25TH FECAVA EUROCONGRESS

5TH SEPTEMBER 2019 / ST. PETERSBURG, RUSSIA

PROCEEDINGS

OF THE

FECAVA SYMPOSIUM ON VECTOR-BORNE DISEASES

FECAVA

Federation of European Companion Animal Veterinary Associations

CANINE LEISHMANIOIS – DIAGNOSIS, TREATMENT AND PREVENTION

PROF. GAD BANETH, DVM, PHD, DIPL. ECVCP
KORET SCHOOL OF VETERINARY MEDICINE, THE HEBREW UNIVERSITY OF JERUSALEM, REHOVOT, ISRAEL

Canine leishmaniosis (CanL) is a major zoonotic disease endemic in more than 70 countries in the world. It is enzootic in regions of southern Europe, Northern Africa, the Middle East, Central Asia, China, South and Central America and has sporadically emerged also in the USA. CanL is also an important concern in non-endemic countries where imported disease constitutes a veterinary and public health problem. Dogs are the main animal reservoir for human visceral leishmaniosis and the disease is usually fatal if not treated in people. Phlebotomine sand flies are the vectors of *Leishmania infantum.* Surveys employing the polymerase chain reaction (PCR) method for the detection of leishmanial DNA in canine tissues, or combining serology and DNA detection, have revealed infection rates approaching 70% in some foci. It has been estimated based on seroprevalence studies from Italy, Spain, France and Portugal that 2.5 million dogs in these countries are infected. The number of infected dogs in South America is also estimated in millions with high infection rates in some areas of Brazil and Venezuela. CanL with the dog as a major reservoir for the parasite is the main form of the disease in many parts of the world including Brazil, the Mediterranean region and China.

Leishmania is a diphasic parasite that complete its life cycle in two hosts, a sand fly which harbors the flagellated extracellular promastigotes and a mammal where the intracellular amastigote parasite forms develop. Dogs are infected by *Leishmania* promastigotes deposited in the skin during the bites of infected female sand fly vectors. The promastigotes invade host cells and replicate as intracellular amastigotes. The disease incubation period prior to the appearance of clinical signs may last months to years, during which the parasite disseminates from the skin throughout the host's body primarily to the hemolympoid organs.

Transmission of *L. infantum* through blood products has been reported in humans and also in dogs that received blood transfusions from infected donors. Although natural transmission of *L. infantum* takes place by the bite of sand flies, vertical in-utero transmission from dam to its offspring has been documented. Direct transmission without involvement of an hematophageous vector has been suspected in North America and also in some cases of infection in areas of Europe where vectors of the disease are apparently absent.

Population studies in *Leishmania-endemic* areas have shown that a proportion of the canine population develops a clinical disease, another fraction has persistent asymptomatic infection, while yet another fraction is resistant to the infection or intermittently resolves it without developing clinical signs. The immune responses mounted by dogs at the time of infection and thereafter appear to be an important factor in determining if they will develop a lasting infection and whether and when it will progress from an asymptomatic state into a symptomatic disease. Specific immune responses play a major role in susceptibility to infection. During infection, dogs become increasingly immunosupressed and may develop decreased CD4+ lymphocyte counts and a decrease in the CD4+/CD8+ ratio. Moreover, it has been demonstrated that the infectiousness of dogs with leishmaniosis to sand flies increases with the decrease in CD4+ counts. Immune-mediated mechanisms are responsible for much of the pathological findings in CanL. Circulating immune complexes and antinuclear antibodies have been detected in animals with CanL. Glomerulonephritis associated with the deposition of immune complexes in the kidneys is a hallmark of the disease. Renal pathology is present, even if not manifested clinically, in the majority of dogs with this disease.

Susceptibility or resistance to CanL is influenced by genetics. The presence of overt CanL among Ibizian hounds in the Balearic islands is rare and significantly lower than among other breeds and it has been shown that this breed mounts a predominantly cellular immune response against *L. infatnum*. Other breeds that originate from regions that are not enzootic for leishmaniosis such as the Boxer, Rottweiler and German shepherd are overrepresented in CanL surveys. A study on the polymorphism of the canine Slc11a1(NRAMP1) gene which encodes an iron transporter protein involved in the control of intraphagosomal replication of parasites and macrophage activation, has implied that susceptible dogs have mutations in this gene. A DLA class II DLA-DRB1

genotype, which is a dog major histocompatibility complex (MHC) class II allele has been linked to the risk of being infected in an endemic area in Brazil.

The typical history reported by owners of dogs with CanL includes the appearance of skin lesions, ocular abnormalities, or epistaxis. These are commonly accompanied by weight loss, exercise intolerance and lethargy. Dogs from all breeds can be infected with leishmaniosis. The age distribution of the disease is bimodal with a peak of prevalence at 2-4 years and a secondary peak from the age of 7 years. The incubation period prior to the appearance of clinical signs may last 3 months up to several years. On physical examination, the main clinical signs associated with CanL are dermal lesions, lymphadenomegaly, splenomegaly, abnormal nails growth (onychogryposis) and poor body condition. Additional findings include: epistaxis, renal failure, decreased appetite, polyuria and polydypsia, vomiting, melena and lameness. Sixteen to 80% of the dogs with clinical leishmaniosis have ocular or pericolar lesions including keratoconjunctivitis and uveitis. The dermal lesions associated with CanL include exfoliative dermatitis which can be generalized or localized over the face, ears and limbs. Nodular dermatitis has been reported and cutaneous ulceration is frequently found with bleeding from pinnal and other local ulceration sites. A mild form of papular dermatitis has also been described. The most consistent serum biochemistry findings in dogs with clinical CanL are serum hyperproteinemia with hyperglobulinemia and hypoalbuminemia resulting in a decreased albumin/globulin ratio. Grossly elevated activities of liver enzymes or azotemia are found in only a minority of dogs with CanL. However, proteinuria and some degree of renal pathology is frequently present in dogs with CanL and subsequent renal failure due to immune-complex glomerulonephritis eventually develops and is believed to be the main cause of death in dogs with CanL. Epistaxis, ocular abnormalities or real failure may be the only presenting clinical findings in CanL and this disease should be considered among the differential diagnoses for these conditions in endemic areas or in dogs that have traveled or were imported from an endemic region. Marked hyperglobulinemia with no apparent cause in dogs from endemic regions should also be investigated for CanL.

The diagnosis of CanL is performed for different indications and using different methods for these different indication. CanL is a good example of a disease in which infection does not equal clinical illness due to the high prevalence of subclinical infection. This makes CanL a diagnostic challenge for the veterinary practitioner, clinical pathologist and public health official in endemic countries as well as non-endemic regions where imported infection is a concern. *Leishmania* amastigotes can be demonstrated by cytology from the skin, lymph nodes, spleen or bone marrow stained with Giemsa stain or a quick commercial stain. Detection of amastigotes by cytology is frequently unrewarding due to a low number of detectable parasites present even in dogs with a full blown clinical disease. *Leishmania* parasites may also be viewed in histophathologic formalin-fixed, parafifin-embedded biopsy sections of the skin or other infected organs. Definite identification of parasites within tissue macrophages may be difficult and an immunohistichemical staining method can be employed to detect or verify the presence of *Leishmania* in the tissue. Various serological methods for the detection of anti-*Leishmania* antibodies have been developed. These include indirect immunofluoresence assays (IFA), enzyme-linked immunosorbent assay (ELISA), direct agglutination assays (DAT) and western blotting. A purified recombinant antigen for ELISA, rK39, has been used for detection of visceral leishmaniosis in humans and dogs. In general, good sensitivities and specificities are gained with these methods for the diagnosis of clinical CanL cases. Detection of parasite-specific DNA in tissues by PCR allows sensitive and specific diagnosis. Several different assays with various target sequences using genomic or kinetoplast DNA (kDNA) have been developed for CanL. PCR can be performed on DNA extracted from tissues, blood or even from histopathologic specimens. Assays based on kDNA appear to be the most sensitive for direct detection in infected issues.

The main drugs used for treatment of CanL include the pentavalent antimony meglumine antimoniate (Glucantime®) which selectively inhibits leishmanial glycolysis and fatty acid oxidation and allopurinol that acts by inhibiting protein translation through interfering with RNA synthesis. Treatment with these drugs is frequently combined with meglumine antimoniate administered for 4 weeks and allopurinol used for long term therapy. Miltefosine (Milteforan®) is an additional oral anti-leishmanial drug that can be used for the first month of treatment in combination with allopurinol instead of meglumine antimoniate. Anti-leishmanial treatment may only achieve only temporary clinical improvement in dogs with leishmaniosis and it is sometimes not associated with the elimination of the parasite. Treated dogs often remain carriers of the disease, may be infectious to sand flies and commonly experience clinical relapses. Owners must receive a thorough and realistic explanation about the disease, its zoonotic potential, the prognosis for their dog, and what should be expected from treatment. Treatment of CanL can be stopped when the following three conditions are all met: (1) disappearance of clinical signs; (2) normalization of the hematology, blood biochemistry profile and urinalysis; (3) serology should become negative (below the cut-off titer of quantitative serological assays).

Disease relapse of dogs with CanL during allopurinol treatment has been described and associated with allopurinol

resistance of *L. infantum* isolated from these animals. *Leishmania infantum* strains isolated in culture from relapsed dogs were significantly less susceptible to allopurinol in comparison to isolates from dogs before treatment and those from dogs under treatment with no clinical relapse. Resistance was consistent in three forms of the parasite strains tested including intracellular amastigotes, promastigotes and axenic amastigotes. These findings indicate that resistance to allopurinol may develop in dogs experiencing clinical disease relapse which may transmit resistant parasite to other dogs and also enhance the danger of transmission the parasite to other animal species and humans

Ancilliary treatment of CanL includes treatment with domepridone (Leishguard®) which is registered in Europe for prophylaxis of the disease. Its a dopamine D2 receptor antagonist reported to have immunostimulant properties via the stimulation of prolactin secretion which acts as a pro-inflammatory cytokine. Domperidone is claimed to reduce the risk of developing active leishmaniosis infection and clinical disease by stimulating specific cellular immunity. An additional ancilliary treatment includes a dietary supplement of nucleotides and active hexose as an adjunctive therapy for canine leishmaniosis.

Commercial vaccines against CanL have been approved in Brazil and Europe, however they do not prevent infection but rather decrease the occurrence of clinical disease. The use of topical insecticides against CanL in collars or spot-on formulation containing pyrethroids has been shown to be effective in reducing disease transmission. Delthamethrin-impregnated collars and permethrin with imidacloprid spot on drops have been shown to significantly reduce the number of sand fly bites to dogs under experimental transmission and demonstrated decreased infection transmission in field studies.