, the veterinary practitioners are obliged to communicate all new cases of leishmaniosis to the appropriate authorities (Despacho nº 6453/2019; ESCCAP 2019).

Section 3 – Clinical management of Canine Leishmaniosis in Portugal: the veterinary community perspective

1. Introduction

Canine leishmaniosis is endemic in more than 70 countries (Solano-Gallego et al. 2011), including Portugal (Maia et al. 2015a; ESCCAP 2019). Research on CanL has allowed developments in the management of this disease, including the publication of international guidelines for diagnosis, staging, treatment, follow-up and prognosis establishment, namely by the CLWG (Oliva et al. 2010; Roura et al 2013; Paltrinieri et al. 2016) and LeishVet (Solano-Gallego et al. 2009, 2011; LeishVet 2018) working groups. The main goals of these recommendations were to provide practical, easy-to-use tools for routine clinical practice and to help uniformizing the clinical and epidemiological approach for controlling the dissemination of this zoonosis. Despite some differences, there is a good correlation and both guidelines are considered adequate (Proverbio 2016).

Although these guidelines have been available for approximately 10 years, some research has shown discrepancies on the management and control of CanL (Bourdeau et al. 2014; le Rutte et al. 2018). A large study conducted over 2004-2011 in 2099 veterinary clinics of 6 southern European countries (including Portugal) revealed a wide variation in the clinical management among countries, namely in terms of dosing regimens, therapeutic drugs and diagnostic approach (Bourdeau et al. 2014). Another survey conducted in 2016 indicated that around 70% of the Spanish and French veterinarians were not familiar with any guidelines for controlling zoonotic visceral leishmaniosis (ZVL), although around 60% were aware of the European spread of CanL and ZVL and were implementing measures similar to those recommended (Le Rutte et al. 2018).

Questionnaire-based surveys have been increasingly used as a research tool to assess the current status and clinical management of CanL, since they allow to survey large geographical areas in an accurate, rapid and cost-reduced manner. Research has been frequently held in Spain (Ruiz de Ybáñez et al. 2009; Gálvez et al. 2011; Alcover et al. 2013; Ballart et al. 2013; Bourdeau et al. 2014; Mattin et al. 2014; Lladró et al. 2017; le Rutte et al. 2018; Montoya et al. 2020). However, scarce information exists regarding this topic in Portugal (Oliveira et al. 2010; Bourdeau et al. 2014; Mattin et al. 2014), being unknown whether Portuguese veterinary surgeons follow the international recommendations. Although both CLWG and LeishVet guidelines are generally accepted, the ones from the LeishVet group have been the most widely used. Furthermore, despite the existence of several published recommendations for the management of renal disease (Lees et al. 2005; IRIS-CGDSG et al.

2013a, 2013b, 2013c, 2013d; IRIS 2019a, 2019b; Roura et al. 2020), little is known about the current medical approach to renal disease in dogs with CanL.

2. Objectives

In this study, an online questionnaire-based survey, mainly consisting of closed-type questions, was conducted among Portuguese veterinarians, via social media, with the aims of:

1) assessing the diagnostic approach, current clinical prevention and treatment of CanL in Portugal, ascertaining whether guidelines are followed in clinical practice and updating the existing scarce information on this specific field;

2) detailing the specific therapeutic approach of renal disease, highly prevalent and considered the main cause of death in dogs with CanL.

3. Materials and methods

3.1. Survey development and distribution

An online questionnaire was developed in Google Forms ® (Annexe 8), including 24 to 64 questions, depending on the answering pathway of each respondent. The content was divided into three parts.

The first part surveyed the general features of the respondent veterinary clinician, including job location, working environment, academic degree, period of clinical experience with dogs and number of CanL confirmed cases in the previous 12 months.

The second part assessed the medical approach (regarding diagnosis, treatment and prevention) to six clinical cases. Five of them were strategically elaborated in order to reflect theoretical scenarios of either infected but clinically healthy patients (*i.e.*, subclinical infection) or infected dogs with clinical signs compatible with the stages II (including substages a and b), III and IV of the LeishVet clinical classification system. Part of the cases showed clinical and laboratory findings that were supportive of a glomerulonephritis. A sixth clinical case was focused on the preventive approach of a healthy dog moving to an endemic zone. All scenarios described dogs which were living in, moving to or coming from an endemic area and none of them was taking any preventive measures for CanL such as vaccination, insecticides/repellents or immunomodulators. The descriptions aimed to highly suggest a possible infection by *L. infantum*, with or without clinical signs, and thus guide the clinician towards the management of CanL. All clinical cases had information regarding the serological titres for anti-*Leishmania* antibodies, in variable concentrations. The need to perform further diagnostic techniques (especially, using parasitological and molecular methods) was surveyed, as well as which were the preferred tools used. Furthermore, it was mentioned in the questionnaire that other vector-borne disease (VBD) pathogens such as *Anaplasma, Babesia, Erlichia, Borrelia, Rickettsia, Hepatozoon, Hemobartonella, Bartonella, Dirofilaria* and

Trypanosoma, had been discarded. The order of presentation of the clinical cases was randomized, first being presented the stage III, followed by subclinical infection, stages IIa, IIb, IV and, finally, the clinical case describing a healthy dog moving to an endemic area. Table 2 summarizes the main characteristics of each case.

In the third part, “other questions” approached the awareness on the existing guidelines, with emphasis on the ones provided by the LeishVet group (Solano-Gallego et al. 2011; LeishVet 2018). The diagnostic tools and samples most frequently used in daily practice and the prompt to use immunosuppressants in a case of a suspected glomerulonephritis secondary to CanL were surveyed as well, in this part.

The questionnaire was primarily distributed exclusively through the mailing list of Hospital Escolar Veterinário from FMV-ULisboa, from 7 November to 10 December 2019, for internal validation. In a second phase, from 11 December 2019 to 8 January 2020, it was also diffused via Portuguese social network veterinary groups.

Table 2. Summary of the information given in the questionnaire about each clinical case

	Signal-ment	**Living in, moving to or coming from an endemic area?**	**Iatrotro-pic stimulus**	**Physical examination**	**CBC, biochemi-cal profile, urinalysis**	**Serum protein electropho-resis**	**Crea-tinine** (mg/dL)	**Urinalys is (UPC, sedi-ment, USG)**	**Sero-logi-cal titre**	**Other exams**
Subcli-nical infecti on	Male, 5 years old	Living	Vaccina-tion	Normal	Normal	-	-	-	1:80	-
Stage IIa	Female spayed, 8 years old	Coming (travelled 6 months before)	Lethargy, weight loss	Periorbital alopecia, footpad exfoliative dermatitis, generalised lymphadeno-megaly	Mild non-regenerative anaemia Hyperproteinemia Hypoalbuminemia	Hyperglobuli-nemia with polyclonal gammopathy.	Normal	Normal	1:160	-
Stage IIb	Male, 6 years old	Living	Epistaxis	Epistaxis	Mild non-regenerative anaemia	Hyperglobuli-naemia without hypoalbumi-nemia	<1.4	UPC=0.5 Inactive sediment	1:640	-

Stage III	Male, 7 years old	Living	Lethargy, anorexia, weight loss, PU/PD, auricular lesions	Pale mucosae, generalised lymphadenomegaly, mucocutaneous ulcerative lesions, ears' crusts	Moderate non-regenerative anaemia Hyperproteinemia Hypoalbuminemia	Hyperglobulinemia with polyclonal gammopathy	1.9	USG=1018; UPC=1.2 inactive sediment	1:320	Ocular: blepharitis, uveitis; US: splenomegaly; SBP: normal
Stage IV	Male, 12 years old	Living	Lethargy, anorexia, weight loss, skin wounds, PU/PD	Pale mucosae, facial and plantar exfoliative dermatitis, onychogryphosis, nasal hyperkeratosis and ulceration	Moderate non-regenerative anaemia Hyperproteinemia Hypoalbuminemia	Hyperglobulinemia with polyclonal gammopathy	3.5	UPC=6.2 inactive sediment	1:640	Corneal opacification
Clinically healthy	Male, 3 years old	Moving to	Screening for CanL, prophylaxis	Normal	-	-	-	-	-	-

3.2. Data processing and statistical analysis

All data were collected using Google Forms and downloaded in a database (Microsoft Excel 2016) for statistical analysis. Descriptive statistics were performed. Since no major abnormalities were detected during internal validation, the questionnaire content used in both first and second phases was the same. Having this into account, answers from HEV-FMV/ULisboa were included in the global statistical analysis.

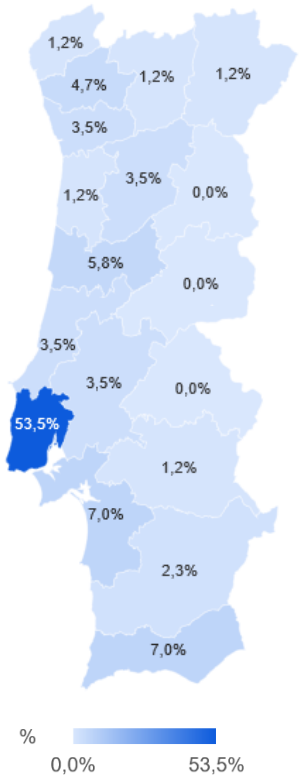
4. Results

The questionnaire was completed by 86 veterinary surgeons.

4.1. General characteristics

The questionnaire was replied by veterinarians working in 15 of the 18 official Portuguese continental districts. Among the 86 respondents, 46 were working in Lisbon (53.5%), six in Setubal (7.0%), six in Faro (7.0%), five in Coimbra (5.8%) and 23 (26.7%) were distributed among 11 other districts, one to four people per district. Replies were not obtained from veterinary practitioners from the districts of Castelo Branco, Guarda, Portalegre and autonomous regions of Azores and Madeira (Graphic 1).

Graphic 1. Geographical distribution of the surveyed clinicians.



Further results on characterization of the respondents, including working environment, academic degree, period of clinical practice with dogs and number of confirmed cases of CanL in the previous 12 months, are summarized in Table 3.

Regarding the type of working environment, 48 clinicians (55.8%) worked in urban areas, 28 (32.6%) in mixed urban/rural environments and 10 (11.6%) worked in rural settings. Concerning the academic degree, all 86 respondents (100%) had a Doctor in Veterinary Medicine (DVM) or a DVM and a Master of Science (MSc) degree and none had a Doctor of Philosophy (PhD) degree in Veterinary Sciences. Moreover, nine clinicians (10.5%) had less than 2 years of practice with dogs, 18 (20.9%) had 2 to 5 years, 27 (31.4%) had 6 to 10, 12 (14.0%) had 11 to 15, 13 (15.1%) had 16 to 20, and seven (8.1%) had more than 20 years of clinical experience with that species (Table 3). Finally, 38 respondents(44.2%) had confirmed less than 5 cases of CanL in the previous 12 months, 25 (29.1%) mentioned 5 to 10 cases, 16 (18.6%) had 11 to 15 cases, five (5.8%) had 16 to 20 and two (2.3%) had confirmed more than 20 cases of CanL during that period (one working in Faro and other in Viseu) (Table 3).

Table 3. General characteristics of the respondents.

	n (%)
Working environment	
Rural	10 (11.6%)
Urban	48 (55.8%)
Mixed rural/urban	28 (32.6%)
Academic degree	
DVM or DVM/MSc degree	86 (100.0%)
PhD degree in Veterinary Sciences	0 (0.0%)
Period of clinical practice with dogs (years)	
<2	9 (10.5%)
2-5	18 (20.9%)
6-10	27 (31.4%)
11-15	12 (14.0%)
16-20	13 (15.1%)
>20	7 (8.1%)
Number of confirmed cases of CanL in the past 12 months	
<5	38 (44.2%)
5-10	25 (29.1%)
11-15	16 (18.6%)
16-20	5 (5.8%)
>20	2 (2.3%)

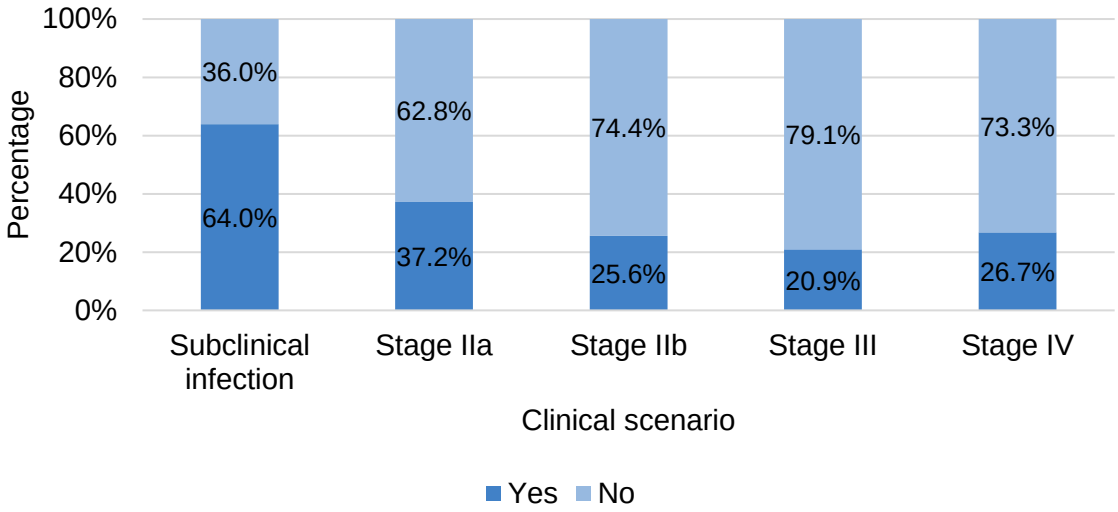
4.2. Diagnostic approach

4.2.1. Additional complementary exams

Having into account the clinical signs, clinicopathological abnormalities and serological titres mentioned in the utterance, the willingness for using further diagnostic methods was surveyed.

From the 86 inquired veterinary surgeons, 55 (64.0%) would require them when faced with a scenario of subclinical infection, 32 (37.2%) in stage IIa of disease, 22 (25.6%) in stage IIb, 18 (20.9%) in stage III and 23 (26.7%) in stage IV (Graphic 2).

Graphic 2. Considerations on performing (or not) more complementary diagnostic tests considering different scenarios of CanL.



4.2.2. First-choice diagnostic tools

The multiple-choice items available in the questions were: “LN cytology”, “BM cytology”, “PCR on BM or LN”, “PCR on blood” and “Other” techniques, which could be detailed thereafter. Among the clinicians considering necessary to apply further diagnostic tests for confirmation or exclusion of CanL in the theoretical clinical scenarios, a summary of the chosen diagnostic tools is available in Table 4. Given that the respondents selecting “Other techniques” could describe them thereafter, and that most of them mentioned more than one, the sum of the percentages regarding those alternative techniques will be higher than 100%.

Table 4. Diagnostic tools used, considering dog suspected of CanL in different stages.

	Subclinical infection (n=55)	Stage IIa (n=32)	Stage IIb (n=22)	Stage III (n=18)	Stage IV (n=23)
Technique	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
LN Cytology	2 (3.6%)	14 (43.8%)	1 (4.5%)	11 (61.1%)	3 (13.0%)
BM Cytology	3 (5.5%)	2 (6.3%)	2 (9.1%)	2 (11.1%)	2 (8.7%)
PCR on BM/LN	26 (47.3%)	9 (28.1%)	5 (22.7%)	2 (11.1%)	2 (8.7%)
PCR on blood	12 (21.8%)	2 (6.3%)	2 (9.1%)	0 (0.0%)	1 (4.3%)
"Other" diagnostic tests (one or more options)	12 (21.8%)[1]	5 (15.6%)[1]	12 (54.5%)[1]	3 (16.7%)[1]	15 (65.2%)[1]
Serum protein electrophoresis	8 (14.5%)		2 (9.1%)[2]	1 (5.6%)[2]	
Repeat serology in 2-3 months	3 (5.5%)				
LN cytology (combined with other exams)	1 (1.8%)				1 (4.3%)
PCR on both blood and BM/LN	1 (1.8%)				
Exclusion of other skin parasitic infections		2 (6.3%)			
Exclusion of dirofilariosis			1 (4.6%)[2]		
Measurement of SBP		1 (3.1%)	1 (4.6%)		2 (8.7%)
Exploring the nasal cavity (rhinoscopy)			2 (9.1%)		
Coagulation tests			2 (9.1%)		
Thoracic radiography			1 (4.6%)	1 (5.6%)	2 (8.7%)
Abdominal US		1 (3.1%)	3 (13.6%)	1 (5.6%)[2]	14(60.9%)
Urinalysis				1 (5.6%)[2]	
Urine culture				1 (5.6%)	2 (8.7%)
CBC and/or biochemical parameters		2 (6.3%)[2]	1 (4.6%)[2]		1 (4.3%)[2]

([1]) Some respondents choosing "Other" techniques detailed more than one diagnostic tool. Therefore, the sum of those alternative techniques is often higher than the total; ([2]) Tests which were detailed in the description of that clinical case, in the questionnaire.

4.2.2.1. Subclinical infection

Among the 55 veterinarians admitting using further diagnostic methods, 26 (47.3%) opted for PCR on BM or LN, 12 (21.8%) for PCR on blood, three (5.5%) for BM cytology and two (3.6%) for LN cytology. Moreover, 12 respondents (21.8%) mentioned “Other” diagnostic techniques, such as serum protein electrophoresis (8; 14.5%), repeating serology in 2-3 months (3; 5.5%), or other cytological/molecular tests (2; 3.6%) (Table 4).

4.2.2.2. Stage IIa

In this scenario, from the 32 clinicians proceeding with more diagnostic tools, 14 (43.8%) chose LN cytology, nine (28.1%) would prefer PCR on BM or LN, two (6.3%) picked BM cytology and two (6.3%) preferred PCR on blood.

Moreover, five respondents (15.6%) would accomplish “Other” exams, including CBC and/or biochemical parameters (2; 6.3%), exclusion of other skin parasitic infections (skin scraping, periocular trichogram or external deworming with fluralaner) (2; 6.3%), abdominal US (1; 3.1%) and measurement of SBP (1; 3.1%) (Table 4).

4.2.2.3. Stage IIb

From the 22 clinicians asking for more complementary exams, five (22.7%) selected PCR on BM or LN, two (9.1%) opted for BM cytology, two (9.1%) invoked PCR on blood and one (1; 4.5%) would prefer LN cytology. In addition, 12 practitioners (54.6%) nominated several other approaches, such as: abdominal US (3; 13.6%), serum protein electrophoresis (2; 9.1%), exploring the nasal cavity (2; 9.1%), coagulation tests (2; 9.1%), exclusion of dirofilariosis (1; 4.6%), SBP measurement (1; 4.6%), thoracic radiographs (1; 4.6%), and biochemical parameters (1; 4.6%) (Table 4).

4.2.2.4. Stage III

Among the 18 veterinarians requesting additional diagnostic tools, 11 (61.1%) voted on LN cytology, two (11.1%) selected BM cytology and two (11.1%) would prefer PCR on BM or LN. Besides, three (16.7%) clinicians invoked “Other” methods: serum protein electrophoresis (1; 5.6%), thoracic radiography (1; 5.6%), abdominal US (1; 5.6%), urinalysis (1; 5.6%) and urine culture (1; 5.6%) (Table 4).

4.2.2.5. Stage IV

The 23 practitioners which considered doing further diagnostic tests for confirmation of CanL included: three veterinarians favouring LN cytology (13.0%), two (8.7%) choosing BM cytology (8.7%), other two (8.7%) electing PCR on BM or LN and one (4.3%) selecting PCR

on blood. Finally, 15 (65.2%) respondents designated alternative diagnostic methods, especially abdominal US (14; 60.9%). Measurement of SBP (2; 8.7%), thoracic radiograpy (2; 8.7%), urine culture (2; 8.7%), CBC and biochemical parameters (1; 4.3%) or LN cytology (combined with other exams) (1; 4.3%) were mentioned as well (Table 4).

4.3. Treatment

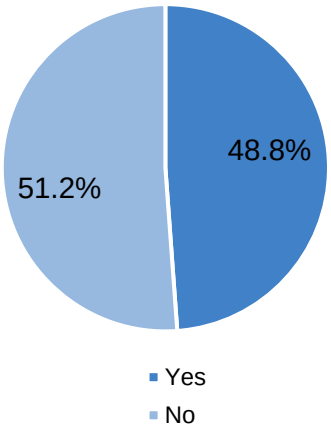
4.3.1. Antileishmanial treatment (or euthanasia)

The preferred antileishmanial therapeutic protocols were surveyed as well. The multiple-choice items which were available were: “allopurinol”, “allopurinol and nucleotide analogues”, “allopurinol and MA”, “allopurinol and domperidone”, “allopurinol and miltefosine”, “nucleotide analogues and AHCC”, “MA”, “domperidone”, “miltefosine” and “Other” protocols. Besides these antileishmanial protocols, the option “euthanasia” was among the options, as well.

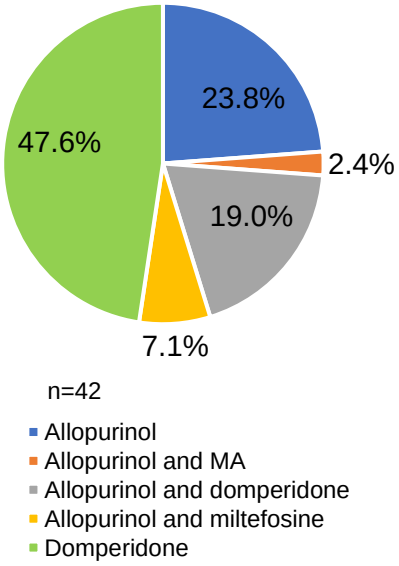
4.3.1.1. Subclinical infection

Faced with an infected but clinically healthy dog, 44 of the 86 respondents (51.2%) would not apply any treatment (Graphic 3). Among the 42 people considering it, 20 (47.6%) elected domperidone as first-choice protocol, 10 (23.8%) chose monotherapy with allopurinol, eight (19.0%) preferred using allopurinol and domperidone, three (7.1%) selected allopurinol with miltefosine, and one (2.4%) preferred the combined therapy of allopurinol and MA (Graphic 4).

Graphic 3. Considerations on applying (or not) treatment considering a dog suspected of subclinical infection.



Graphic 4. First-choice treatment protocols considering a dog suspected of subclinical infection.



4.3.1.2. Stage IIa

As a first-line treatment of this scenario, 78 of the 86 respondents (90.7%) opted for the association of allopurinol with MA or miltefosine: 60 (69.8%) preferred the association of allopurinol and MA, while 18 (20.9%) selected allopurinol with miltefosine. Four clinicians (4.7%) elected single therapy with allopurinol, and the remaining four respondents (4.7%) have chosen treatments such as the combination of allopurinol and domperidone (1/86; 1.2%), the single use of MA (1/86; 1.2%) or miltefosine (1/86; 1.2%), or “other” protocols (1/86; 1.2%) (Graphic 5).

4.3.1.3. Stage IIb

Considering etiological treatment, 80 of the 86 veterinary surgeons (93.0%) elected the combined therapies of allopurinol with MA or miltefosine: 63 (73.3%) preferred allopurinol with MA and 17 (19.8%) chose allopurinol with miltefosine. Two clinicians (2.3%) would use the monotherapy with allopurinol, and the remaining four (4.7%) opted for the combination of allopurinol and domperidone (1/86; 1.2%), single treatment with MA (1/86; 1.2%) or miltefosine (1/86; 1.2%), or “other” protocols (1/86; 1.2%) (Graphic 5).

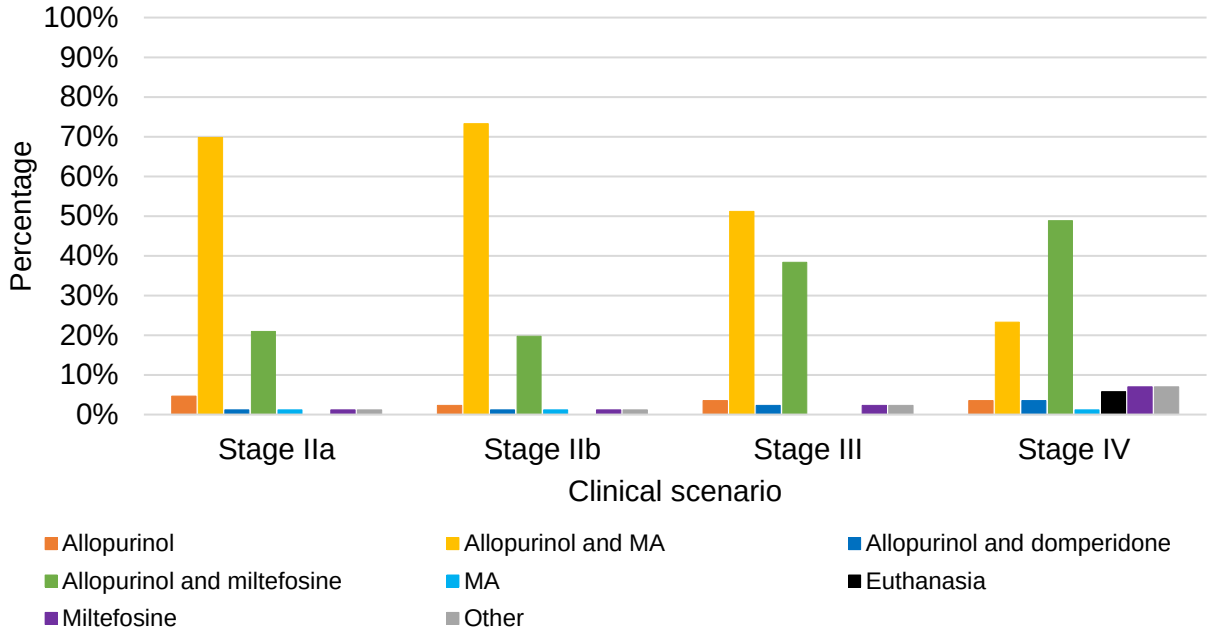
4.3.1.4. Stage III

Regarding the first-line treatment of the stage III clinical case, 77 of the 86 veterinarians (89.5%) have chosen the combination of allopurinol with MA or miltefosine: 44 (51.2%) chose allopurinol with MA, while 33 (38.4%) preferred allopurinol and miltefosine. Furthermore, three practitioners (3.5%) opted for monotherapy with allopurinol, and other six answers (7.0%) included the use of allopurinol and domperidone (2/86; 2.3%), monotherapy with miltefosine (2/86; 2.3%), among “other” protocols (2/86; 2.3%) (Graphic 5).

4.3.1.5. Stage IV

For this stage, 62 of the 86 respondents (72.1%) admitted prescribing the association of allopurinol with MA or miltefosine: 42 (48.8%) elected allopurinol with miltefosine and 20 (23.3%) preferred allopurinol and MA. Furthermore, three respondents (3.5%) considered single therapy with allopurinol and five (5.8%) elected euthanasia. The remaining 16 clinicians (18.6%) preferred other treatments, such as the single use of miltefosine (6/86; 7.0%), combination of allopurinol and domperidone (3/86; 3.5%), single use of MA (1/86; 1.2%), or “other” protocols (6/86; 7.0%) (Graphic 5).

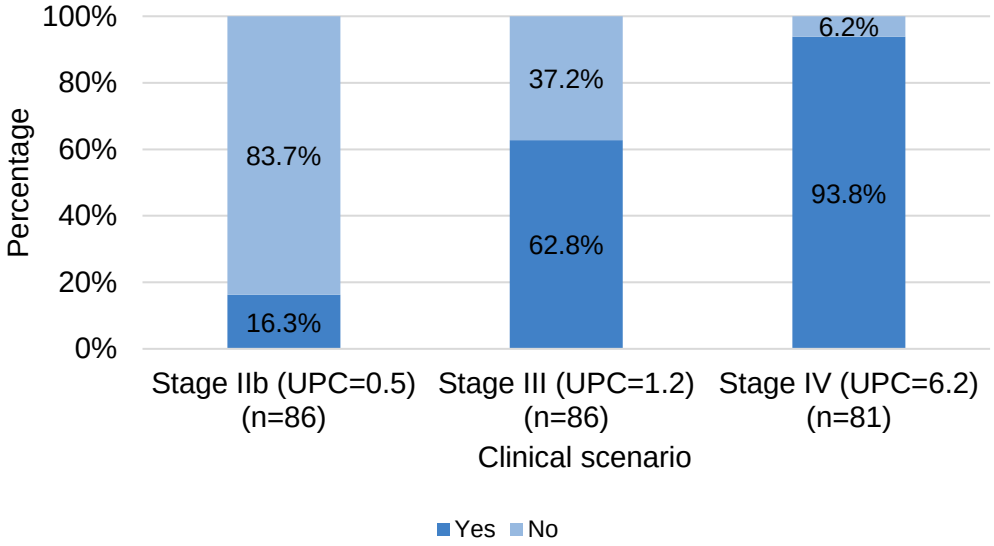
Graphic 5. First-choice treatment protocols (or euthanasia), considering dogs suspected of clinical CanL in different stages.



4.3.2. Antiproteinuric treatment

Given that five clinicians would perform euthanasia rather than treating stage IV CanL dogs (Graphic 5), those respondents were excluded from the results concerning treatment of renal disease in that scenario. Thus, antiproteinuric treatment would be applied: by 76 of the 81 respondents (93.8%) treating stage IV of CanL (UPC=6.2, creatinine=3.5 mg/dL); by 54 of the 86 respondents (62.8%) in stage III of CanL (UPC=1.2, creatinine=1.9 mg/dL); and by 14 of the 86 veterinarians (16.3%) in stage IIb of CanL (UPC=0.5, creatinine<1.4 mg/dL) (Graphic 6).

Graphic 6. Considerations on treating (or not) proteinuria in stages IIb, III and IV of CanL.



4.3.2.1. Antiproteinuric protocols

Among the clinicians considering treating proteinuria, a summary of the preferred antiproteinuric drugs is available in Graphic 7. The multiple-choice items were: “ACEI”, “ARB”, “CCB”, “aldosterone receptor blockers”, “antithrombotic therapy” and “other” compounds. Graphic 8 provides information which includes not only the first-line drugs mentioned in Graphic 7, but also the association of other compounds by some respondents.

4.3.2.1.1. Stage IIb

Considering this clinical case (creatinine<1.4 mg/dL; UPC=0.5), all of the 14 clinicians accepting to treat proteinuria have chosen ACEI as their preferential treatment (Graphic 7).

Concerning the association of other compounds to ACEI, nine out of these 14 practitioners (64.3%) would still use ACEI as single-therapy, four (28.6%) did not detail it (NA), and one (7.14%) would add ARB (Graphic 8).

Besides pharmacological treatment, the introduction of a renal diet was accepted by four veterinarians (28.6%) (Graphic 9).

4.3.2.1.2. Stage III

Among the 54 respondents who would proceed with antiproteinuric treatment in this scenario (creatinine=1.9 mg/dL; UPC=1.2), 46 (85.2%) elected ACEI as first choice drug, five (9.3%) selected ARB, two (3.7%) preferred CCB, and one (1.9%) chose antithrombotic therapy (Graphic 7).

Considering the addition of more compounds, 22 clinicians (40.7%) still preferred single therapy with ACEI and seven (13.0%) elected ACEI but did not detail whether would use or not more compounds. Other less frequently chosen protocols were: ACEI with antithrombotic therapy (6/54; 11.1%), ACEI with “other” (not detailed) compounds (5/54; 9.3%), monotherapy with ARB (4/54; 7.4%), ACEI with ARB (4/54; 7.4%), ACEI with CCB (2/54; 3.7%), ACEI with aldosterone receptor blockers (1/54; 1.85%), and ARB with antithrombotic therapy (1/54; 1.85%). Finally, two clinicians (2/54; 3.5%) have chosen CCB but did not discriminate whether would use or not more compounds (Graphic 8).

Besides pharmacological treatment, the introduction of a renal diet was accepted in 45 of the 54 replies (83.3%) (Graphic 9).

4.3.2.1.3. Stage IV

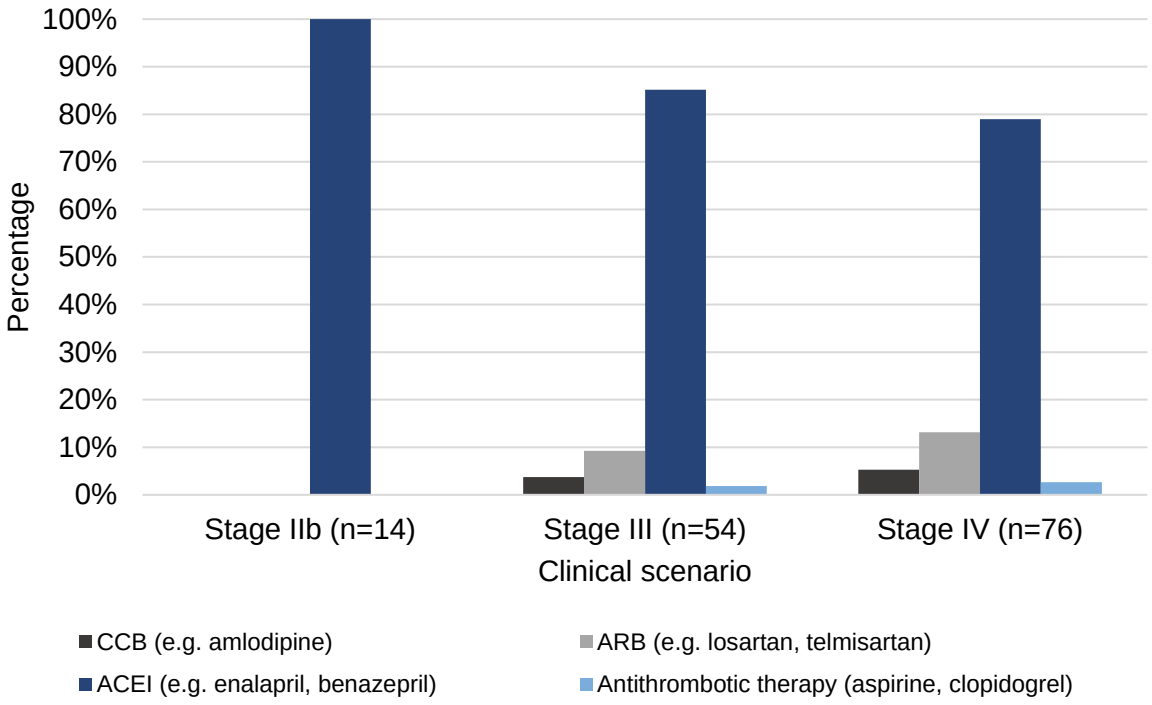
Given that the five respondents who opted for euthanasia in this clinical scenario were discarded from the subsequent statistics, only 81 answers were analysed.

In this case (creatinine=3.5 mg/dL; UPC=6.2), from the 76 veterinarians treating proteinuria, 60 (78.9%) chose ACEI As first-line medical therapy, 10 (13.2%) mentioned ARB, four (5.3%) preferred CCB, and two (2.6%) prioritized antithrombotic therapy (Graphic 7).

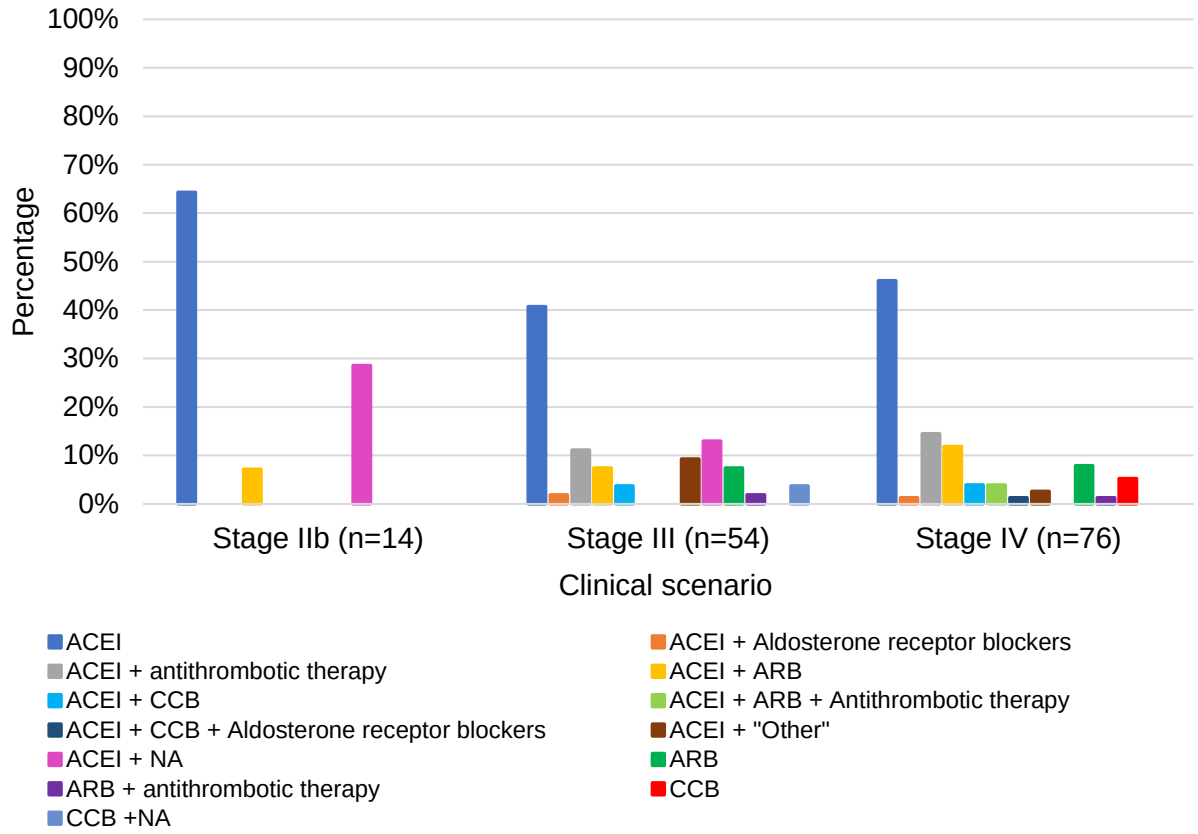
Considering both single and combined therapies, 35 veterinary surgeons (46.1%) prioritized the single use of ACEI. The use of ACEI with antithrombotic therapy was selected by 11 clinicians (14.5%), followed by the association of ACEI with ARB (9/76; 11.8%), monotherapy with ARB (6/76; 7.9%), monotherapy with CCB (4/76; 5.3%), combination of ACEI with CCB (3/76; 3.9%), ACEI with ARB and antithrombotic therapy (3/76; 3.9%), ACEI with aldosterone receptor blockers (1/76; 1.3%), ACEI with CCB and aldosterone receptor blockers (1/76; 1.3%), ACEI with “Other” drugs (1/76; 1.3%) and, finally, ARB with antithrombotic compounds (1/76; 1.3%) (Graphic 8).

From the 76 respondents who would treat proteinuria, 74 (97.4%) would change for a renal diet and 2 (2.6%) either would not do it or did not detail the answer (Graphic 9).

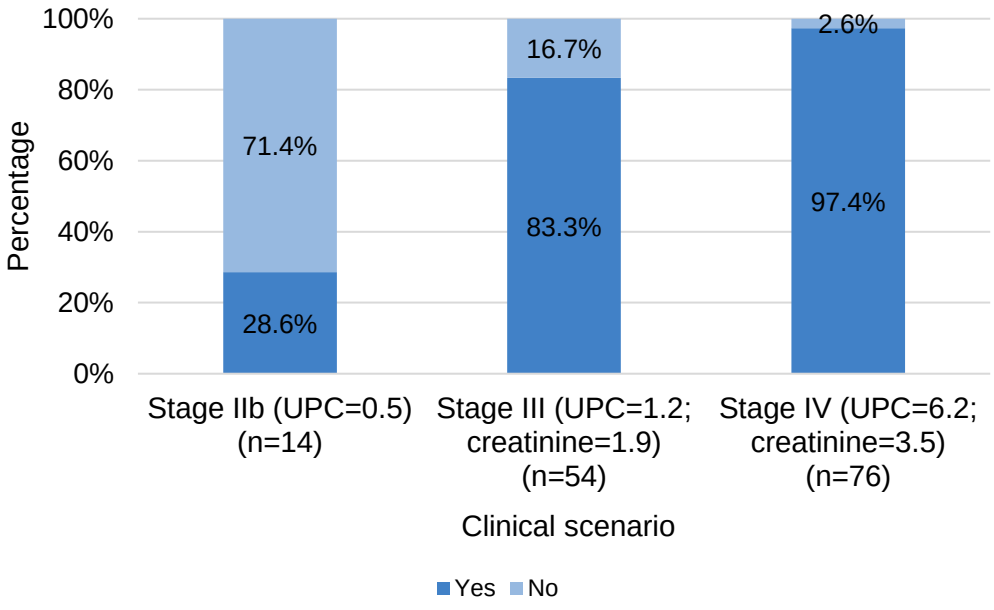
Graphic 7. First-choice antiproteinuric drugs in dogs suspected of CanL.



Graphic 8. Management of proteinuria, including single-drug and combined therapies.



Graphic 9. Considerations on changing (or not) for a renal diet in proteinuric dogs suspected of CanL.



4.4. Prevention and control

4.4.1. Management of subclinical infection

Faced with the scenario of a subclinical infection, the respondents could choose one or more options among the following: 1) only monitoring (including repeating serology within 3-6 months), 2) only applying appropriate preventive measures, depending on the patient, or 3) both measures. From the 86 veterinarians, 85 (98.8%) admitted conducting both measures, and one person (1.2%) would only monitor that patient.

4.4.2. Prevention/control in healthy populations

Concerning prevention and control measures applied on a healthy dog planning to move to an endemic area of CanL, the respondents could choose one or more options among the following: domperidone, repellents/insecticides and vaccination. Among the 86 respondents, 85 (98.8%) chose repellents/insecticides, 67 (77.9%) opted for vaccination and 33 (38.4%) selected domperidone.

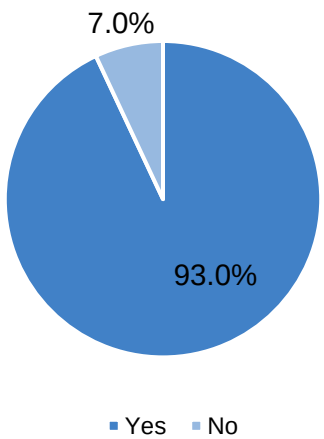
4.5. Other questions

4.5.1. Awareness about the guidelines on CanL

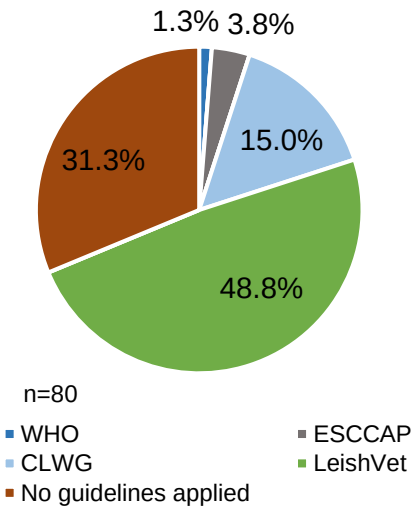
Among the 86 responses, 80 (93.0%) alleged to know about existing guidelines for the management of CanL (Graphic 10).

From these 80, 39 (48.8%) admitted using preferentially the LeishVet guidelines, 12 (15.0%) referred to use those from the CLWG group, three (3.8%) mentioned the ESCCAP recommendations, one (1.3%) elected the guidelines from WHO and the remaining 25 (31.3%) stated that, although recognizing their existence, they do not apply any specific recommendations (Graphic 11).

Graphic 10. Awareness on the existence of guidelines for leishmaniosis.



Graphic 11. Guidelines accessed by the veterinarians for the management of CanL.



4.5.2. LeishVet Staging

The 39 respondents who declared applying the LeishVet guidelines were asked to stage each of the clinical cases according to those recommendations. The substaging of stage II in a) or b) was not surveyed.

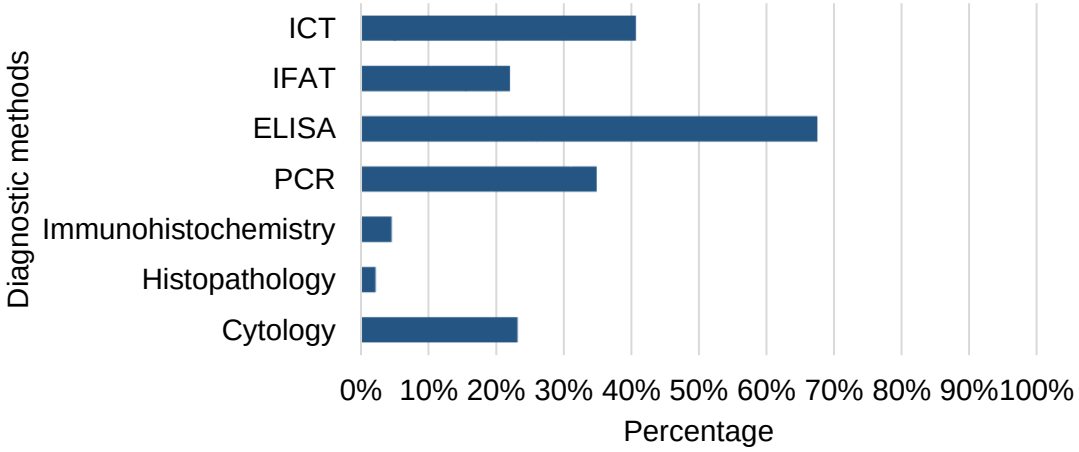
The scenario representing a subclinical infection was classified as Stage I CanL by 36 of the 39 respondents (92.3%); the scenarios corresponding to stages IIa and IIb were considered as stage II by 33 (84.6%) and 22 veterinary surgeons (56.4%), respectively; and, finally, the scenarios representing stages III and IV of CanL were classified in accordance by 31 (79.5%) and 36 (92.3%) clinicians, respectively.

4.5.3. Diagnostic tools used on a routine basis

4.5.3.1. Diagnostic methods

Besides the observation of clinical signs and/or clinicopathological abnormalities compatible with disease, the 86 clinicians were surveyed on their preferred diagnostic methods and samples generally used for confirmation of parasite infection, regardless of the clinical scenario. It was allowed the choice of more than one option and several combinations were mentioned, among the ones shown in Graphic 12. Therefore, ELISA was selected by 58 of the 86 practitioners (67.4%), followed by ICT tests (35/86; 40.7%), PCR (30/86; 34.9%), cytology (20/86; 23.3%), IFAT (19/86; 22.1%) and, in smaller proportions, immunohistochemistry (4/86; 4.7%) and histopathology (2/86; 2.3%) (Graphic 12).

Graphic 12. Diagnostic methods routinely used for diagnosis of *L. infantum* infection.

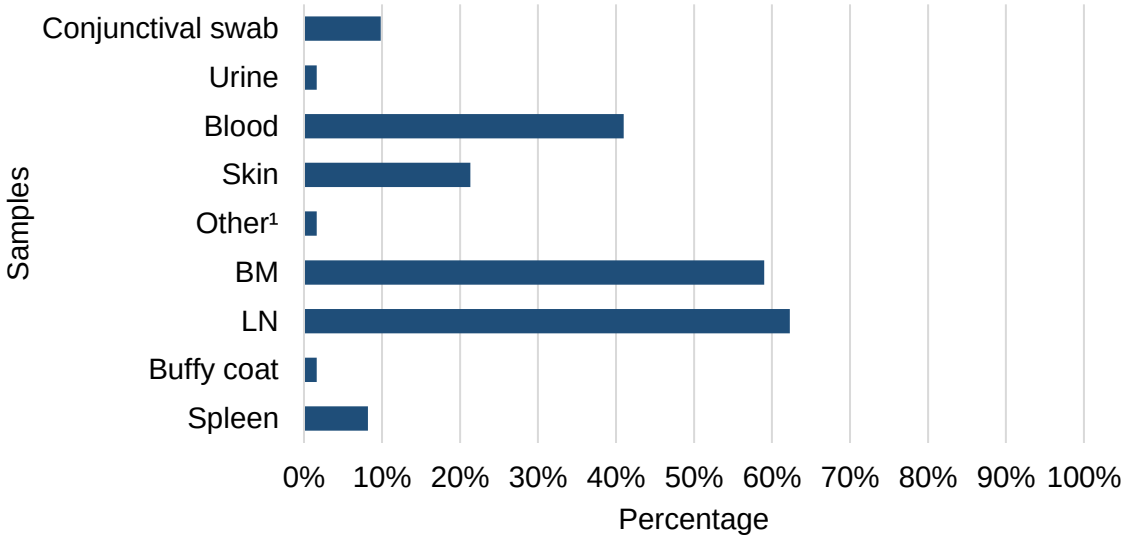


4.5.3.2. Diagnostic samples elected for PCR

The selection of multiple options was allowed, as for the previous topic. The 86 replies included 25 (29.1%) which declared not using PCR. Among the 61 veterinarians performing PCR, LN samples were evoked by 38 respondents (62.3%), followed by BM samples (36/61;

59.0%), blood (25/61; 41.0%), skin (13/61; 21.3%), conjunctival swabs (6/61; 9.8%), spleen aspirates (5/61; 8.2%), buffy coat (1/61;1.6%) and urine (1/61;1.6%). Lastly, one respondent stated using “any tissues containing lesions compatible with CanL” (1.6%) (Graphic 13).

Graphic 13. Samples used for PCR.

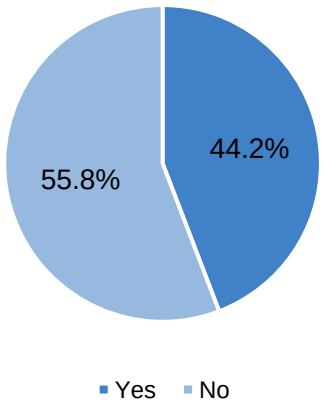


([1]) “Any tissues containing lesions compatible with CanL”. n=61.

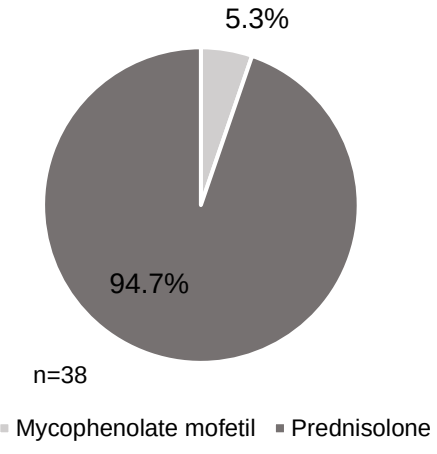
4.5.4. Immunosuppressants in renal disease secondary to CanL

The use of immunosuppressants to treat renal disease secondary to CanL was considered by 38 of the 86 professionals (44.2%) (Graphic 14), from which 36 (36/38; 94.7%) cited prednisolone as first-line drug, and the other two (2/38; 5.3%) prioritized mycophenolate mofetil (Graphic 15).

Graphic 14. Considerations on the use of immunosuppressants for the treatment of renal disease secondary to CanL.



Graphic 15. First-choice immunosuppressive drugs for the treatment of renal disease secondary to CanL.



5. Discussion

5.1. General characteristics

This study explored several features concerning the current trends for CanL management among Portuguese veterinary surgeons.

Clinicians working exclusively in urban areas were the most represented (55.8%), and Lisbon offered the highest number of replies (53.5%). These results may be correlated with the higher density of clinics in those areas, compared to the rural, less populated regions (Ordem dos Médicos Veterinários [OMV] 2020).

The fact that all respondents had a DVM or DVM/MSc degree but none had a PhD may be correlated with the reality of most Portuguese clinicians, such as those working in first-opinion practices. The bias resultant from veterinarians specialised in this area may be, therefore, less likely to exist.

Concerning the clinical experience with the canine species, the fact that most veterinary surgeons have more than 2 years of clinical experience (89.5%) may result in a lower influence of inexperience of the respondent, and, on the other side, give information on whether these clinicians have been aware of the most recent developments in this area since their graduation.

Regarding the frequency of CanL diagnosis, almost three quarters of the veterinary surgeons (73.3%) had less than 11 CanL cases per year. These values may be highly variable accordingly with the dimension of the clinical practice where the veterinarian is inserted, as well as the prevalence of CanL in the regions which were surveyed, or the clinical experience of the practitioner. Oliveira et al. (2010) registered that 65% of the Portuguese clinics consulted less than 10 dogs per day and approximately 40% had less than 10 cases of CanL per year, 20% had 10-20 and 40% consulted more than 20 dogs with leishmaniosis. Furthermore, Mattin et al. (2014) estimated that the percentage of practice-attending dogs diagnosed with CanL was 2.92% in Portugal. Since these values are in clinical practices and not in individual veterinarians, it is difficult to compare the presented results with those from the current literature. Nevertheless, having into account the previous reports and the increasing prevalence of CanL in Portugal, a higher proportion of veterinarians diagnosing more than 20 cases per year was expected. That number of cases was only reported by two clinicians (2.3%) who worked in Faro and Viseu, two endemic regions for CanL (Oliveira et al. 2010).

The number of replies (n=86) was, apparently, relatively small, considering the global Portuguese veterinary community, estimated in 6420 members (OMV 2020). However, the proportion of those clinicians working in clinical practice is unknown, and these numbers were actually in line with those of previous similar surveys conducted in Portugal (with 57 to 141 answers) (Oliveira et al. 2010, Mattin et al. 2014, Bourdeau et al. 2014).

5.2. Diagnostic approach

The results showed that the need to perform further diagnostic tests generally decreased in line with the increasing severity of clinicopathological findings compatible with the disease. The general recommendations, namely those from CLWG (Paltrinieri et al.2010) and LeishVet (Solano-Gallego et al. 2011) groups, indicate that high serological titres (*i.e.* 3-4 times higher than the cut-off value of the laboratory) accompanying the clinical signs and/or laboratory abnormalities compatible with CanL should provide a definitive diagnosis of CanL. However, when serology is not high enough, further work-up is recommended, including cytological/histological evaluation in target organs and PCR (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

Given that the laboratory cut-off value for a positive serology was 1:80, titres of 1:320 and higher would be less doubtful for general practitioners. That was the case of stages IIb, III and IV, showing serological titres of 1:640, 1:320 and 1:640, respectively. In spite of these high serologies, those three cases had more than 20% of the respondents asking for more diagnostic exams. In fact, the identification of the parasite through direct observation or molecular detection may provide valuable information for diagnosis and follow-up during treatment and the exclusion of other causes should be considered as well, since more than one agent may be causing illness (*e.g.* other VBD agents, autoimmune diseases, etc) (Paltrinieri et al. 2016). In addition, subclinical infection and stage IIa scenarios presented serological titres which were not high, but 36.0% and 62.8%, respectively, would not perform further diagnosis for confirmation of infection. Several European surveys have revealed that 90 to 100% of the veterinarians employ etiological diagnostic tests to confirm infection by *L. infantum* in dogs suspected of CanL, including serological, parasitological and molecular methods (Ruiz de Ybáñez et al. 2009; Gálvez et al. 2011; Alcover et al. 2013; Ballart et al. 2013; Bourdeau et al. 2014; Lladró et al. 2017; le Rutte et al. 2018). Serological data – the most recommended technique for diagnosis of CanL (along with cytology of collectable lesions, when possible) (Paltrinieri et al. 2016; LeishVet 2018) – was available in the utterance, which may explain higher proportions not requesting further diagnostic tools.

Besides clinical and serological data, the preferred diagnostic methods were PCR on BM or LN in subclinical infection, PCR on BM or LN and LN cytology in stage II scenarios (IIa and IIb) and LN cytology in the stage III case. The most elected tool for stage IV was US, followed by LN cytology, being this the preferred tool for etiological diagnosis. Stages IIa and III had lymphadenomegaly, which may justify this choice, in opposition to stage IV. The results reflect a high variability in the selection of the diagnostic tools among the different clinical presentations of infection and disease due to *L. infantum*, which may correlate with the wide range of clinical manifestations.

The case representing a subclinical infection consisted in a clinically healthy dog revealing low seropositivity (1:80 [cut-off=1:80]) in a screening test. For this case, over two-thirds would follow more diagnostic procedures and PCR was elected by the great majority, especially PCR on BM/LN. These results are in line with the LeishVet guidelines, which recommend using serology alone or combined with PCR for screening healthy dogs. However, over 20% selected PCR on blood, which is considered a less sensitive technique (LeishVet 2018). Cytology of LN or BM were barely selected, probably due to the lower sensitivity of microscopic evaluation, especially in asymptomatic patients (Paltrinieri et al. 2016). Serum protein electrophoresis was mentioned by a small percentage, probably to evaluate the existence of hypergammaglobulinemia or hypoalbuminemia, frequent in dogs with CanL, although generally in more advanced stages of disease (Paltrinieri et al. 2016). Other alternative techniques mentioned by few respondents included: the serological monitoring within some months, which is also in line with the guidelines, as part of a medium-long term approach (Solano-Gallego et al. 2011); or the combination of multiple parasitological/molecular techniques or samples, probably to increase the diagnostic performance.

The scenario reflecting the stage IIa CanL disease was characterized by various clinicopathological abnormalities compatible with the disease, but a normal renal profile and serological titres theoretically not sufficiently high to be conclusive of CanL (1:160 [>1:80]). In this clinical case, only 37.2% declared needing more complementary exams. The fact that the majority would deny them may be justified by the several clinical and laboratory abnormalities compatible with the disease, despite that the serological values were not conclusive. In this scenario, the preference for LN cytology by almost half of the respondents may be justified by the presence of lymphadenomegaly. Despite the non-regenerative anaemia being present as well, the clear preference for LN samples over BM for cytology is likely because collection of BM is a more invasive and expertise-dependent test (Paltrinieri et al. 2016). The selection of PCR on BM or LN may be associated with those signs as well. Furthermore, PCR on blood was rarely selected, in line with the recommendations. The existing skin lesions would also be further evaluated by a small percentage (6.3%) of the inquired clinicians. Despite requiring expertise and generally presenting lower diagnostic sensitivity, this is also a suitable and recommended test that allows the confirmation of the parasite presence when amastigotes are seen (Solano-Gallego et al. 2011; Paltrinieri et al. 2016). Few people mentioned alternative techniques (*e.g.* measurement of SBP, exclusion of skin parasitic infections, US, etc.), to access the clinical status of the patient, but not leading to a definitive diagnosis.

The stage IIb CanL scenario presented few clinicopathological abnormalities (mild nonregenerative anaemia, epistaxis, hyperglobulinemia and borderline proteinuria) but high serological titres (1:640 [>1:80]). According to the guidelines, these values combined with the clinicopathological alterations should confirm the diagnosis of CanL (Solano-Gallego et al.

2011; Paltrinieri et al. 2016). Most respondents answered accordingly to this and declared not requiring more complementary exams. However, the existence of few and unspecific alterations could explain the need of further diagnostic exploration declared by a quarter of the practitioners, with PCR on BM/LN as the favourite option. Each of the other diagnostic means (LN cytology, BM cytology, PCR on blood) was mentioned by few respondents, probably due to the few, unspecific clinical signs described. Alternative diagnostic means, such as coagulation tests or the observation of the nasal cavity (probably to explore the cause of epistaxis described in this case), imaging and SBP measurement were mentioned as well by over a half of the veterinarians. Although none of these various methods would allow a definitive diagnosis of CanL, they may help to better stage the disease, emphasising the complexity of the diagnosis and the importance of an individual approach.

The stage III CanL diseased patient presented various manifestations compatible with the disease, including high serological titres (1:320 [1:80]) and renal values suggestive of kidney compromise (creatinine=1.9 mg/dL and UPC=1.2) (IRIS 2019a). According to the LeishVet guidelines (Solano-Gallego et al. 2011), these data could be enough to confirm CanL disease and, accordingly, only 20.9% of the respondents accepted performing further complementary exams. Among them, almost two-thirds would employ LN cytology, perhaps due to the presence of generalized lymphadenomegaly. Cytology on BM and PCR on BM/LN were equally mentioned by few respondents and could be legit as well, considering the clinical picture. Similarly to the previous cases, none of the other methods detailed (*e.g.* urine culture, imaging, among others) could provide a definitive diagnosis, but could be useful as a staging procedure by exploring possible concurrent conditions that can affect therapeutic efficacy and prognosis.

The stage IV CanL clinical case had various similarities with the stage III scenario, but higher levels of azotaemia and proteinuria (creatinine=3.5 mg/dL [<1.4]; UPC=6.2 [<0.5]), and higher serological titres (1:640). In line with the previously stated, this case would not leave space for doubt on a CanL diagnosis, and few respondents asked for further complementary exams. However, clinical data about ultrasonography was absent (in contrast with stage III), which may justify the significant proportions requiring further diagnostic tests (26.7%), especially US, mentioned by 60.9% of those respondents. This exam was likely required to further access the clinical status of the patient (as part of the CKD work-up, for example) rather than to confirm disease. In addition, few people invoked: parasitological and molecular techniques, which could increase the diagnostic performance, but are not essential for diagnosis at this stage; and other tools useful access the clinical status of the patient.

5.3. Treatment

5.3.1. Antileishmanial treatment (or euthanasia)

According to the guidelines (Solano-Gallego et al. 2011; Oliva et al. 2010), subclinical infection does not require treatment, but routine monitoring should be performed to early assess a possible evolution of infection towards disease. However, faced with this scenario, almost half of the respondents admitted proceeding with treatment. To be considered a clinical diseased patient requiring therapy, the dog should have, at least, mild clinical signs, which was not the case (only low anti-*Leishmania* antibody titres were detected). However, the use of immune stimulators such as domperidone has been studied not only as a promising preventive measure but also for the treatment of mild CanL disease (Miró et al. 2017). In fact, almost a half of the respondents who considered medical treatment mentioned the use of domperidone as first-choice approach. Nevertheless, there was a significant number of clinicians using other protocols consisting of allopurinol, either as monotherapy or combined with other compounds such as MA, miltefosine or domperidone. This reflects some inconsistency regarding the management of subclinically infected cases such as this one, probably misclassified as sick patients at stage I of CanL (LeishVet 2018).

The combined therapy of allopurinol and MA was the preferred protocol for the scenarios of CanL at stages II and III, followed by the combination of allopurinol with miltefosine. Single therapy with allopurinol was the third most selected protocol in those stages as well, although in a much lesser extent. These results demonstrated various similarities in the treatment approach of these three stages and are generally in line with the literature recommendations (Oliva et al. 2010; Solano-Gallego et al. 2011; LeishVet 2018) and international tendencies reported by other surveys (Mattin et al. 2014; Lladró et al. 2017; Montoya et al. 2020). However, the survey conducted by Bourdeau et al. (2014) revealed that Portuguese veterinarians (n=141) preferred allopurinol alone to the combination of allopurinol with antimonials, contrarily to the other veterinarians in endemic countries such as Spain, Italy and France, which prioritized the allopurinol/antimonials combination. By the time of that survey, miltefosine was not legalized for treatment of CanL, therefore its use was not surveyed. Besides these protocols, in the present study, few respondents preferred other treatments such as monotherapy with MA or miltefosine, allopurinol with domperidone, among others. These protocols are not recommended due to the lack of compelling scientific evidence of their efficacy/safety (Roura et al. 2020) and some have even shown discouraging results, such as the potential nephrotoxicity of MA (Bianciardi et al. 2009), the lower efficacy of monotherapies compared with combined protocols (Manna et al. 2015), or the development of drug resistance (Yasur-Landau et al. 2016).

The treatment protocols surveyed for the clinical scenario of a stage IV CanL disease displayed a different tendency from that of the remaining stages, with the combined therapy of

allopurinol and miltefosine being prioritized to that of allopurinol and MA. These results may probably correlate with the assumed lower nephrotoxic effect of miltefosine when compared with MA (Bianciardi et al. 2009). The single use of allopurinol was still considered by a small percentage of the respondents, which may be correlated to its safety and relative efficacy in treatment of dogs with renal disease (Plevraki et al. 2006), despite lower than the other combined therapies. Non-scientific evidence-based protocols were considered by several respondents, including allopurinol with domperidone, single use of miltefosine or MA, among others. Considering the severity stage IV cases, an individual approach is recommended (LeishVet 2018; IRIS 2019b). This stage was the only one in which euthanasia was considered. Bearing in mind that the only clinicopathological parameters which were worse compared with stage III CanL were the renal values (creatinine=3.5 mg/dL and UPC=6.2 in stage IV, compared with 1.9 mg/dL and 1.2 in stage III), these results are in agreement with previous research reports which mention that severity of renal condition (especially proteinuric values) is one of the most important prognostic factors (LeishVet 2018), and that CKD is the main cause of death or euthanasia in dogs with leishmaniosis (Costa et al. 2003; Zatelli et al. 2003).

5.3.2. Antiproteinuric treatment

Besides the control of the etiological cause (*L. infantum)*, the importance of renal disease in CanL is widely recognized (Koutinas AF and Koutinas CK 2014). The clinical management of concomitant renal disease was studied as well, in three of the six scenarios described: the ones corresponding to stage IIb, III and IV. These cases presented variable magnitudes of proteinuria, with or without azotaemia.

Assuming that proteinuria in those three scenarios was classified as “persistent, renal proteinuria” and CKD was diagnosed (Lees et al. 2005), it would be staged, respectively, as: CKD stage 1 with borderline proteinuria (creatinine<1.4 mg/dL, UPC=0.5), CKD stage 2 with proteinuria (creatinine=1.9 mg/dL, UPC=1.2) and CKD stage 3 with proteinuria (creatinine=3.5 mg/dL, UPC=6.2) (IRIS 2019a).

The proportion of respondents using antiproteinuric treatment increased in line with the increasing magnitude of proteinuria (and azotaemia). According to the guidelines, dogs with CanL and borderline proteinuria (UPC between 0.2 and 0.5, such as the scenario of stage IIb CanL) require close monitoring of the associated disease (CanL, in this case) and response to the anti-infective treatment (antileishmanial drugs), through measurement of creatinine and UPC values. In addition, dogs with UPC>0.5 should follow antiproteinuric treatment, including both pharmacological and dietary management (Lees et al. 2005; IRIS-CGDSG et al. 2013a; IRIS 2019b). Some authors have stated that, since proteinuria decreases in 4–8 weeks following antileishmanial treatment (Pierantozzi et al. 2013; Proverbio et al. 2016), antiproteinuric drugs should be considered thereafter (Paltrinieri et al. 2016; Roura et al. 2020).

The most recent recommendations on treatment of leishmaniosis-induced kidney disease reinforce this idea, stating that, independently of creatinine concentration, those cases should immediately start specific treatment for leishmaniosis, alone (if UPC≤3.0) or associated with the standard treatment for the renal disease, and the patient should be closely followed up to evaluate the efficacy of treatment and renal parameters. If, at follow-up, proteinuria remains (UPC>0.5), then antiproteinuric therapy should be performed (Roura et al. 2020). Therefore, following the most recent guidelines, the clinical scenarios of stage IIb and III should first follow only antileishmanial treatment and, if at follow-up proteinuria remains, then start antiproteinuric treatment. However, 16.3% and 62.8% of the veterinarians would apply antiproteinuric treatment along with antileishmanial drugs.

Regarding the most elected antiproteinuric protocols, several similarities were found among the three scenarios. The preferred therapeutic choice consisted, by far, in the use of ACEI, especially as monotherapy. In a much lesser extent, some veterinarians selected ARB, CCB and antithrombotic drugs. An increasing use of these compounds accompanied the increasing severity of renal (and CanL) disease among the clinical scenarios. Globally, the results have shown that the most employed antiproteinuric treatments are in line with those mentioned by the international recommendations (Lees et al. 2005; IRIS-CGDSG et al. 2013a, 2013b, 2013c, 2013d; IRIS 2019b; Roura et al. 2020). All guidelines prioritize ACEI as first-choice antiproteinuric treatment, along with the change for a renal diet. Then, ARB should be implemented as second-line drugs in case of persistent proteinuria, despite the use of ACEI. The use of CCB is generally recommended as adjunctive to ACEI and ARB in the presence of hypertension (IRIS-CGDSG et al. 2013a; IRIS 2019b).

The selection of antithrombotic therapy in stages III and IV may be correlated with the hypoalbuminemia described in those scenarios. However, the guidelines recommend the use of antithrombotic therapy when there is severe hypoalbuminemia, which was not described. Despite the results being generally accordingly with the recommendations, the use of non-recommended compounds (such as ARBs when UPC=0.5 (stage IIb), therapies without ACEI, combined therapies of ACEI with CCB/aldosterone receptor blockers/others) highlights some inconsistency regarding the management of proteinuria in dogs with leishmaniosis, particularly in those cases with a lower magnitude. This may be correlated with some misinformation and/or lack of awareness and may raise some concern, since renal disease is likely the most common and important concomitant complication in these sick dogs (Koutinas AF and Koutinas CK 2014).

Besides the pharmacological treatment, the change for a renal diet was also increasingly accepted, accompanying the severity of the renal disease. However, stage IIb CanL would only require monitoring (IRIS 2019b; Roura et al. 2020), and according to the most recent guidelines (Roura et al. 2020), stage III CanL should follow monitoring as well, given

that UPC was lower than 3.0, and diet (along with antiproteinuric drugs) should be considered at follow-up, four weeks later. Nevertheless, Roura et al. (2020) emphasised that protocols may vary accordingly with the clinical status of the patient and should be performed individually.

5.4. Prevention and control

This topic was evaluated in two different clinical scenarios: one concerning the management of a subclinical infection; other concerning a healthy dog planned to move from a non-endemic to an endemic area.

The results concerning the first case surveyed the willing to apply monitoring, prevention or both measures. Almost all respondents would employ both, while only 1.2% would perform only monitoring. These results are in line with the recommendations, which indicate that these dogs should be monitored on a regular basis (every 3-6 months) to assess the progression of infection towards disease (LeishVet 2018). Besides, prevention of dissemination of infection to other animals should be performed as well. Some measures include using repellents/insecticides, using nets, avoiding permanence in the outside during the high-risk season, among others (Miró et al. 2017).

The second case surveyed the specific use of immunomodulators (domperidone), immune prophylaxis (vaccination) and repellents/insecticides in a dog planned to move from a non-endemic to an endemic area. The results are in line with the recommendations (Miró et al. 2017) and are similar to previous national and international reports on this topic, showing that repellents/insecticides are the most frequently used by the veterinarians, followed by vaccination and, less frequently used, domperidone (Ruiz de Ybáñez et al. 2009; Oliveira et al. 2010; Gálvez et al. 2011; Alcover et al. 2013; Ballart et al. 2013; Bourdeau et al. 2014; Mattin et al. 2014; Lladró et al. 2017; le Rutte et al. 2018; Montoya et al. 2020). The literature reports that the use of topical insecticides with proven efficacy against sand flies is the best way to avoid *L. infantum* infection, but owner compliance is crucial as well. Vaccination is a more recent and promising tool for preventing CanL disease, as well as domperidone, being recommended as complementary measures to the use of repellents/insecticides (Miró et al. 2017). However, vaccination raises some controversy, given that the administration of certain vaccines may interfere with serological screening. Furthermore, the frequent use of ICT tests (known for their variable sensitivities) previously to vaccination may contribute for the risk of unintentionally vaccinating seropositive dogs. Therefore, the decision to vaccinate should be considered individually and based on a risk-benefit assessment for an optimal prevention against both infection (with repellents) and development of clinical disease (with vaccination) (Solano-Gallego et al. 2017).

The dog described in the current questionnaire was apparently clinically healthy, however data on quantitative serology was absent. Therefore, some respondents might not have chosen the “vaccination” option, considering that it is only recommended in seronegative dogs. Additionally, it was not mentioned whether the dog had an indoors or outdoors lifestyle. Whilst repellents are always recommended for dogs moving to endemic areas, vaccination is strongly advised for outdoor, but is only considered optional for indoor dogs (LeishVet 2018). Among the three preventive measures available for choice – repellents/insecticides, vaccination and/or domperidone – domperidone was the least preferred, but it was still the choice of over one-third of the respondents. This proportion is lower than that reported in Spain of approximately 45-50% (Lladró et al. 2017; Le Rutte et al. 2018; Montoya et al. 2020), but higher than France, with 1.6% using it (Le Rutte et al. 2018). There is scarce information about the use of this drug in Portugal. Mattin et al. (2014) reported that less than 15% of the Portuguese clinicians (n=57) prescribed domperidone as prophylactic measure in more than 20% of their canine patients and thus, the present survey shows an apparent increase on its use. The administration of domperidone has been the most recently studied and recommended measure for prevention/control of CanL, in seronegative or seropositive mild diseased dogs (LeishVet stage I). Given its quite recent introduction in the market, it would be expectable to be the least commonly prescribed among the veterinarian community (Miró et al. 2017; LeishVet 2018). Moreover, there is still lack of research concerning the safety and effectiveness of this drug, including its potential side effects (Travi and Miró 2018), or the impact of its use in ill animals with a high parasite load and that may have a limited or exhausted T cell response (Miró et al. 2017).

5.5. International guidelines and LeishVet staging

The results revealed that the great majority of the inquired veterinarians is aware about the existence of guidelines for the management of CanL. The LeishVet guidelines are the most recognized ones, followed by those stablished by the CLWG group. However, the fact that almost one-third admitted knowing about their existence but not applying any guidelines in specific should raise some concern, since these people may be missing the most recent developments, available in those guidelines. Most of the respondents who stated applying the LeishVet guidelines, classified the clinical scenarios accordingly. Subclinical infection was considered as stage I by over 90%, which would be expected, since the option “subclinical infection” was not among the multiple-choice items. Furthermore, most respondents would either not treat this case or would apply domperidone, which is in accordance with the recommendations for the management of subclinical infection (LeishVet 2018).

Although stage IIb was properly classified by more than half of the respondents, it showed some divergence, since almost one third of the veterinarians had classified it as a

stage III. These results may be justified by the presence of ambiguous clinicopathological and serological data, which were intentionally selected to raise some doubt and create a less evident clinical case. Although mild non-regenerative anaemia and borderline proteinuria may suggest mild clinical disease, the high anti-*Leishmania* antibody titres (1:640 [<1:80]) and epistaxis might have justified the classification as stage III. However, according to the Leishvet guidelines (LeishVet 2018), “medium to high titres” may be present in stage II of CanL and epistaxis may result of various pathological mechanisms such as nasal ulceration and hyperviscosity syndrome (which may exist in stage II) or immunocomplex deposition and vasculitis, for example (present in stage III) (Koutinas AF and Koutinas CK 2014). Since an increasing severity of CKD is generally highly important for stage III classification and this scenario only had borderline proteinuria without any other signs of immunocomplex deposition (*e.g.* glomerulonephritis, uveitis), the author opted for classifying this case as stage IIb, but considers that stage III could be legit as well, depending on the viewpoint of the clinician. These results stress that cases with discrepancies between clinicopathological and serological assessment are one of the fragilities of the LeishVet classification system (Proverbio 2016).

5.6. Diagnostic tools used on a routine basis

This study showed that ELISA was the preferred complementary exam for routine diagnosis of *L. infantum* infection, followed by ICT, contrasting with the results of previous surveys conducted in Portugal and other European countries, where IFAT or ICT are generally prioritized to ELISA (Oliveira et al. 2010; Bourdeau et al. 2014; Montoya et al. 2020). Although IFAT has generally being considered the gold standard diagnostic test, ELISA has shown higher diagnostic performance, compared to IFAT or ICT tests (Solano-Gallego et al. 2014), which may justify the increasing use of this method. Additionally, the interpretation of ELISA tests is less subjective and operator-dependent than that of IFAT (Paltrinieri et al. 2016). ICT tests have been frequently employed as screening tools for its easy to use, rapid in-clinic results and high specificities. However, the single qualitative data and reduced sensitivities reported have discouraged their use on a routine basis (Solano-Gallego et al. 2011), especially considering the risk of vaccinating false-negative dogs (Solano-Gallego et al. 2017).

PCR was the third most elected tool for routine diagnosis, elected by more than one third of the respondents, after ELISA and ICT, and followed by IFAT. These results show an increasing use of PCR, compared with reports of Bourdeau et al. (2014) and Oliveira et al. (2010), which reported around 5-6% “always” performing PCR in their practices, thus suggesting an increasing use of this tool on daily veterinary practice in Portugal. Despite confirming infection by the parasite and having high sensitivity and specificity, PCR performance depends on the methods/samples used, requires expertise (thus being an expensive technique) and does not reveal the immunological status of the dog, thus always

requiring further evaluation with clinicopathological and serological data (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

The routine practice of cytology has increased (23.3%), comparing with previous surveys by Oliveira et al. (2010) (1.7 to 11%, varying with the samples used) and Bourdeau et al. (2014) (2.4% to 10.6%). This diagnostic tool confirms infection, allows definitive diagnosis and is a rapid and non-invasive technique (when performed in LN or skin lesions). However, these low proportions may be correlated with the fact that this method shows variable/low sensitivities, does not give information about the immunological status of the patient and is operator-dependent, requiring expertise (Solano-Gallego et al. 2011; Paltrinieri et al. 2016).

Immunohistochemistry and histopathology were rarely selected, similarly to previous results from Oliveira et al. (2010), but in lower proportions compared with reports from Bourdeau et al. (2014), either for the Portuguese (66% and 9%, respectively), or SW European countries in general (36% and 37%, respectively). These techniques are generally costly, laborious, time-consuming, have variable/low sensitivities and do not reveal the immunological status of the patient. Nevertheless, may help improving the diagnosis of CanL through providing detailed data (Paltrinieri et al. 2016). In addition, these results may also reflect some lack of familiarization with potential techniques such as those.

Concerning the PCR samples, to the author's knowledge, this is the first report to date surveying the most frequently used samples for PCR. The results are partially in line with the recommendations stated on the LeishVet consensus and previous literature research, according which the most sensitive samples (and, consequently, the most recommended) are BM, LN, spleen, skin and conjunctival swabs (LeishVet 2018). This survey showed that the most elected samples were LN and BM. The use of "any tissues containing lesions compatible with CanL" is also a legit answer given that PCR should, indeed, be collected from any tissue or body fluid with "lesions eligible for FNA or biopsy" (Paltrinieri et al. 2016). However, blood samples were the third favourite choice, elected by over one-third of the veterinarians using PCR, even though associated with less sensitive results. These findings may be correlated with the fact that the collection of blood is an easier, less invasive and frequently performed technique, which may be often seized to conduct further tests such as this one. It can also reflect some lack of awareness regarding recent scientific research and international recommendations (Paltrinieri et al. 2016; LeishVet 2018).

5.7. Immunosuppressants

Almost half of the respondents admitted using immunosuppressants when glomerulonephritis secondary to CanL is suspected, probably bearing in mind an immune-mediated origin. However, almost all of them prioritize prednisolone and a small percentage elected mycophenolate mofetil. On one hand, these results are in opposition to the Consensus recommendations for treatment of immune-mediated glomerular disease (IRIS-CGDSG et al. 2013d), given that glucocorticoids have significant adverse effects (such as worsening of proteinuria and hypertension), and mycophenolate mofetil is recommended as first choice immunosuppressant for these cases (IRIS-CGDSG et al. 2013d). On the other hand, there is some accordance with the latest recommendations which, based on the opinion of experts, admitted the use of prednisone at anti-inflammatory dosage, to reduce the immune-mediated renal inflammation rather than decreasing the formation/circulation of immune complexes (Roura et al. 2020).

The findings of the present study stress the important lack of scientific evidence supporting the use of specific immunosuppressive drugs in dogs with immune-mediated glomerular disease, especially when there is a concurrent infectious cause. The main concern relies in the possibility that the immunosuppressive treatment directed at the glomerular disease may compromise control of the predisposing infection (IRIS-CGDSG et al. 2013d). However, according to the IRIS study group, the glomerular disease and the infection must be considered and managed as separate diseases, since some dogs may die from consequences of their glomerular disease rather than nonrenal alterations (IRIS-CGDSG et al. 2013b). The balance between benefits and adverse effects should be carefully and individually pondered and discussed with the client, followed by a cautious administration and close monitoring (IRIS-CGDSG et al. 2013b, 2013d; Roura et al. 2020).

In sum, although some interesting information was collected from this study, further larger surveys would be useful in order to obtain more representative results and to establish some interesting, representative correlations, such as the relationship between the profile of the practitioner and the answering trends. A correlation between the working area and the number of dogs diagnosed with CanL per clinician, between the time of graduation or academic degree and the type of diagnostic/treatment/preventive tools used, or a deeper statistical analysis per region and identification of regional patterns, are some examples of interesting features to investigate.

6. Limitations

Although this questionnaire-based survey allowed to obtain several data and some interesting conclusions, there are some limitations that could be considered for further similar studies.

First, it was not possible to calculate the rate of replies, for the following reasons: 1) besides the mailing list of the scholar hospital, the questionnaire was uploaded in a Facebook group, which has an unknown proportion of veterinary surgeons working in clinical practice; 2) the link used to spread the questionnaire was shareable, hampering the estimates on the number of clinicians having access to it. A shorter, simpler formulary would likely have more replies, and a wider promotion through e-mail to other clinical practices or through personal visits could have increased its coverage as well. Nevertheless, the complexity and the length of the questionnaire might have prevented answers from people to whom it was not directed, such as non-veterinarians or veterinarians not working in clinical practice.

Furthermore, the variability of the clinical signs and clinicopathological abnormalities described among the various scenarios may, on one hand, reflect the complexity of CanL, but on the other hand, increase the subjectivity of the interpretation of each scenario, by the respondent (and, consequently, the analysis of the results, by the author).

Another limitation was noted while analysing the questions about prevention and control, in which an option such as “I would not apply any measure” was not available for choice. Consequently, some respondents who are less sensitised for this issue and generally do not apply any control/preventive measures might have been biased towards one of the items available in the multiple-choice questions. In addition, the inclusion of other preventive measures such as the use of shampoo could have been surveyed as well, or the “insecticides/repellents” option subdivided in “collars”, “spot-ons” or “insecticidal sprays” to obtain more specific data.

There is a similar observation concerning diagnosis: an option including the use of skin as sample for techniques such as cytology or PCR was absent. Indeed, the collection of skin samples is recommended by the international guidelines, along with quantitative serology, when skin lesions are detected in a patient with suspicion of CanL (Paltrinieri et al. 2016; LeishVet 2018).

Moreover, the staging of the scenario representing a subclinical infection was likely biased towards the option “Stage I”, instead of “subclinical infection”, given that this option was missing among the multiple-choice items. Regardless of that, the treatments applied by most respondents (either none or domperidone) were, indeed, in line with those recommended for subclinical infection (Oliva et al. 2010; LeishVet 2018).

In addition, the question about the use of additional antiproteinuric drugs was not replied by few people (represented as NA in Graphic 9), given that it was not mandatory. In

stage IV, the option “I would not add any other antiproteinuric drug” was absent, and therefore, whoever skipped the question was considered as using single therapy only.

Concerning the preferred first-choice diagnostic tools for each clinical case, some respondents mentioned diagnostic tools which were already detailed in the information about each clinical case. This may reflect not only some lack of attention by the respondent while reading that information, but also the need to perform shorter, easier-to-read utterances.

Furthermore, some information about the clinical scenarios was not detailed, to avoid extending too much the questionnaire. For example, the characterization of the renal abnormalities only included the magnitude of azotaemia/proteinuria, but not the persistence nor location (pre-renal, renal or post-renal) of proteinuria. Therefore, despite being probable, CKD could not be confirmed with certainty as the cause of proteinuria.

7. Conclusion and future perspectives

This survey provides further information regarding the clinical management of CanL and secondary renal disease by the Portuguese veterinary community.

In terms of diagnosis, the first-line diagnostic tools to confirm CanL (besides serological analysis) were generally in line with those recommended by the guidelines. However, PCR on blood was a relevant choice, despite blood being considered a less sensitive sample. Also, other complementary exams, such as imaging, were often invoked, emphasising their utility to assess the clinical status of the patients.

Concerning antileishmanial treatment, most veterinarians also mentioned treatment protocols which are in line with the guidelines and reports from other international surveys. Those include not treating subclinical infection, or applying domperidone in those cases, and prescribing the combination of allopurinol with MA or miltefosine in dogs with clinical disease. The first combination was preferred in stages II and III, while stage IV was more frequently treated with the allopurinol/miltefosine protocol, probably due to the potential nephrotoxic effect of MA reported by some studies. Euthanasia was only considered for stage IV CanL scenarios, highlighting the severity of renal condition as a determinant factor for prognosis and decision for euthanasia.

Regarding antiproteinuric treatment, the prescription of pharmacological and dietary therapeutic protocols increased in line with the worsening of proteinuria. Most veterinary surgeons apparently follow the international guidelines and consensus for the treatment of kidney disease: the prescription of a renal diet along with the single use of ACEI was, by far, the preferred protocol in all cases presenting proteinuria, and the use of ARB or CCB increased in line with the increasing severity of the clinical status of the patient.

The great majority of the respondents have agreed on conducting both prevention and monitoring measures in dogs with subclinical infection.

Moreover, repellents/insecticides were the most elected preventive measure, followed by vaccination and, less frequently, domperidone.

The existence of guidelines for the management of CanL – especially those from the LeishVet and CLWG groups – was recognised by the great majority. However, around one-third of the clinicians who were mindful of them, admitted not to consult any guidelines in daily practice, which may result in the application of different measures from those that are most adequate and recommended. Nevertheless, most practitioners properly staged the clinical cases in accordance with the LeishVet classification.

In a daily basis, preferred diagnostic tools routinely used (regardless of the clinical scenario) were quantitative serology by ELISA, followed by ICT tests, PCR and IFAT in the fourth place. The use of PCR and cytology has been increasing in Portugal, in opposition to that of histopathology and immunohistochemistry.

The use of immunosuppressants was considered by around half of the respondents, who visibly preferred prednisolone to mycophenolate mofetil. These results reinforce the important lack of information regarding the use of immunosuppressive drugs in immune-mediated renal disease, and the recent publication of guidelines based on scientific evidence and clinical experience of experts might help minimizing this problem. Therefore, this survey may be useful for further comparative studies.

Further studies conducted in a larger scale and in other countries would be useful to extrapolate these conclusions to other European countries. Although the most widely recognized guidelines have good agreement between them, the creation of a common clinical staging system would enable the uniformization of the clinical management of CanL and an easier comparison of clinical and epidemiological studies.

Section 4 – References

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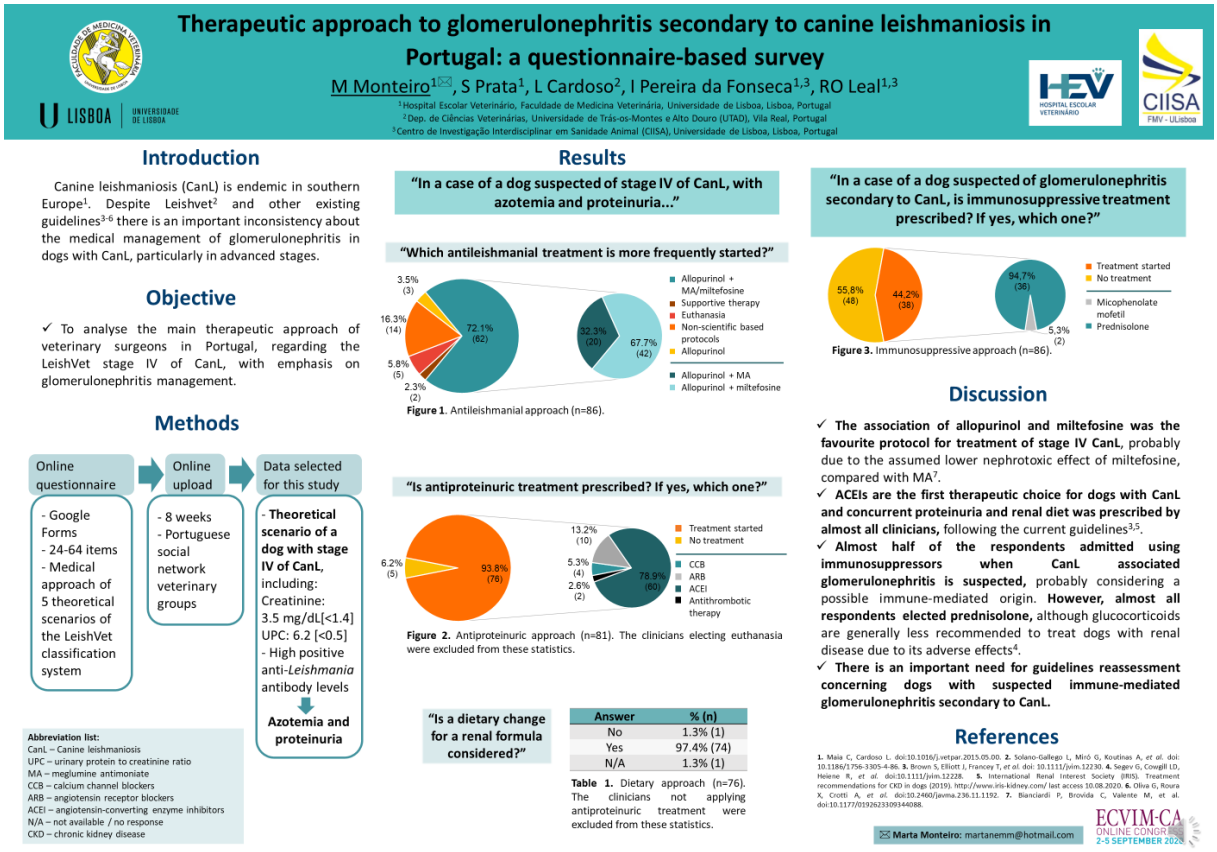
Section 5 – Annexes

Annexe 1. Poster presented at the Online Congress of the European College of Veterinary Internal Medicine (ECVIM) – Companion Animals (2-5 September 2020)



Therapeutic approach to glomerulonephritis secondary to canine leishmaniosis in Portugal: a questionnaire-based survey

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Introduction

Canine leishmaniosis (CanL) is endemic in southern Europe[1]. Despite Leishvet[2] and other existing guidelines[3-6] there is an important inconsistency about the medical management of glomerulonephritis in dogs with CanL, particularly in advanced stages.

Objective

✓ To analyse the main therapeutic approach of veterinary surgeons in Portugal, regarding the LeishVet stage IV of CanL, with emphasis on glomerulonephritis management.

Methods

Online questionnaire → Online upload → Data selected for this study

- Google Forms
- 24-64 items
- Medical approach of 5 theoretical scenarios of the LeishVet classification system

- 8 weeks
- Portuguese social network veterinary groups

- **Theoretical scenario of a dog with stage IV of CanL**, including:
  Creatinine: 3.5 mg/dL[<1.4]
  UPC: 6.2 [<0.5]
- High positive anti-*Leishmania* antibody levels

→ **Azotemia and proteinuria**

Abbreviation list:
CanL – Canine leishmaniosis
UPC – urinary protein to creatinine ratio
MA – meglumine antimoniate
CCB – calcium channel blockers
ARB – angiotensin receptor blockers
ACEI – angiotensin-converting enzyme inhibitors
N/A – not available / no response
CKD – chronic kidney disease

Results

"In a case of a dog suspected of stage IV of CanL, with azotemia and proteinuria..."

"Which antileishmanial treatment is more frequently started?"

Figure 1. Antileishmanial approach (n=86).

"Is antiproteinuric treatment prescribed? If yes, which one?"

Figure 2. Antiproteinuric approach (n=81). The clinicians electing euthanasia were excluded from these statistics.

"Is a dietary change for a renal formula considered?"

Answer	% (n)
No	1.3% (1)
Yes	97.4% (74)
N/A	1.3% (1)

Table 1. Dietary approach (n=76). The clinicians not applying antiproteinuric treatment were excluded from these statistics.

"In a case of a dog suspected of glomerulonephritis secondary to CanL, is immunosuppressive treatment prescribed? If yes, which one?"

Figure 3. Immunosuppressive approach (n=86).

Discussion

✓ **The association of allopurinol and miltefosine was the favourite protocol for treatment of stage IV CanL**, probably due to the assumed lower nephrotoxic effect of miltefosine, compared with MA[7].

✓ **ACEIs are the first therapeutic choice for dogs with CanL and concurrent proteinuria and renal diet was prescribed by almost all clinicians,** following the current guidelines[3,5].

✓ **Almost half of the respondents admitted using immunosuppressors when CanL associated glomerulonephritis is suspected,** probably considering a possible immune-mediated origin. **However, almost all respondents elected prednisolone,** although glucocorticoids are generally less recommended to treat dogs with renal disease due to its adverse effects[4].

✓ **There is an important need for guidelines reassessment concerning dogs with suspected immune-mediated glomerulonephritis secondary to CanL.**

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ECVIM-CA ONLINE CONGRESS 2-5 SEPTEMBER 2020

Annexe 2. Staging of canine CKD based on blood creatinine and SDMA concentrations.

Stage	Blood creatinine mg/dL (µmol/L)	SDMA µg/dL	Other remarks
1	<1.4 (<125)	<18	Normal blood creatinine or normal/mildly increased blood SDMA. Some other renal abnormalities present (*e.g.* abnormalities in renal palpation, renal biopsy or imaging findings, renal proteinuria, inadequate urine concentration, etc). Persistently elevated blood SDMA (>14ug/dL) may be used to indicate early CKD. No clinical signs
2	1.4–2.8 (125–250)	18 - 35	Normal/mildly increased creatinine, mild renal azotaemia. Mildly increased SDMA. Clinical signs usually mild/absent
3	2.9 – 5.0 (251 – 440)	36 - 54	Moderate renal azotaemia. Various extrarenal signs may be present. This stage is considered either as early or late stage 3, depending on the severity/extent of those signs.
4	>5.0 (440)	>54	Increasing risk of systemic clinical signs and uremic crises

When SDMA values correspond to a higher stage classification than creatinine (*e.g.* SDMA >18 µg/dL, but creatinine is <1.4 mg/dL), the patient should be staged and treated according to the SDMA value (stage 2, in this example). Adapted from IRIS (2019a).

Annexe 3. IRIS substaging of CKD by proteinuria.

UPC value	Substage
<0.2	Non-proteinuric
0.2-0.5	Borderline proteinuric
0.5	Proteinuric

Adapted from IRIS (2019a).

Annexe 4. IRIS substaging of CKD by SBP.

SBP (mm Hg)	Substage	Risk of target organ damage
<140	Normotensive	Minimal
140-159	Prehypertensive	Low
160 – 179	Hypertensive	Moderate
≥180	Severely hypertensive	High

Adapted from IRIS (2019a).

Annexe 5. Summary of the LeishVet guidelines for staging, treatment and prognosis of CanL.

Clinical stage	Serology (antibody levels)	Clinical signs	Clinicopathological abnormalities	Treatment	Prognosis
Stage I, mild disease	Negative to low positive	Mild clinical signs (*e.g.* solitary lymphadenomegaly, papular dermatitis)	Usually no clinicopathological abnormalities observed. Normal renal profile: creatinine < 1.4 mg/dL; non-proteinuric: UPC < 0.2	Scientific neglect[1]/ Monitoring of disease progression	Good
Stage II, moderate disease	Low to high positive	Besides stage I clinical signs, may present: diffuse or symmetrical cutaneous lesions such as exfoliative dermatitis/onychogryphosis, ulcerations, generalized lymphadenomegaly, hyporrhexia and weight loss	Mild non-regenerative anemia, hypergammaglobulinemia, hypoalbuminemia, serum hyperviscosity syndrome. Substage a: Normal renal profile: -creatinine<1.4 mg/dL; -non-proteinuric: UPC<0.5 Substage b: -Creatinine <1.4 mg/dL; -UPC= 0.5-1	Allopurinol + MA or miltefosine	Good to guarded
Stage III, severe disease	Medium to high positive	Clinical signs of Stages I and II, plus signs caused by immune-complex deposition (*e.g.* uveitis, glomerulonephritis)	Abnormalities listed in stage II; CKD IRIS stage I with UPC=1-5 or stage II	Allopurinol + MA or miltefosine Follow IRIS guidelines for CKD	Guarded to poor
Stage IV, very severe disease	Medium to high positive	Clinical signs of Stage III, plus pulmonary thromboembolism, nephrotic syndrome and end-stage kidney disease	Abnormalities listed in stage III; CKD IRIS stage III and IV or nephrotic syndrome (marked proteinuria: UPC>5)	Specific and individual treatment Follow IRIS guidelines for CKD	Poor

([1]) Dogs in Stage I (mild disease) are likely to need shorter treatments with one or two combined drugs (allopurinol, domperidone, MA or miltefosine) or alternatively monitoring with no treatment. Adapted from LeishVet (2018).

Annexe 6. Summary of the CLWG guidelines for staging, treatment and prognosis of CanL.

Stage, definition	Etiological diagnosis	Clinical signs and clinicopathological abnormalities compatible with CanL	Treatment	Prognosis
A, Exposed	Negative direct diagnostic assays (microscopy, culture or PCR) and low-positive antibody titres	Absent	Antileishmanial treatment not required.	Favourable
B, Infected	Detection of the parasite through direct diagnostic assays (microscopy, culture or PCR), regardless of serology results	Absent	Antileishmanial treatment not required.	Favourable
C, Clinically sick	Detection of the parasite through direct diagnostic assays (microscopy, culture or PCR) and positive antibody levels (regardless of extent); **OR** High antibody titres, regardless of direct diagnosis results	Present	Antileishmanial treatment required: MA/miltefosine with allopurinol	Favourable to guarded
D, Severely sick	-	Severe clinical and laboratory signs, such as: -Severe proteinuria associated with nephropathy (UPC>3) -Severe kidney disease (IRIS CKD stage 3-4) -Severe ocular and/or joint disease that lead to functional loss. -Severe concomitant diseases	Antileishmanial treatment required: MA/miltefosine with allopurinol These dogs may require immunosuppressive drugs in addition to antileishmanial treatment	Guarded to poor
Ea, Unresponsive to treatment **Eb, Early relapse**	-	Clinically unresponsive to antileishmanial treatment Clinical relapse following cessation of antileishmanial treatment	Similar to stage D. - Rule out other concomitant conditions influencing response to treatment - Consider alternative treatment: allopurinol alone or miltefosine with allopurinol.	Guarded to poor

Adapted from Roura et al. (2013, 2020).

Annexe 7. Recommended treatment protocols for CanL.

Drug	Recommended dosages (LeishVet 2018)	Potential side effects
Allopurinol (in combination with meglumine antimoniate or miltefosine)	10 mg/kg PO, twice daily for at least 6-12 months	- Xanthinuria/urolithiasis (Miró et al. 2009; Torres et al. 2016)
Meglumine antimoniate (in combination with allopurinol)	100 mg/kg subcutaneously, once daily or divided in two doses for 4-6 weeks	- Potential nephrotoxicity (Bianciardi et al. 2009) - Pain and inflammation at site of injection (Bourdeau et al. 2014) - Anorexia, vomiting, diarrhoea, lethargy (Ikeda-Garcia et al. 2007a; Bourdeau et al. 2014) - Transient increasing in liver enzymes (Ikeda-Garcia et al. 2007a)
Miltefosine (in combination with allopurinol)	2 mg/kg *per os* (PO), once daily for 28 days	- Nausea, vomiting, diarrhoea (Manna et al. 2009; Solano-Gallego et al. 2011)
Domperidone (only for stage I)	0.5 mg/kg PO, once daily for 30 days, every 4 months.	- Vomiting, diarrhoea, dysorexia, polyuria (Travi and Miró 2018) - Potential cardiotoxicity (Travi and Miró 2018) - Galactorrhoea (Solano-Gallego et al. 2011)

Adapted from LeishVet (2018).

Annexe 8. Questionnaire provided online – Portuguese (original) version

Maneio da Leishmaniose canina em Portugal - diagnóstico e tratamento

Olá! Se é Médico(a) Veterinário(a) e exerce atividade em Portugal, na área de Clínica de Animais de Companhia - nomeadamente, na espécie canina - então este questionário é para si!

Este questionário faz parte de um projeto de dissertação de Mestrado integrado em Medicina Veterinária, abordando em específico o diagnóstico e tratamento da leishmaniose canina em Portugal.

É constituído, essencialmente, por três partes: "Perfil do Médico Veterinário", "Casos Clínicos" e "Outras questões", sempre referentes à leishmaniose canina.

O questionário contém cerca de 50 questões e o seu preenchimento demorará aproximadamente 15 minutos. Importa referir que as respostas são anónimas e serão tratadas com a devida confidencialidade, pelo que solicitamos que responda o mais honestamente possível, de forma a serem obtidas conclusões válidas e representativas. A privacidade dos dados obtidos será igualmente respeitada no caso de os resultados serem utilizados em comunicações escritas ou orais, ou em publicações que possam resultar do estudo.

Se tiver quaisquer questões referentes a este questionário, poderá contactar, via correio eletrónico, a aluna Marta Monteiro (martanemm@hotmail.com) ou o Professor Doutor Rodolfo Oliveira Leal (rleal@fmv.ulisboa.pt).

Mais informações relativas ao estudo serão enviadas aos participantes, via correio eletrónico, se assim o desejarem. Nesse caso, será necessário que enviem um e-mail demonstrando o interesse para martanemm@hotmail.com.

Agradecemos desde já a sua colaboração e o tempo despendido no preenchimento deste questionário!

PRIMEIRA PARTE - PERFIL DO MÉDICO VETERINÁRIO

1. Qual a sua formação académica? Selecione a habilitação mais elevada que possui, entre os indicados.
□ Doutor em Ciências Veterinárias
□ Licenciado em Medicina Veterinária (pré-Bolonha)
□ Mestre em Medicina Veterinária (pós-Bolonha)

2. Quantos anos tem de prática clínica em cães?
□ Menos de 2 □ 2 a 5 □ 6 a 10
□ 11 a 15 □ 16 a 20 □ Mais de 20

3. Selecione o distrito onde exerce maioritariamente a sua atividade médico veterinária.
(Lista de distritos portugueses)

4. Exerce atividade médico veterinária em meio:
□ Rural □ Urbano □ Ambos

5. Aproximadamente, quantos casos confirmados de leishmaniose teve nos últimos 12 meses?
□ Menos de 5 □ 5 a 10 □ 11 a 15 □ 16 a 20 □ Mais de 20

SEGUNDA PARTE - CASOS CLÍNICOS
Seguem-se 6 casos clínicos, sempre relativos à leishmaniose canina, perante os quais se pede que sejam respondidos tendo em conta o contexto da sua prática clínica e conhecimento relativamente ao tema.

Caso clínico 1
Cão macho, de 7 anos de idade, que habita em zona geográfica onde a leishmaniose é endémica e não faz prevenção para a doença (repelente, vacina ou imunomoduladores).

Motivo de consulta: prostração, anorexia, perda de peso, poliúria/polidipsia, lesões auriculares.

Exame de estado geral (EEG): mucosas pálidas, linfadenomegália generalizada, lesões ulcerativas mucocutâneas, lesões crostosas nos pavilhões auriculares.

Exame oftalmológico: com blefarite e indicativo de uveíte (diminuição bilateral da pressão intraocular, eritema).

Ecografia abdominal: esplenomegália.

Exames laboratoriais: anemia não regenerativa moderada; hiperproteinémia, hipoalbuminémia, hiperglobulinémia e gamopatia policlonal. Azotémia ligeira (creatinina: 1.9mg/dL); urianálise: densidade urinária: 1.018, proteinúria (RPCU: 1.2) com sedimento inativo.
Sem hipertensão arterial.

Serologia (imunofluorescência):
Título de anticorpos anti-*Leishmania* positivo na diluição 1:320.
Serologia para outros agentes vetoriais concomitantes (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)* negativa.

Valores serológicos de referência:
Limiar de positividade: título de anticorpos anti-*Leishmania* positivo na diluição 1:80

6. Considerando a leishmaniose como um dos principais diagnósticos diferenciais deste caso, consideraria necessária a realização de mais exames complementares de diagnóstico?
□ Não □ Sim

7. Se sim, qual faria preferencialmente?
□ Citologia de linfonodo(s)
□ Citologia de medula óssea
□ PCR para *Leishmania* de baço
□ PCR para *Leishmania* a partir de medula óssea ou de linfonodo(s)
□ PCR para *Leishmania* a partir de sangue
□ Outro

8. Se selecionou "Outro", a qual se refere?

9. Que tratamento faria neste caso?
□ Alopurinol
□ Alopurinol e análogos de nucleótidos
□ Alopurinol e antiamoniato de meglumina
□ Alopurinol e domperidona
□ Antiamoniato de meglumina
□ Domperidona
□ Eutanásia
□ Miltefosina

□ Alopurinol e miltefosina
□ Análogos de nucleótidos + AHCC (composto correlacionado com hexose ativa)
□ Outro

10. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

11. Considerando que este animal tinha um RPCU de 1.2 com sedimento inativo, trataria a proteinúria neste caso?
□ Não □ Sim

12. Se sim, que tratamento faria preferencialmente?
□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

13. Se selecionou "Outro", qual o fármaco a que se refere?

14. Além do que assinalou como preferencial, associaria algum(ns) outro(s) fármaco? Se sim, qual(is)?
□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Não associaria nenhum outro fármaco
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

15. Faria dieta formulada para doença renal?
□ Não □ Sim

<u>Caso clínico 2</u>
Cão, macho, com 5 anos de idade, habita em zona geográfica onde a leishmaniose é endémica e não faz prevenção para a doença (repelente, vacina ou imunomoduladores).

Motivo de consulta: vacinação.

EEG: Sem alterações consideráveis.

Exames laboratoriais: Hemograma, bioquímicas e urianálise sem alterações.

Serologia (imunofluorescência):
Título de anticorpos anti-*Leishmania* positivo na diluição 1:80.
Serologia para outros agentes vetoriais concomitantes (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)* negativa.
Valores serológicos de referência:
Limiar de positividade: título de anticorpos anti-*Leishmania* positivo na diluição 1:80

16. Consideraria necessária a realização de mais exames complementares de diagnóstico neste caso?
□ Não □ Sim

17. Se sim, qual faria preferencialmente?
□ Citologia de linfonodo(s)
□ Citologia de medula óssea
□ PCR para *Leishmania* a partir de medula óssea ou de linfonodo(s)
□ PCR para *Leishmania* a partir de sangue
□ Outro

18. Se selecionou "Outro", a qual se refere?

19. Faria tratamento neste caso?
□ Não □ Sim

20. Se sim, que tratamento faria?
□ Alopurinol
□ Alopurinol e análogos de nucleótidos
□ Alopurinol e antiamoniato de meglumina
□ Alopurinol e domperidona
□ Alopurinol e miltefosina
□ Análogos de nucleótidos + AHCC (composto correlacionado com hexose ativa)
□ Antiamoniato de meglumina
□ Domperidona
□ Miltefosina
□ Outro

21. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

22. Independentemente de fazer ou não tratamento, faria monitorização e/ou prevenção?
□ Apenas monitorização clínica (incluindo repetição da serologia em 3-6 meses)
□ Apenas prevenção contra Leishmaniose adequada ao caso clínico
□ Prevenção adequada e monitorização clínica (incluindo repetição da serologia em 3-6 meses)

Caso clínico 3
Cadela, com 8 anos de idade, esterilizada. Viajou com os tutores há cerca de 6 meses para zona geográfica onde a leishmaniose é endémica, mas não fez prevenção (repelente, vacina ou imunomoduladores) para a doença, antes ou durante a viagem.

Motivo de consulta: prostração e perda de peso.

EEG: alopecia periorbital e focos de dermatite esfoliativa nas almofadas plantares, linfadenomegalia generalizada.

Exames laboratoriais: anemia não regenerativa ligeira, hiperproteinemia (hiperglobulinemia com gamopatia policlonal e hipoalbuminemia). Sem azotemia, sem proteinúria.

Serologia (imunofluorescência):
Título de anticorpos anti-*Leishmania* positivo na diluição 1:160.
Serologia para outros agentes vetoriais concomitantes (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)* negativa.
Valores serológicos de referência: Limiar de positividade: título de anticorpos anti-*Leishmania* positivo na diluição 1:80

23. Considerando a leishmaniose como um dos principais diagnósticos diferenciais deste caso, consideraria necessária a realização de mais exames complementares de diagnóstico?
□ Não □ Sim

24. Se sim, qual faria preferencialmente?
□ Citologia de linfonodo(s)
□ Citologia de medula óssea
□ PCR para *Leishmania* a partir de medula óssea ou de linfonodo(s)
□ PCR para *Leishmania* a partir de sangue
□ Outro

25. Se selecionou "Outro", a qual se refere?

26. Assumindo o diagnóstico definitivo de leishmaniose, que tratamento faria neste caso?
□ Alopurinol
□ Alopurinol e análogos de nucleótidos
□ Alopurinol e antiamoniato de meglumina
□ Alopurinol e domperidona
□ Alopurinol e miltefosina
□ Análogos de nucleótidos + AHCC (composto correlacionado com hexose ativa)
□ Antiamoniato de meglumina
□ Domperidona
□ Miltefosina
□ Outro

27. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

<u>Caso clínico 4</u>
Cão, macho, com 6 anos de idade. Habita em zona geográfica onde a leishmaniose é endémica e não faz prevenção para a doença (repelente, vacina ou imunomoduladores).

Motivo de consulta: Epistaxis.

EEG: Sem alterações consideráveis.

Exames laboratoriais: Anemia não regenerativa ligeira, hiperglobulinémia sem hipoalbulinémia, creatinina <1.4 mg/dL (normal), ligeira proteinúria (RPCU: 0.5) com sedimento inativo.

Serologia (imunofluorescência):
Título de anticorpos anti-*Leishmania* positivo na diluição 1:640.
Serologia para outros agentes vetoriais concomitantes (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)* negativa.
Valores serológicos de referência: Limiar de positividade: título de anticorpos anti-*Leishmania* positivo na diluição 1:80

28. Considerando a leishmaniose como um dos principais diagnósticos diferenciais deste caso, consideraria necessária a realização de mais exames complementares de diagnóstico?
□ Não □ Sim

29. Se sim, qual faria preferencialmente?
□ Citologia de linfonodo(s)
□ Citologia de medula óssea
□ PCR para *Leishmania* a partir de medula óssea ou de linfonodo(s)
□ PCR para *Leishmania* a partir de sangue
□ Outro

30. Se selecionou "Outro", a qual se refere?

31. Assumindo agora o diagnóstico definitivo de leishmaniose, que tratamento faria neste caso?

□ Alopurinol
□ Alopurinol e análogos de nucleótidos
□ Alopurinol e antiamoniato de meglumina
□ Alopurinol e domperidona
□ Alopurinol e miltefosina
□ Análogos de nucleótidos + AHCC (composto correlacionado com hexose ativa)
□ Antiamoniato de meglumina
□ Domperidona
□ Eutanásia
□ Miltefosina
□ Outro

32. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

33. Considerando que este animal tinha um RPCU de 0.5 com sedimento inativo, trataria a proteinúria neste caso?

□ Não □ Sim

34. Se sim, que tratamento faria preferencialmente?

□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

35. Se selecionou "Outro", qual o fármaco a que se refere?

36. Além do que assinalou como preferencial, associaria algum(ns) outro(s) fármaco? Se sim, qual(is)?

□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Não associaria nenhum outro fármaco
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

37. Faria dieta formulada para doença renal?

□ Não □ Sim

<u>Caso clínico 5</u>

Cão, macho, com 12 anos de idade. Habita em zona geográfica onde a leishmaniose é endémica e não faz prevenção para a doença (repelente, vacina ou imunomoduladores).

Motivo de consulta: prostração, anorexia, perda de peso, feridas na pele, PU/PD.

EEG: mucosas pálidas, lesões de dermatite exfoliativa facial, assim como nas almofadas plantares, opacidade na córnea, onicogrifose, hiperqueratose e ulceração nasal.

Exames laboratoriais: Anemia não regenerativa moderada; hiperglobulinémia com gamopatia policlonal e hipoalbuminémia. Azotémia (Creatinina 3.5mg/dL), proteinúria (RPCU: 6.2) com sedimento inativo.

Serologia (imunofluorescência): título de anticorpos anti-*Leishmania* positivo na diluição 1:640. Serologia para outros agentes vetoriais concomitantes (*Anaplasma* spp.*, Babesia* spp.*,*

Ehrlichia spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)* negativa.
Valores serológicos de referência:
Limiar de positividade: título de anticorpos anti-*Leishmania* positivo na diluição 1:80

38. Considerando a leishmaniose como um dos principais diagnósticos diferenciais deste caso, consideraria necessária a realização de mais exames complementares de diagnóstico?
□ Não □ Sim

39. Se sim, qual faria preferencialmente?
□ Citologia de linfonodo(s)
□ Citologia de medula óssea
□ PCR para *Leishmania* a partir de medula óssea ou de linfonodo(s)
□ PCR para *Leishmania* a partir de sangue
□ Outro

40. Se selecionou "Outro", a qual se refere?

41. Que tratamento faria neste caso?
□ Alopurinol
□ Alopurinol e análogos de nucleótidos
□ Alopurinol e antiamoniato de meglumina
□ Alopurinol e domperidona
□ Alopurinol e miltefosina
□ Análogos de nucleótidos + AHCC (composto correlacionado com hexose ativa)
□ Antiamoniato de meglumina
□ Domperidona
□ Eutanásia
□ Miltefosina
□ Outro

42. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

43. Considerando que este animal tem um RPCU de 6.2 com sedimento inativo, trataria a proteinúria neste caso?
□ Não □ Sim

44. Se sim, que tratamento faria preferencialmente?
□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

45. Se selecionou "Outro", qual o fármaco a que se refere?

46. Além do que assinalou como preferencial, associaria algum(ns) outro(s) fármaco? Se sim, qual(is)?
□ Bloqueadores de canais de cálcio (ex. amlodipina)
□ Bloqueadores dos recetores de aldosterona (ex. espironolactona)
□ Bloqueadores dos recetores da angiotensina (BRA) – ex. losartan, telmisartan
□ Inibidores da enzima de conversão da angiotensina (IECA, ex. enalapril, benazepril)
□ Outro
□ Terapia antitrombótica (aspirina, clopidogrel)

47. Faria dieta formulada para doença renal neste caso?
□ Não □ Sim

Caso clínico 6

Cão, macho, com 3 anos de idade. Habita em zona geográfica onde a leishmaniose não é endémica, mas irá mover-se para zona endémica, pelo que o motivo da consulta foi a realização de rastreio da doença para posterior aplicação de profilaxia.

EEG: normal, sem alterações.

48. Que profilaxia faria? Assinale uma ou mais opções.

□ Domperidona □ Repelente/inseticida □ Vacinação

TERCEIRA PARTE - OUTRAS QUESTÕES

Esta terceira e última parte refere-se às normas orientadoras ("guidelines") existentes relativas ao diagnóstico e tratamento da leishmaniose canina.

49. Tem conhecimento de normas orientadoras para o diagnóstico e tratamento da leishmaniose?

□ Não □ Sim

50. Se sim, quais as normas orientadoras que utiliza preferencialmente?

□ World Health Organization (WHO)
□ European Food Safety Authority (EFSA)
□ European Scientific Counsel Companion Animal Parasites (ESCCAP)
□ World Organization for Animal Health (OIE)
□ Canine Leishmaniosis Working Group (CLWG)
□ Associação LeishVet
□ Outro
□ Tenho conhecimento, mas não aplico nenhuma norma orientadora em específico.

51. Se selecionou "Outro", a qual se refere?

ESTADIAMENTO SEGUNDO AS NORMAS DA ASSOCIAÇÃO LEISHVET

Considerando as linhas orientadoras formuladas pelo grupo LeishVet, em que grau classificaria cada um dos casos clínicos?

52. Caso clínico 1
□ Grau I
□ Grau II
□ Grau III
□ Grau IV
□ Prefiro não responder/não sei

53. Caso clínico 2
□ Grau I
□ Grau II
□ Grau III
□ Grau IV
□ Prefiro não responder/não sei

54. Caso clínico 3
□ Grau I
□ Grau II
□ Grau III
□ Grau IV
□ Prefiro não responder/não sei

55. Caso clínico 4
□ Grau I
□ Grau II
□ Grau III
□ Grau IV
□ Prefiro não responder/não sei

56. Caso clínico 5
□ Grau I
□ Grau II
□ Grau III
□ Grau IV
□ Prefiro não responder/não sei

MEIOS COMPLEMENTARES DE DIAGNÓSTICO. TRATAMENTO.

57. Para além da observação dos sinais clínicos, que meios utiliza para diagnosticar leishmaniose de forma rotineira? Assinale uma ou mais opções.

□ Citologia
□ Histopatologia
□ Imuno-histoquímica
□ Outro
□ PCR
□Serologia – ELISA
□Serologia – IFAT
□Serologia – imunocromatografia (teste rápido)

58. Se selecionou "Outro", a qual se refere?

59. Caso utilize PCR, que tipo de amostras utiliza preferencialmente? Assinale uma ou mais opções.

□ Baço
□ Camada leucocitária (“buffy coat”)
□ Linfonodos
□ Medula óssea
□ Não utilizo PCR
□ Outro
□ Pele
□ Sangue
□ Urina
□ Zaragatoas conjuntivais

60. Se selecionou "Outro", a que se refere?

61. Usa imunossupressores aquando de uma suspeita de glomerulonefrite secundária a leishmaniose clínica?

□ Não
□ Sim

62. Se sim, qual a sua primeira escolha?

□ Azatioprina
□ Ciclofosfamida
□ Ciclosporina
□ Clorambucil
□ Micofenolato de Mofetil
□ Prednisolona
□ Outro

63. Se selecionou "Outro" na alínea anterior, a que tratamento se refere?

64. Em que dose? Se for a recomendada na posologia, refira apenas "dose recomendada".

Annexe 9. Questionnaire provided online – English version

Management of canine Leishmaniosis in Portugal – diagnosis and treatment

Hello! If you are a veterinarian working in Portugal, in small animal clinics – namely, with the canine species – then, this questionnaire is for you!

This questionnaire is part of a dissertation to obtain a Master's degree in Veterinary Medicine, approaching the diagnosis and treatment of Canine Leishmaniosis in Portugal.

It consists, essentially, of three parts: "Veterinarian profile", "Clinical cases" and "Other questions", always related to canine leishmaniosis.

The questionnaire contains around 50 questions and will take approximately 15 minutes to answer. It is worth noting that the questions are anonymous and will be analysed with proper confidentially, thus we kindly ask you to answer honestly, to allow obtaining valid and representative conclusions. Data privacy will be equally preserved in case those being used in written or oral communications, or in publications that may result from the study.

In case of any questions related to this questionnaire, please contact, via email, the student Marta Monteiro (martanemm@hotmail.com) or Professor Doctor Rodolfo Oliveira Leal (rleal@fmv.ulisboa.pt).

More information concerning the study will be sent to the participants, via email, if it is their willing. In that case, please send an email showing your interest to martanemm@hotmail.com.

Thank you for your collaboration and time spent replying this questionnaire!

FIRST PART – VETERINARIAN PROFILE
PRIMEIRA PARTE - PERFIL DO MÉDICO VETERINÁRIO

1. Which is your academic degree in veterinary medicine? Select the highest you have, among the following.
□ Doctor of Philosophy (PhD) in Veterinary Sciences
□ Doctor in Veterinary Medicine (DVM) (pre-Bolonha)
□ Master of Science (MSc) (post-Bolonha)

2. How many years of clinical practice with dogs do you have?
□ Less than 2 □ 2 to 5 □ 6 to 10
□ 11 to 15 □ 16 to 20 □ More than 20

3. Select the district in which you mostly work as a veterinarian.
(List of Portuguese districts).

4. The type of area you work in, is:
□ Rural □ Urban □ Both

5. Approximately, how many confirmed cases of leishmaniosis did you have in the past 12 months?
□ Less than 5 □ 5 to 10 □ 11 to 15 □ 16 to 20 □ More than 20

SECOND PART – CLINICAL CASES
Six clinical cases will be presented, always related to canine leishmaniosis. Answers should be given bearing in mind your clinical practice context and knowledge on this topic.

1st Clinical case
Dog, male, 7 years old, living in a geographic area where leishmaniosis is endemic and does not follow any prevention measures for the disease (repellents, vaccine, immunomodulators).

Reason for consultation: prostration, anorexia, weight loss, polyuria/polydipsia, auricular lesions.

General examination: Pale mucosae, generalised lymphadenomegaly, mucocutaneous ulcerative lesions, ears' crusts.

Ophtalmologic exam: blepharitis, uveitis (bilateral intraocular pressure reduction, erythema).

Abdominal ultrasound: splenomegaly.

Laboratory tests: Moderate nonregenerative anaemia, hyperproteinemia, hypoalbuminemia, hyperglobulinemia with polyclonal gammopathy.
Mild azotaemia (creatinine: 1.9mg/dL); urinalysis: USG: 1.018, proteinuria (RPCU: 1.2) with inactive sediment.
Normal systemic blood pressure.

Serology (immunofluorescence):
Anti-*Leishmania* antibody titre: positive for 1:320 dilution.
Negative serology for other concomitant vectorial agents (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)*.
Reference serological values:
Cut-off: positive anti-*Leishmania* antibody titres at 1:80 dilution.

6. Admitting leishmaniosis as one of the main differential diagnostics in this case, would you consider necessary doing more complementary diagnostic exams?
□ No □ Yes

7. If yes, which one would you prefer to do?
□ Lymph node(s) cytology
□ Bone marrow cytology
□ *Leishmania* PCR on spleen
□ *Leishmania* PCR on bone marrow or lymph node(s)
□ *Leishmania* PCR on blood
□ Other

8. If you selected "Other", which one is it?

9. Which treatment would you do in this case?
□ Allopurinol
□ Allopurinol and nucleotide analogues
□ Allopurinol and meglumine antimoniate
□ Allopurinol and domperidone
□ Allopurinol and miltefosine
□ Nucleotide analogues and active hexose correlated compounds (AHCC)
□ Meglumine antimoniate
□ Domperidone
□ Eutanasia
□ Miltefosine
□ Other

10. In case you selected "Other", which treatment is it?

11. Considering that this animal had a UPC of 1.2 with inactive sediment, would you treat proteinuria in this case?
□ No □ Yes

12. If yes, which treatment would you prefer to apply?
□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)

□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

13. In case you selected "Other", which drug is it?

14. Besides the one selected as preferential, would you add any other drug(s)? If yes, which one(s)?
□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)
□ I would not add any other drug
□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

15. Would you start a renal diet?
□ No □ Yes

2nd Clinical case
Dog, male, 5 years old, living in a geographic area where leishmaniosis is endemic and does not follow any prevention measures for the disease (repellents, vaccine, immunomodulators).

Reason for consultation: vaccination.

General examination: No abnormalities.

Laboratory tests: CBC, biochemical profile and urinalysis without abnormalities.

Serology (immunofluorescence):
Anti-*Leishmania* antibody titre: positive for 1:80 dilution.
Negative serology for other concomitant vectorial agents (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)*.
Reference serological values:
Cut-off: positive anti-*Leishmania* antibody titres at 1:80 dilution.

16. Would you consider necessary to perform more complementary exams in this case?
□ No □ Yes

17. If yes, which one would you prefer to do?
□ Lymph node(s) cytology
□ Bone marrow cytology
□ *Leishmania* PCR on bone marrow or lymph node(s)
□ *Leishmania* PCR on blood
□ Other

18. In case you selected "Other", which one is it?

19. Would you treat this case?
□ No □ Yes

20. If yes, which treatment would you do?
□ Allopurinol
□ Allopurinol and nucleotide analogues
□ Meglumine antimoniate
□ Domperidone

□ Allopurinol and meglumine antimoniate
□ Allopurinol and domperidone
□ Allopurinol and miltefosine
□ Nucleotide analogues and active hexose correlated compounds (AHCC)
□ Miltefosine
□ Other

21. In case you selected "Other", which treatment is it?

22. Regardless of applying treatment or not, would you monitor and/or apply preventive measures in this case?
□ Only clinical monitoring (including repeating serology in 3-6 months)
□ Only prevention against leishmaniosis, adequate to the clinical case
□ Adequate prevention and clinical monitoring (including repeating serology in 3-6 months)

3rd Clinical case
Dog, female, 8 years old, spayed. Travelled 6 months before to an area where leishmaniosis is endemic but did not perform any preventive measure for the disease (repellent, vaccination or immunomodulators), before or during the trip.

Reason for consultation: lethargy and weight loss.

General examination: Periorbital alopecia, footpad exfoliative dermatitis, generalised lymphadenomegaly.

Laboratory tests: Mild nonregenerative anaemia, hyperproteinemia, hypoalbuminemia, hyperglobulinemia with polyclonal gammopathy. No azotaemia neither proteinuria.

Serology (immunofluorescence):
Anti-*Leishmania* antibody titre: positive for 1:160 dilution.
Negative serology for other concomitant vectorial agents (*Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Borrelia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Hemobartonella canis, Bartonella* spp., *Dirofilaria* spp., *Trypanosoma* spp.).
Reference serological values:
Cut-off: positive anti-*Leishmania* antibody titres at 1:80 dilution.

23. Admitting leishmaniosis as one of the main differential diagnostics in this case, would you consider necessary doing more complementary diagnostic exams?
□ No □ Yes

24. If yes, which one would you prefer to do?
□ Lymph node(s) cytology
□ Bone marrow cytology
□ *Leishmania* PCR on bone marrow or lymph node(s)
□ *Leishmania* PCR on blood
□ Other

25. In case you selected "Other", which one is it?

26. Assuming a definitive diagnosis of leishmaniosis, which treatment would you apply?
□ Allopurinol
□ Allopurinol and nucleotide analogues
□ Allopurinol and meglumine antimoniate
□ Allopurinol and domperidone
□ Allopurinol and miltefosine
□ Meglumine antimoniate
□ Domperidone
□ Miltefosine
□ Other

□ Nucleotide analogues and active hexose correlated compounds (AHCC)

27. In case you selected "Other", which treatment is it?

4th clinical case

Dog, male, 6 years old. Lives in a geographic area where leishmaniosis is endemic and does not follow any prevention measures for the disease (repellents, vaccine, immunomodulators).

Reason for consultation: Epistaxis.

General examination: No abnormalities.

Laboratory tests: Mild nonregenerative anaemia. Hyperglobulinaemia without hypoalbuminemia. Creatinine <1.4 mg/dL (normal), borderline proteinuria (RPCU: 0.5), inactive sediment.

Serology (immunofluorescence):
Anti-*Leishmania* antibody titre: positive for 1:640 dilution.
Negative serology for other concomitant vectorial agents (*Anaplasma* spp., *Babesia* spp., *Ehrlichia* spp., *Borrelia* spp., *Rickettsia* spp., *Hepatozoon* spp., *Hemobartonella canis, Bartonella* spp., *Dirofilaria* spp., *Trypanosoma* spp.).
Reference serological values:
Cut-off: positive anti-*Leishmania* antibody titres at 1:80 dilution.

28. Admitting leishmaniosis as one of the main differential diagnostics in this case, would you consider necessary doing more complementary diagnostic exams?
□ No □ Yes

29. If yes, which one would you prefer to do?
□ Lymph node(s) cytology
□ Bone marrow cytology
□ *Leishmania* PCR on bone marrow or lymph node(s)
□ *Leishmania* PCR on blood
□ Other

30. In case you selected "Other", which one is it?

31. Assuming a definitive diagnosis of leishmaniosis, which treatment would you apply?
□ Allopurinol
□ Allopurinol and nucleotide analogues
□ Allopurinol and meglumine antimoniate
□ Allopurinol and domperidone
□ Allopurinol and miltefosine
□ Meglumine antimoniate
□ Domperidone
□ Eutanasia
□ Miltefosine
□ Other
□ Nucleotide analogues and active hexose correlated compounds (AHCC)

32. In case you selected "Other", which treatment is it?

33. Considering that this animal had a UPC of 0.5 with inactive sediment, would you treat proteinuria in this case?
□ No □ Yes

34. If yes, which treatment would you prefer to apply?

□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)
□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

35. In case you selected "Other", which drug is it?

36. Besides the one selected as preferential, would you add any other drug(s)? If yes, which one(s)?
□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)
□ I would not add any other drug
□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

37. Would you start a renal diet?
□ No □ Yes

5th Clinical case
Dog, male, 12 years old. Lives in a geographic area where leishmaniosis is endemic and does not follow any prevention measures for the disease (repellents, vaccine, immunomodulators).

Reason for consultation: lethargy, anorexia, weight loss, skin wounds, polyuria/polydipsia.

General examination: Pale mucosae, facial and plantar exfoliative dermatitis, corneal opacity, onychogryphosis, nasal hyperkeratosis and ulceration.

Laboratory tests: Moderate nonregenerative anaemia; hyperglobulinemia with polyclonal gammopathy, hypoalbuminemia. Azotaemia (creatinine: 3.5mg/dL), proteinuria (UPC: 6.2), inactive sediment.

Serology (immunofluorescence):
Anti-*Leishmania* antibody titre: positive for 1:640 dilution.
Negative serology for other concomitant vectorial agents (*Anaplasma* spp.*, Babesia* spp.*, Ehrlichia* spp.*, Borrelia* spp.*, Rickettsia* spp.*, Hepatozoon* spp.*, Hemobartonella canis, Bartonella* spp.*, Dirofilaria* spp.*, Trypanosoma* spp.*)*.
Reference serological values:
Cut-off: positive anti-*Leishmania* antibody titres at 1:80 dilution.

38. Admitting leishmaniosis as one of the main differential diagnostics in this case, would you consider necessary doing more complementary diagnostic exams?
□ No □ Yes

39. If yes, which one would you prefer to do?
□ Lymph node(s) cytology
□ Bone marrow cytology
□ *Leishmania* PCR on bone marrow or lymph node(s)
□ *Leishmania* PCR on blood
□ Other

40. In case you selected "Other", which one is it?

41. Which treatment would you apply?

□ Allopurinol
□ Allopurinol and nucleotide analogues
□ Allopurinol and meglumine antimoniate
□ Allopurinol and domperidone
□ Allopurinol and miltefosine
□ Nucleotide analogues and active hexose correlated compounds (AHCC)
□ Meglumine antimoniate
□ Domperidone
□ Eutanasia
□ Miltefosine
□ Other

42. In case you selected "Other", which treatment is it?

43. Considering that this animal had a UPC of 6.2 with inactive sediment, would you treat proteinuria in this case?

□ No □ Yes

44. If yes, which treatment would you prefer to apply?

□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)
□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

45. In case you selected "Other", which drug is it?

46. Besides the one selected as preferential, would you add any other drug(s)? If yes, which one(s)?

□ Calcium channel blockers (*e.g.* amlodipine)
□ Aldosterone receptor blockers (*e.g.* spironolactone)
□ Angiotensin receptor blockers (ARB, *e.g*. losartan, telmisartan)
□ Angiotensin-converting enzyme inhibitors (ACEI, *e.g.* enalapril, benazepril)
□ Other
□ Antithrombotic therapy (*e.g.* aspirin, clopidogrel)

47. Would you start a renal diet?

□ No □ Yes

6th Clinical case

Dog, male, 3 years old. Lives in a geographic area where leishmaniosis is not endemic but will move to an endemic zone. Therefore, came for screening for leishmaniosis and, then, apply prophylaxis.

General examination: normal, without abnormalities.

48. Which prophylaxis would you do? Select one or more options.

□ Domperidone □ Repellent/insecticide □ Vaccination

THIRD PART – OTHER QUESTIONS

This third and last part refers to the current guidelines concerning diagnosis and treatment of canine leishmaniosis.

49. Do you know about any guidelines for diagnosis and treatment of leishmaniosis?

□ No □ Yes

50. If yes, which ones do you prefer to use?

□ World Health Organization (WHO)
□ European Food Safety Authority (EFSA)
□ European Scientific Counsel Companion Animal Parasites (ESCCAP)
□ World Organization for Animal Health (OIE)
□ Canine Leishmaniosis Working Group (CLWG)
□ LeishVet group
□ Other
□ I have knowledge, but I do not apply any specific guidelines.

51. In case you selected "Other", which one is it?

STAGING ACCORDING WITH THE LEISHVET GUIDELINES

Bearing in mind the LeishVet guidelines, in which stage would you include each of the following clinical cases?

52. 1st clinical case
□ Stage I
□ Stage II
□ Stage III
□ Stage IV
□ I prefer not to answer/I do not know

53. 2nd clinical case
□ Stage I
□ Stage II
□ Stage III
□ Stage IV
□ I prefer not to answer/I do not know

54. 3rd clinical case□ **Stage I**
□ Stage II
□ Stage III
□ Stage IV
□ I prefer not to answer/I do not know

55. 4th clinical case
□ Stage I
□ Stage II
□ Stage III
□ Stage IV
□ I prefer not to answer/I do not know

56. 5th clinical case
□ Stage I
□ Stage II
□ Stage III
□ Stage IV
□ I prefer not to answer/I do not know

COMPLEMENTARY DIAGNOSTIC METHODS. TREATMENT.

57. Besides the observation of clinical signs, which methods do you use to diagnose leishmaniosis in a routine basis? Select one or more options.

□ Cytology
□ Histopathology
□ Immunohistochemistry
□ Other
□ PCR
□Serology – ELISA
□Serology – IFAT
□Serology – immunochromatography (rapid test)

58. In case you selected "Other", which one is it?

59. In case you use PCR, which samples do you prefer touse? Select one or more options.

□ Spleen
□ Buffy coat
□ Lymph nodes
□ Bone marrow
□ I do not use PCR
□ Other
□ Skin
□ Blood
□ Urine
□ Conjunctival swabs

60. In case you selected "Other", which one is it?

61. Do you use immunosuppressants when suspecting of glomerulonephritis secondary to clinical leishmaniosis?

□ No □ Yes

62. If yes, which would be your first choice?

□ Azatioprine □ Cyclosporine □ Micophenolate mofetil □ Other
□ Cyclophosphamide □ Chlorambucil □ Prednisolone

63. In case you selected "Other", which treatment is it?

64. In which dosage? In case it is the recommended in the package leaflet, just mention "recommended dosage".