Leishmaniosis (*Leishmania infantum* infection) in dogs

M. Gharbi [(1)*], M. Mhadhbi [(1)], A. Rejeb [(2)], K. Jaouadi [(1)], M. Rouatbi [(1)] & M.A. Darghouth [(1)]

(1) Laboratoire de Parasitologie, Université de la Manouba, École Nationale de Médecine Vétérinaire de Sidi Thabet, 2020 Sidi Thabet, Tunisia
(2) Laboratoire d'Anatomie Pathologique, Université de la Manouba, École Nationale de Médecine Vétérinaire de Sidi Thabet, 2020 Sidi Thabet, Tunisia
*Corresponding author: gharbim@yahoo.fr

Summary

The authors present an overview of canine leishmaniosis due to *Leishmania infantum*. This protozoan is transmitted by sandflies and the disease is frequently characterised by chronic evolution. Cutaneous and visceral clinical signs appear as the infection progresses. Lymph node enlargement, emaciation and skin lesions are the main signs observed in the classical forms of the disease. Control is difficult since infected dogs remain carriers for years and may relapse at any time. The mass screening of infected animals and their treatment or euthanasia represent the best way to reduce the prevalence of this disease in endemic regions. Further research is needed to improve the efficiency of the vaccines available to protect dogs against infection. This disease is zoonotic; in humans, clinical cases are reported mainly in elderly people, the young and those whose immune systems have been compromised.

Keywords

Canine – Dog – *Leishmania infantum* – Leishmaniosis – Parasite – Sandfly – Zoonosis.

Introduction

Canine visceral leishmaniosis (CvL) is a complex of infectious, vector-borne, zoonotic diseases caused by the presence and multiplication of a protozoan belonging to the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). These infections are due to several species with varying virulence, they have different epidemiological and clinical patterns, and some are zoonotic. The most important cycle of *Leishmania infantum* takes place between dogs and sandflies but other canid species (both domestic and wild) are involved. While acknowledging the increasing number of emerging zoonotic diseases, the authors would like to point out that we must not forget the importance of the 'classical' diseases, such as leishmaniosis, rabies, tuberculosis and brucellosis. The implementation of an effective CvL control programme requires a good knowledge of *L. infantum*, sandflies, the host and the regional epidemiological pattern of this disease. Indeed, the interactions of the parasite, host and vector lead to a specific epidemiological pattern. The principal aim of this paper is to review the information gathered on CvL caused by *L. infantum*.

Sandflies

Taxonomy of sandflies

The current classification of sandflies remains controversial and cumbersome. Lewis *et al.* (1977) proposed two genera of Old World species (*Phlebotomus* spp. and *Sergentomyia* spp.) and three genera of New World species (*Lutzomyia* spp., *Brumptomyia* spp. and *Warileya* spp.) (1). The genus *Phlebotomus* consists of 11 subgenera, 96 species and 17 subspecies (2). Galati (2003) recognised 464 species of Neotropical sandflies grouped into 23 genera, 20 subgenera and three groups (3, 4).

Geographic distribution of sandflies

Sandflies are principally present in the warm zones of Asia, Africa, Australia, southern Europe and the Americas (5). Their distribution extends northwards to just above a latitude of 50°N in south-west Canada (6) and just below this latitude in northern France and Mongolia (2). Their southernmost distribution ends at a latitude of 40°S, but they are absent from New Zealand and the Pacific Islands (the New Zealand 'sandflies' are actually black flies of the

Austrosimulium species) (7). Their altitudinal distribution extends from below sea level to 3,300 m above sea level in Afghanistan (8).

Biology of sandflies

Sandflies are holometabolous (they undergo complete metamorphosis through four developmental stages): the egg; four larva instars; the pupa; and imagos. The duration of the life cycle is variable; it depends on the species concerned and abiotic factors, such as temperature, rainfall and hygrometry. Imagos are small (1.5–4 mm) and not noisy when they fly. The seasonal activity of sandflies is mainly determined by temperature and rainfall. Females take blood meals during the early morning, evening and night but they can also bite during the day if they are disturbed. After a blood meal, the female lays 30 to 70 eggs, spreading them around a suitable breeding site. When inactive, adult sandflies have species-specific resting sites. These are often similar or near to the larval sites, which are cool, humid and dark environments. Adult sandflies can survive in dry environments by moving to cool, humid resting sites during the day and becoming active at night, when the temperature drops and humidity increases. Eggs hatch within one to two weeks. The larvae feed on organic materials. They are found in leaf litter and in damp environments containing organic substances, such as caves, animal burrows and shelters, and cracks in walls and rocks. If the temperature falls, the fourth larva instar diapauses. The pupal stage of development takes between five and ten days.

Leishmania infantum

Taxonomy of *Leishmania*

The genus *Leishmania* Ross, 1903, includes several obligate protozoan parasites of humans and domestic and wild mammals. Based on their location in the vector's intestine, Lainson and Shaw (1987) distinguished two *Leishmania* subgenera, *Leishmania* and *Viannia* (9). Since the 1970s, immunological, biochemical and genetic criteria have been used to define *Leishmania* species (Fig. 1) (10).

Life cycle of *Leishmania infantum*

Leishmania is transmitted during the blood meal of infected female sandflies. The infectious *Leishmania* stage (promastigotes) is inoculated from the vector's proboscis into the host's skin. The promastigotes are phagocytised by macrophages and other mononuclear phagocytic cells, where they transform into amastigotes, which multiply by bipartition and infect other mononuclear phagocytic cells. The relationship between the parasite and the host determines the outcome of the infection. *Leishmania infantum*

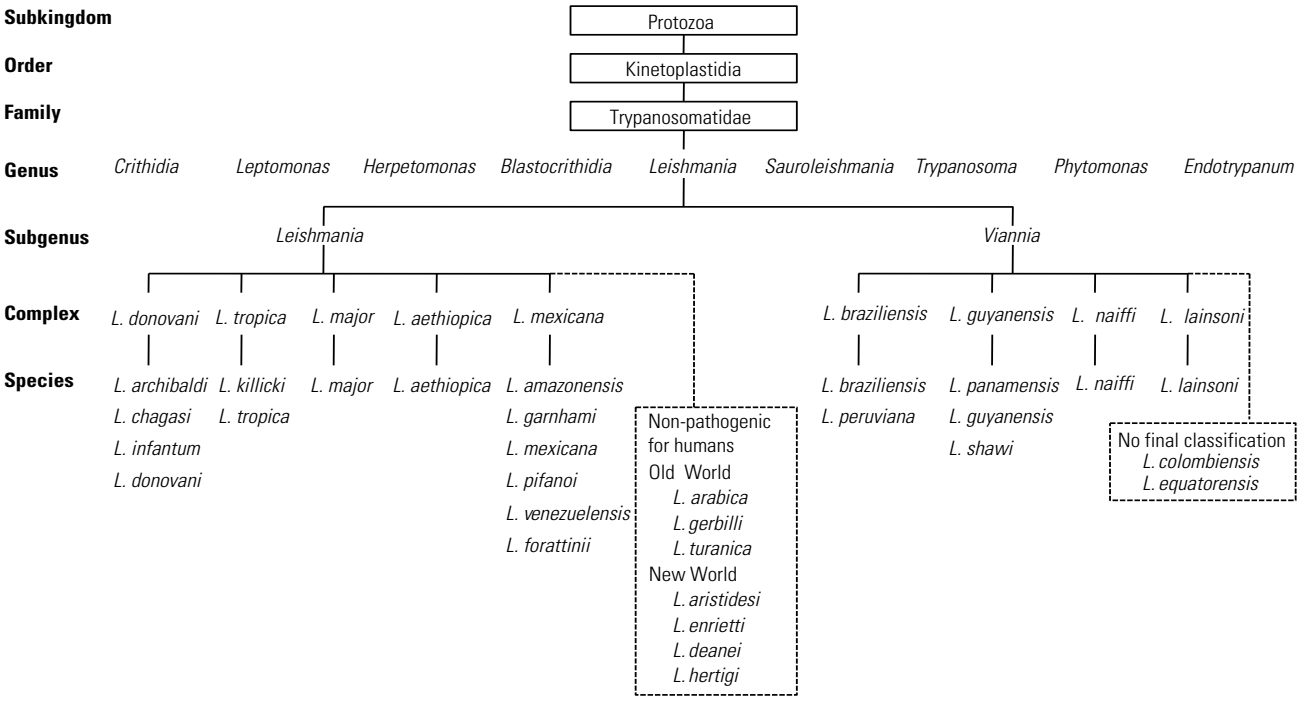

Fig. 1
Classification of *Leishmania* parasites (10)

may invade all the host's organs. However, its presence in the host's dermis is necessary for the transmission of infection to the vector. In sandflies, amastigotes transform into promastigotes, develop in the hindgut (*Viannia* subgenus parasites) or in the midgut (*Leishmania* subgenus parasites), and turn back to the proboscis (Fig. 2).

Epidemiology of canine visceral leishmaniosis

The epidemiology of CvL is diverse; this is the result of intricate interactions between the vector (biomass, species, vector capacity, behaviour, etc.), the host (biomass, species, age structure, breed, breeding type, control measures), the parasite (biomass, zymodem) and ecological factors (such as temperature, hygrometry, the ecological niches of the vector, mainly the larvae, etc.). The epidemiological patterns of *L. infantum* infection are dynamic, with a clear trend towards geographical extension, an increase of listed host and vector species, and the description of new zymodems (11). The life style of the dog is very important, since dogs that live outdoors or have no insecticide protection (mainly collars) are more exposed. The geographic distribution of CvL corresponds to the distribution of its vectors. This disease is present in 50 countries in Europe, Africa, Asia and the Americas (12), including new endemic areas where outbreaks are occasionally reported, such as the United States (13), Canada (14) and Europe (Fig. 3) (15, 16). In the United States, CvL is considered an emerging disease in dogs (17). Within countries with historical foci of leishmaniosis, the disease seems to be spreading geographically; for example, as reported in Tunisia (18, 19). The number of infected dogs is much higher than the number of clinical cases. This aspect drives the epidemiology of this infection since non-clinically infected dogs are the main reservoirs of the parasites. The main epidemiological cycle of canine leishmaniosis is domestic; it is concentrated in suburban regions, while the sylvatic cycle is cryptic (Fig. 4). Cats can express clinical signs of visceral leishmaniosis (20) but their role as reservoirs is still unknown and needs to be explored (21).

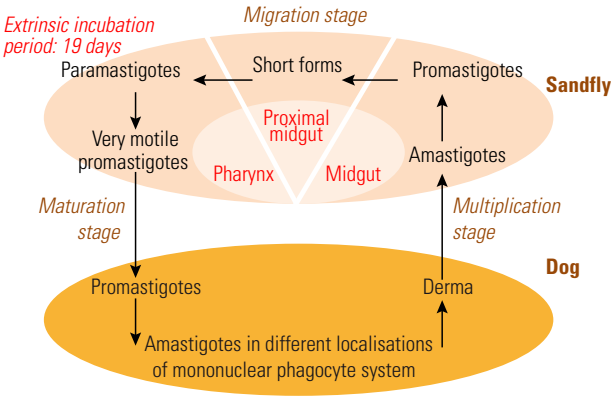

Fig. 2
Life cycle of *Leishmania infantum*

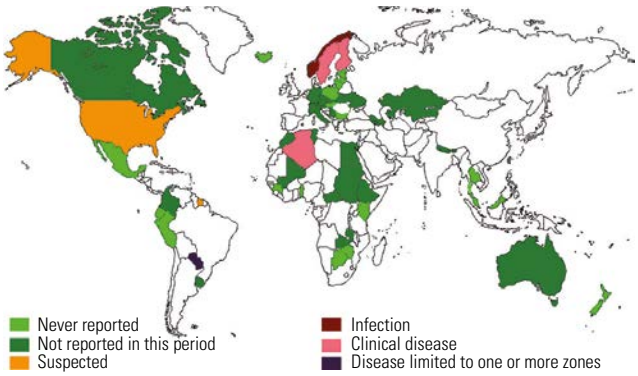

Fig. 3
Geographic distribution of visceral canine leishmaniosis (12)

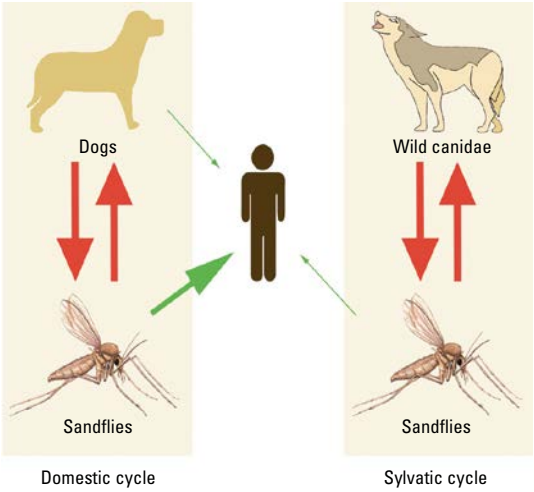

Fig. 4
General epidemiological cycle of canine leishmaniosis

Clinical signs of canine visceral leishmaniosis

Canine leishmaniosis is generally a chronic and multi-systemic disease. As a result of the complexity of the immunopathogenic interactions, the diversity of the affected tissues and organs, and the strain of the protozoan, clinical signs are very polymorphic and, in many cases, can lead to misdiagnosis (22, 23, 24).

Chronic forms of canine visceral leishmaniosis

General signs

The signs of canine leishmaniosis begin with an inoculation chancre. This is a unique sweating ulcerative lesion, which regresses after a variable number of days, giving way to a 'silent' period (Fig. 5). The clinical expression of infection begins with slight but progressive clinical signs which gain in severity: dullness and increasing reluctance to exercise, poor body condition, hyporexia, weight loss and localised (head) then generalised amyotrophy. However, the early stages of the disease are not associated with clear general signs.

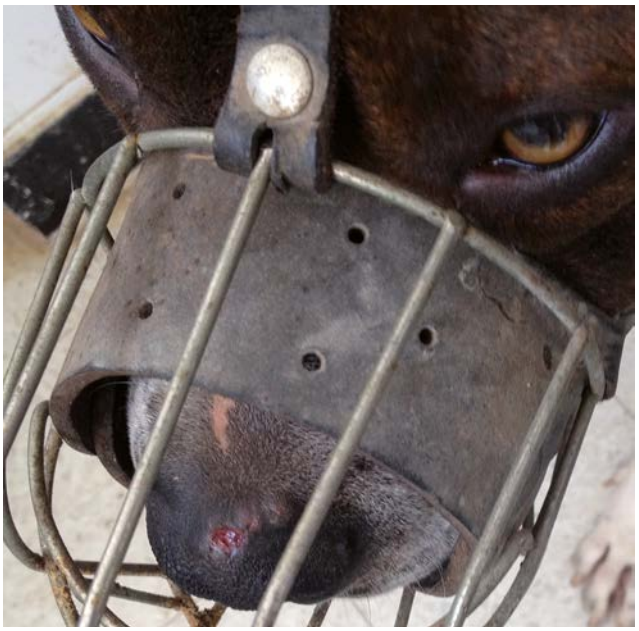

Fig. 5
***Leishmania infantum* inoculation chancre on a dog's nose**

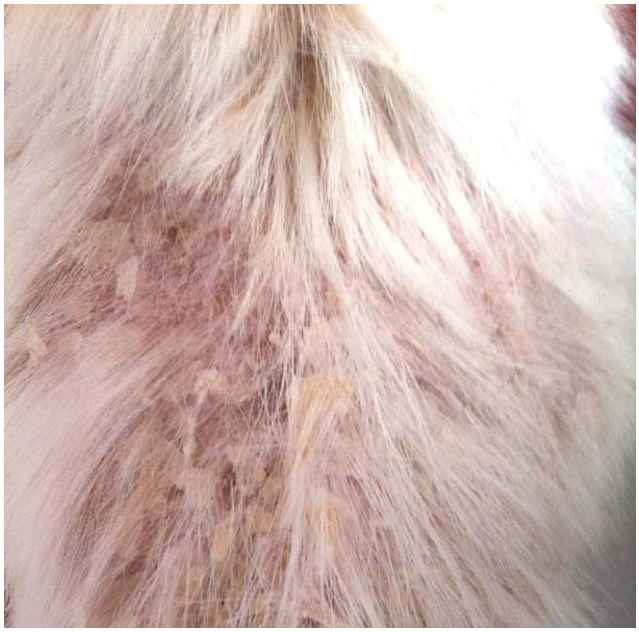

Fig. 6
A dog with severe dorsal hyperkeratosis
Note the size of the dorsal pellicles

Mononuclear phagocyte system signs

The most frequent sign in canine leishmaniosis is enlargement of the lymph nodes, which become mobile and painless (22). This enlargement occurs early on during the course of the disease and, in some cases, may be the only clinical sign. Splenomegaly can be detected but it is inconsistent (25).

Cutaneous signs

Dermatological signs are the second-most-common manifestation of leishmaniosis (23, 25, 26, 27, 28). They may be the only detectable signs or they may be associated with other types of lesions (29). Dermatological signs vary in type and intensity but are not associated with pruritus.

– Non-itchy diffused alopecia may be associated with dry seborrhoea with silver-white scales, which can be localised (mainly on the dorso-lumbar region) or disseminated throughout the entire body (Fig. 6) (30). The dog presents with follicular keratosis and, in some cases, parakeratosis with an inflammatory infiltrate, sometimes associated with histiocytes, plasmocytes and macrophages. The *Leishmania* amastigotes are sometimes present.

– Hyperkeratosis may appear on the head, the nose and footpads (Fig. 7).

– Multifocal cutaneous or mucosal ulcers may be associated with necrosis of the epidermal and dermal layers (Fig. 8). There is a polymorphic infiltrate around the necrosis, consisting of a mixture of histiocytes, lymphocytes, plasmocytes and macrophages infected by *Leishmania* amastigotes.

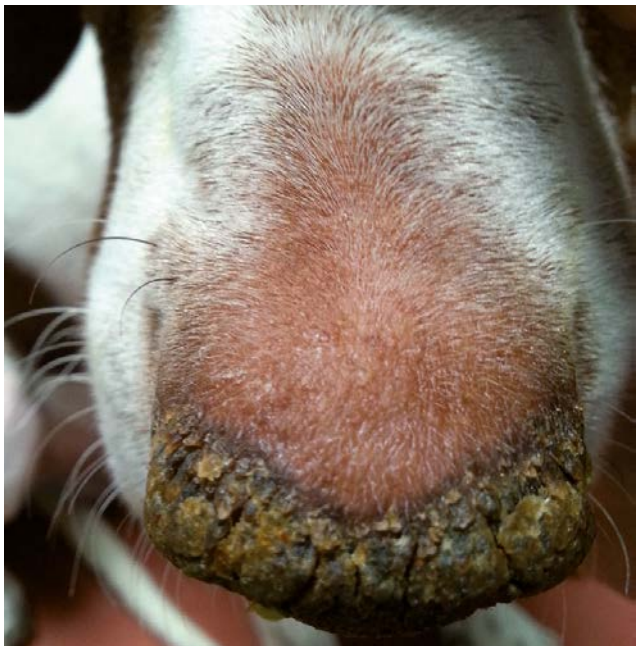

Fig. 7
Hyperkeratosis on the nose of a dog with canine leishmaniosis

– Frequently, superficial and/or deep bacterial (mainly *Staphylococcus* spp.) or fungal (*Malassezia pachydermatis*) infections are reported, which induce pustular dermatitis characterised by the presence of an inflammatory histio-lymphoplasmocytic infiltrate.

Fig. 8
Ulcerative dermatitis at the bony prominences of a dog with canine leishmaniosis

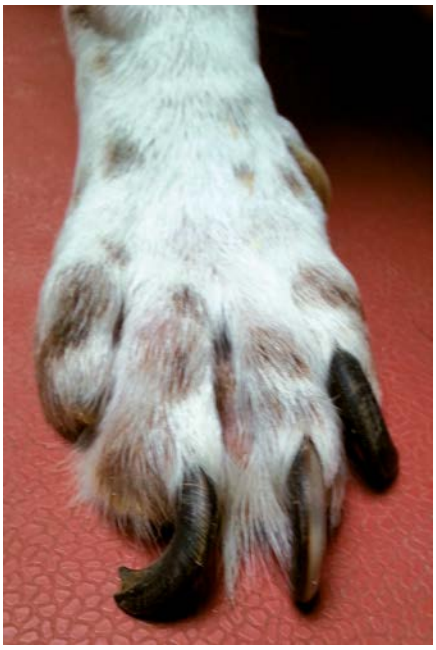

Fig. 9
Onychogryphosis in a dog with canine leishmaniosis

– An increase in the length of the claws may become apparent (Fig. 9).

– Nodules may occur. These can take several forms, from simple papules to tumour-like nodules with peripheral lymphadenopathy. The nodules can be generalised or localised to the interdigital space. In some cases, nodules may become ulcerated (30). Nodular dermatitis with an inflammatory infiltrate, containing macrophages with high parasite burdens and some lymphocytes and plasmocytes, has been reported. The nodular form is more frequently seen in boxer breeds (26, 31).

– Atypical and rare cutaneous lesions may occur: with depigmentation (mainly nasal), panniculitis and nasal hyperkeratosis. These lesions may also be found on the footpads.

Ocular signs

Ocular signs are polymorphic and include: diffuse or nodular conjunctivitis; exfoliative, ulcerative or nodular blepharitis; nodular or diffuse scleritis; anterior or posterior uveitis, which can be granulomatous or diffuse; glaucoma; panophthalmia and keratoconjunctivitis (Fig. 10) (25, 32, 33).

Non-specific forms

Several other atypical forms are reported with CvL, such as sterile pustular dermatitis due to suppurated folliculitis; lameness due to polyarthritis, resulting from type III hypersensitivity (23); and, more rarely, granulomatous myositis (30).

Diagnosis of canine leishmaniosis

In many cases, it is difficult to diagnose CvL, due to its polymorphic expressions and to the presence of banal

Fig. 10
Purulent keratoconjunctivitis with blepharitis in a dog with canine leishmaniosis

clinical signs, which are frequently neglected by the dog's owner. In the classic, cutaneo-visceral form associated with general signs, however, diagnosis is relatively easy. To improve the prognosis and to avoid both human and animal transmission (from false-negative cases) and unnecessary euthanasia (from false-positive cases), the diagnosis should be established as soon as possible, even on the basis of only a few or even a single clinical sign. A summary of the advantages and limitations of the diagnostic techniques available can be found in Table I.

At the clinic

The first step is to carry out a detailed clinical examination to look for signs that could be attributed (or not) to leishmaniosis. A biopsy of the lymph nodes is the first diagnostic technique to implement, especially if enlargement is detected. The biopsy product is smeared on a microscope slide, then Giemsa-stained and examined under an optic microscope at ×1,000 magnification to verify the presence of amastigotes. The parasites are rod-shaped, contain a nucleus and kinetoplast (these two structures are a dark red-purple in colour), and can be present in both intracellular and extracellular compartments, since the cell host is frequently ruptured during smear preparation. Alternatively, other tissues could be biopsied: spleen, bone marrow and skin, but these techniques are laborious. Since the sensitivity of this technique is low (50–60%), negative clinical cases should be further investigated using other techniques.

Table I
Advantages and limitations of diagnostic techniques used for canine leishmaniosis

Direct techniques	Detection	Advantages	Limits	Implementation
Lymph node biopsy	Amastigotes	Rapid Low cost Excellent specificity	Low sensitivity	First technique to implement
Culture in Novy-McNeal-Nicolle medium	Amastigotes	Excellent specificity Quantitative technique Yields a large quantity of parasites that may be used for further study	Takes time (several days)	Research
Polymerase chain reaction (PCR)	Nucleic acids	Excellent specificity Excellent sensitivity Not subjective	Expensive Does not differentiate between carrier and diseased state Requires laboratory equipment	Research To follow-up treated animals
Dual path platform (lateral flow device)	Antigens	Rapid Easy Low cost	Qualitative result Does not differentiate between carrier and diseased state	For orientation More suitable in countries which have not previously reported the presence of infection
Loop-mediated isothermal amplification PCR	Nucleic acids	Relatively rapid Excellent sensitivity Excellent specificity Does not require a thermocycler	Does not differentiate between carrier and diseased state Expensive	Research Mass screening
Lateral flow devices	Antibody	Low cost, rapid and easy to perform	Low sensitivity and specificity Qualitative result	Individual/mass screening
Immunofluorescent antibody test	Somatic antibody	Low cost Simple Does not require expensive equipment Quantitative technique Standard technique	Sometimes not objective Takes time to perform	Mass screening Clinical diagnosis
Enzyme-linked immunosorbent assay	Soluble antibody	Objective Relatively low cost Simple Quantitative technique	Available in kit form	

In the laboratory

Gomes *et al*. (34) published an excellent review of all the laboratory tests; the tests' limitation is that they do not easily differentiate between the carrier state and diseased dogs. Moreover, serological tests are negative when dogs are in anergy, but generally these animals present severe clinical signs. Seropositive results also do not differentiate between diseased and carrier animals. Ideally, if the results are positive, high-sensitivity techniques should be coupled with a highly specific technique.

Immunofluorescent antibody test

The immunofluorescent antibody test (IFAT) using promastigotes is the reference technique (12); its cut-off value is 1/80 for dogs.

Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay has been replacing the IFAT since commercial kits became available. It has higher sensitivity and specificity; it is able to be automated, easy to read and provides objective results.

Polymerase chain reaction

In polymerase chain reaction (PCR), DNA is extracted from the blood buffy coat or from biopsies of different tissues. Several primer sets have been developed, which amplify different parts of the parasite's genome, mainly kinetoplast DNA minicircles, allowing us to identify DNA from the various species of the *Leishmania* genus (35, 36).

Loop-mediated isothermal amplification

Loop-mediated isothermal amplification (LAMP) PCR needs no thermocycler and so can be used in laboratories that are not well equipped. Indeed, the *Bacillus stearothermophilus* (Bst) polymerase used in LAMP PCR works at 60–65°C. Chaouch *et al*. (37) developed a LAMP PCR for the identification of *L. infantum* DNA, targeting a cysteine protease B multi-copy gene. The sensitivity of this technique is comparable to that of conventional PCR. Its specificity is higher than that of the generic 18S LAMP but Adams *et al*. (2010) reported a cross-reaction with *Trypanosoma cruzi* (38).

Rapid tests: dual path platform

Several rapid, ready-to-use, commercially available disposable dual path platforms (DPP) are available. They are based on a reaction between colloidal gold particles coupled with the protein A/G and the antigen bound to antibodies (39). A sample of 5 µL is placed in the device. These tests can be performed on serum, plasma and total blood with anticoagulant but no antibody quantification can be performed.

Canine visceral leishmaniosis lesions

Visceral lesions

Visceral lesions may include the following:

– Lesions on the spleen. Splenomegaly is frequent, due to the infiltration of the spleen by immune cells, mainly monocytes and macrophages, and also to the hyperplasia of the red and white pulps.

– The bone marrow takes on the appearance of a dark pink or red jelly. This is more or less consistent.

– Lesions on the lymph nodes. There is hypertrophy of the cortical and medullary regions. However, in advanced stages, the size of the peripheral lymph nodes may be normal or even hypoplasic. A proliferation of macrophages and lymphocytes is observed, with numerous intra- and inter-macrophagic *Leishmania*.

– The liver is enlarged and calcified during the final stages (24).

Gut lesions

These are due to granulomatous or pyogranulomatous inflammation, leading to chronic diarrhoea in advanced cases (40, 41, 42).

Testes

The testes are atrophied, yellowish-brown or greyish, with thickening of the fibrous septa (43).

Kidney lesions

Kidney signs consist of glomerulonephritis and/or interstitial nephritis and, more rarely, amyloidosis. These explain the presence of proteinuria and can be responsible for the development of hypertension and the establishment of a vicious circle, whose final stage may be nephrotic syndrome and/or chronic renal failure, the most common cause of death during leishmaniosis.

Other lesions

Other lesions are frequent and polymorphic (30). They include: joint distension, sometimes associated with erosive arthritis; proliferative or lytic bone lesions; chronic hepatitis; chronic colitis; meningitis; and atrophic myositis of the masseter muscle and/or polymyositis.

Zoonotic aspects of *Leishmania infantum*

Healthy humans, whose susceptibility to *L. infantum* is low, most frequently experience asymptomatic infections. Clinically expressed infections are more frequent in infants below two years of age (15). The main reservoir of *L. infantum* is dogs; wild carnivores could also be involved but they are of lesser epidemiological importance in the life cycle of this parasite. This is due to their smaller biomass and density and also to their more distant relationship with the human population. The parasite is transmitted from an infected dog to a sandfly, then from the vector to a human being. Statistical data about the global frequency and geographic distribution of leishmaniosis are unreliable and imprecise and need to be further investigated (44). According to the World Health Organization (WHO) (4), this disease is endemic in 98 countries (Fig. 11) and its world incidence is estimated at 2 million cases, consisting of 0.5 million cases of visceral leishmaniosis and 1.5 million cases of the cutaneous form. Factors such as malnutrition, HIV infection (45) and immunodepression predispose humans to clinical forms of the parasitic infection (46). Fever, mucosal pallor and splenomegaly form a clinical triad, which is associated with low blood cell counts (anaemia, leukoneutropenia, and thrombocytopenia) and an inflammatory syndrome (47).

Controlling leishmaniosis

The control of CvL is important because of its severe nature in infected dogs and its zoonotic character. Prevention is far more important than treatment because of constraints associated with treatment and its high cost. Moreover, individual control measures have no impact on the epidemiology of the disease since a high proportion of the canine population will never be affected by such measures (i.e. a large proportion of dogs with owners, stray dogs, and wild canids). Before choosing any control option, it is important to consider two factors:

– The epidemiological pattern of the targeted region. For example, is the infection occurring in sporadic, enzootic or hyperenzootic patterns?

– The impact of canine leishmaniosis on human health in the targeted area.

Treatment of clinical cases

Since there is no legislation concerning dogs with CvL, the decision to treat a diseased dog is the result of a discussion between the dog owner and the veterinarian. One important factor is the owner's ability and/or willingness to comply with the treatment protocol. First, the dog's kidney and liver

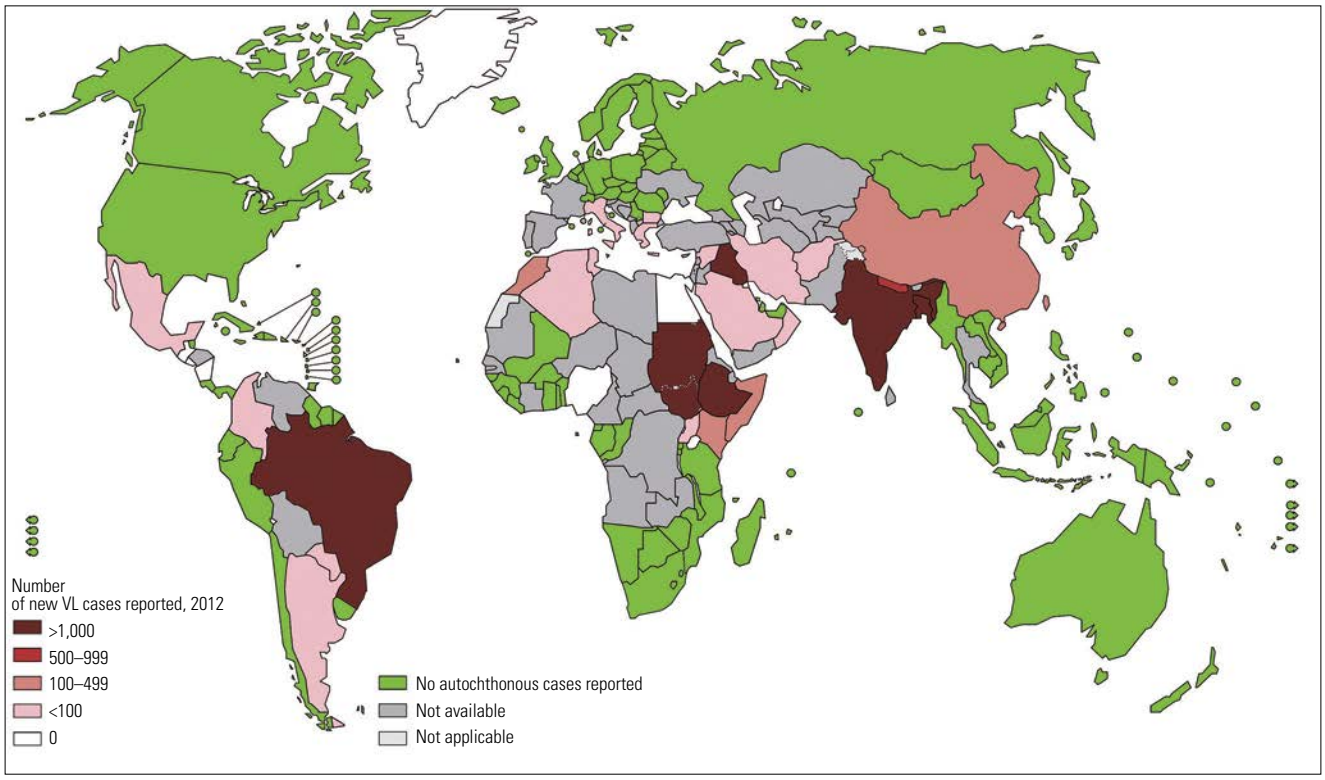

Fig. 11
Geographic distribution of human visceral leishmaniosis (45)

functions should be investigated. If these are affected, then euthanasia is indicated. Each treated animal should wear an insecticidal collar during the season of vector activity to avoid reinfection and abolish the dog's role of reservoir. Several molecules and protocols have been tried to treat CvL; the best approach is to associate leishmanistatic and leishmanicidal molecules. There are several excellent comprehensive reviews and trials of CvL treatment (48, 49, 50). Since a small population of parasites will remain in all treated dogs, serological and clinical monitoring should continue during the entire lifetime of the animal. One treatment method uses meglumine antimoniate (the leishmanicidal molecule) together with allopurinol (the leishmanistatic molecule):

i) Meglumine antimoniate is injected subcutaneously or intramuscularly, at a dose of 75–100 mg/kg once daily, or 40–75 mg/kg twice daily, for 4–6 weeks. Meglumine antimoniate causes nephrotoxicity (51), anorexia, vomiting, diarrhoea and reactions at the site of injection (50).

ii) Allopurinol is given orally at a dose of 10–15 mg/kg twice daily for at least 6–12 months (32, 52). Some 12% of dogs treated with allopurinol develop xanthine urinary crystals, leading, in some cases, to xanthine urolithiosis (53).

Miltefosine, a leishmanicidal alkylphosphocholine used in human dermatology as an antimitotic, can also be used to treat leishmaniosis in dogs when administered orally (2 mg/kg once daily for one month) in association with allopurinol (54). *Leishmania infantum* rapidly develops resistance against miltefosine.

Symptomatic treatment should be implemented to treat bacterial infections, ulcers, keratoconjunctivitis and other signs. Corticoids should be prescribed when immune complexes are deposited in the joints or kidneys.

Vaccination

Despite its huge importance in veterinary medicine and the number of candidates, vaccination against CvL only went into operation during the last decade. Three commercial vaccines are now available, as described below (44).

Leishmune®

This is a fructose mannose ligand (FML)-saponin vaccine; it was licensed in Brazil in 2003 to protect dogs against *L. donovani*. Its efficacy against this parasite was 76%, with a protection rate of 92%. The Brazilian Ministry of Health has not adopted Leishmune® as a tool for preventing leishmaniosis in canines.

Leish-Tec®

This vaccine contains recombinant A2-antigen of *Leishmania* amastigotes and uses saponin as an adjuvant. It was licensed in 2008. There is a lack of evidence for the efficacy of this commercial vaccine (55).

Canileish® (LiESP/QA-21)

This vaccine was licensed in several European countries in 2011. It contains a culture supernatant of *L. infantum* promastigotes (LiESAp), consisting of a 54-kilodalton (kDa) excreted protein of *L. infantum* with muramyl dipeptide (MDP). The efficacy of this vaccine against *L. infantum* was 68.4%, with a protection rate of 92.7%.

After exposure to natural *L. infantum* infection, the probability of vaccinated dogs becoming PCR positive was similar to that of dogs in a control group, but, among PCR positive dogs, the probability of reverting back to a PCR negative state was higher in the vaccinated group than in the group of control animals (55, 56).

Killing of infected dogs

In endemic areas, one method of control would be to carry out mass screening and kill the infected dogs. This control approach has been implemented in Brazil, but it would not gain public support in countries where the dog is considered a member of the family (57). Animal-health decision makers could include different zoonotic diseases in a single control programme (for example, leishmaniosis, echinococcosis and rabies). The role of international organisations, such as the World Organisation for Animal Health (OIE) and WHO, is important in establishing the framework for these programmes, suggesting various options and providing support. Such control programmes should be established after considering several interests: those of the animal itself, the owner, the veterinarian, the animal population as a whole, public health in general and economic interests.

In fact, the Brazilian control programme failed. A mathematical model showed that this failure was due to the high incidence and the severity of the infection among the dog population, the low sensitivity of the diagnostic test, and time delays between diagnosis and culling (58).

Insecticide collars

The use of deltamethrin in insecticidal collars should reduce the risk of infection for dogs in endemic regions. Manzillo *et al.* (59) performed a two-year field study on stray dogs in kennels in areas where leishmaniosis is highly endemic. These authors showed that signs of canine leishmaniosis occurred significantly more frequently (90% versus 36%) in non-collared dogs than in collared dogs, and that infection progressed more rapidly (59). But, in the absence of

other efficient insecticides, resistance to deltamethrin will eventually emerge.

Vector control

Vector control is more or less impossible with sandflies, since the shelters of the immature stages are difficult to identify and the imagos are spread throughout a huge environment. It is important not to make the common error of assuming that draining or otherwise treating standing water is efficient against sandflies.

Extension programmes

An extension programme should target dog owners, particularly those in the rural areas of developing countries. Owners should be encouraged to improve the living conditions of their animals, in particular by providing them with food and water of sufficient quality and quantity and suitable health care. Domestic wastes should be promptly collected, to avoid the development of a large stray dog population, which can act as a reservoir for the parasite. Veterinarians whose skills have been extended in this area should promote three important concepts to the public.

– Leishmaniosis is a zoonotic disease transmitted by sandflies, affecting mainly the immunocompromised, the very young and the elderly.

– Transmission of the infection to dogs occurs mainly outdoors, between sunrise and sunset.

– You should take your dog to a veterinarian if you notice one of these signs: skin lesions, lethargy or abnormal enlargement of the lymph nodes.

Ethical aspects

The ethical aspects of controlling leishmaniosis should be discussed at the national level, taking into account the epidemiological, socioeconomic and religious contexts. The community, in consultation with the national Public and Veterinary Health Services, should decide on the best control option by finding a compromise between protecting human health and respecting both human and animal welfare. Official legislation and/or regulation should prescribe what should be done in cases of clinical canine leishmaniosis and when dogs test seropositive for the infection, in the case of both owned animals and strays. Owners should be encouraged not to abandon a seropositive or clinically affected dog, but to treat or euthanase them.

Conclusion

Canine visceral leishmaniosis is one of the most important zoonotic diseases in dogs, and its epidemiological features are changing. Indeed, both the geographical distribution of the infection and its prevalence are growing. These factors make it of increasing importance in both human and animal pathology. Decision-makers in both human and animal health should collaborate closely to decrease its prevalence in the various regions of the world. Further studies are needed to improve our knowledge about this infection and, in particular, the role played by other animal species (such as cats and wild canids) as reservoirs.

Acknowledgements

This work received financial support from the Epidemiological Laboratory of Enzootic Infections of Herbivores (Laboratoire d'épidémiologie des infections enzootiques des herbivores) in Tunisia, Ministry of Higher Education, Scientific Research and Information, Technology and Communication.

La leishmaniose canine (infection due à *Leishmania infantum*)

M. Gharbi, M. Mhadhbi, A. Rejeb, K. Jaouadi, M. Rouatbi & M.A. Darghouth

Résumé

Les auteurs présentent une synthèse des connaissances sur la leishmaniose canine due à *Leishmania infantum*. Ce protozoaire transmis par les phlébotomes occasionne une maladie à l'évolution souvent chronique. Des signes cliniques cutanés et une atteinte viscérale surviennent à mesure que l'infection s'installe. Les principaux signes observés dans les formes classique de la leishmaniose sont un élargissement des ganglions lymphatiques, un amaigrissement extrême et des lésions cutanées. La maladie est difficile à contrôler car les chiens demeurent porteurs pendant des années et le risque de récidive est permanent. Dans les régions endémiques, la meilleure méthode pour réduire la prévalence consiste à procéder au dépistage systématique des animaux infectés, qui sont ensuite traités ou euthanasiés. Des travaux de recherche complémentaires sont nécessaires pour améliorer l'efficacité des vaccins disponibles contre l'infection canine. Cette maladie est une zoonose ; chez l'homme, les signes cliniques sont principalement rapportés chez les sujets âgés, les jeunes et les patients immunodéprimés.

Mots-clés

Canin – Chien – *Leishmania infantum* – Leishmaniose – Parasite – Phlébotome – Zoonose.

Leishmaniosis (infección por *Leishmania infantum*) en el perro

M. Gharbi, M. Mhadhbi, A. Rejeb, K. Jaouadi, M. Rouatbi & M.A. Darghouth

Resumen

Los autores ofrecen una descripción general de la leishmaniosis canina causada por *Leishmania infantum*. Este protozoo transmitido por flebótomos, engendra una enfermedad que se caracteriza a menudo por una evolución crónica. A medida que la infección progresa aparecen signos clínicos en la piel y las vísceras. En las formas clásicas de la enfermedad, los principales signos observados son hipertrofia de los ganglios linfáticos, caquexia y lesiones cutáneas. El hecho de que los perros infectados sigan siendo portadores durante años y puedan recaer en cualquier momento hace difícil controlar la enfermedad. La mejor fórmula para reducir su prevalencia en las regiones donde es endémica pasa por la detección sistemática masiva de animales infectados y su tratamiento o eutanasia. Es preciso seguir investigando para lograr que las vacunas existentes protejan más eficazmente a los perros de la infección. La enfermedad es zoonótica: en el ser humano se describen casos clínicos sobre todo en personas de edad o jóvenes o en aquellas cuyo sistema inmunitario está debilitado.

Palabras clave

Canino – Flebotomo – *Leishmania infantum* – Leishmaniosis – Parásito – Perro – Zoonosis.

References

1. Lewis D.J., Young D., Fairchild G.B. & Minter D.M. (1977). – Proposals for a stable classification of the Phlebotomine sandflies (Diptera: Psychodidae). *Syst. Entomol.*, **2**, 319–332.

2. Lewis D.J. (1982). – A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull. Br. Mus. Nat. Hist. Entomol.*, **45** (2), 121–209.

3. Galati E.A.B. (2003). – Flebotomíneos do Brasil (E.F. Rangel & R. Lainson, eds). FIO CRUZ, Rio de Janeiro, Brazil, 23–51.

4. World Health Organization (WHO) (2010). – Control of the leishmaniases: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March. WHO Technical Report Series 949. WHO, Geneva, 186 pp.

5. Killick-Kendrick R. (1999). – The biology and control of phlebotomine sandflies. *Clin. Dermatol.*, **17** (3), 279–289.

6. Young D.G. & Perkins P.V. (1984). – Phlebotomine sand flies of North America (Diptera: Psychodidae) [Lutzomyia]. *Mosq. News*, **44** (2), 263–304.

7. Lane R.P. (1993). – Sandflies (Phlebotominae). *In* Medical insects and arachnids (D.R.P. Lane & D.R.W. Crosskey, eds). Springer, Dordrecht, the Netherlands, 78–119.

8. Artemiev M.M. (1980). – A revision of sandflies of the subgenus *Adlerius* (Diptera, Phlebotommae, *Phlebotomus*). *Zool. Zhurnal*, **59** (8), 1177–1192.

9. Aransay A.M., Scoulica E., Tselentis Y. & Ready P.D. (2000). – Phylogenetic relationships of phlebotomine sandflies inferred from small subunit nuclear ribosomal DNA. *Insect Molec. Biol.*, **9** (2), 157–168.

10. Rioux J.A., Lanotte G., Serres E., Pratlong F., Bastien P. & Perieres J. (1990). – Taxonomy of *Leishmania*. Use of isoenzymes. Suggestions for a new classification. *Ann. Parasitol. Hum. Comp.*, **65** (3), 111–125.

11. Chaara D., Haouas N., Dedet J.P., Babba H. & Pratlong F. (2014). – Leishmaniases in Maghreb: an endemic neglected disease. *Acta. Trop.*, **132**, 80–93. doi:10.1016/j.actatropica.2013.12.018.

12. World Organisation for Animal Health (OIE). – World Animal Health Information Database (WAHID) Interface. OIE, Paris. Available at: www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home (accessed on 7 January 2015).

13. Freeman K.S., Miller M.D., Breitschwerdt E.B. & Lappin M.R. (2010). – Leishmaniasis in a dog native to Colorado. *JAVMA*, **237** (11), 1288–1291.

14. Schantz P.M., Steurer F.J., Duprey Z.H., Kurpel K.P., Barr S.C., Jackson J.E., Breitschwerdt E.B., Levy M.G. & Fox J.C. (2005). – Autochthonous visceral leishmaniasis in dogs in North America. *JAVMA*, **226** (8), 1316–1322.

15. Takken W. & Knols B.G.J. (2007). – Emerging pests and vector-borne diseases in Europe. Wageningen Academic Publishers, Wageningen, the Netherlands, 500 pp.

16. Espejo L.A., Costard S. & Zagmutt F.J. (2014). – Modelling canine leishmaniasis spread to non-endemic areas of Europe. *Epidemiol. Infect.*, **143** (9), 1936–1949. E-pub.: 27 October 2014. doi:10.1017/S0950268814002726.

17. Petersen C.A. (2009). – Leishmaniasis, an emerging disease found in companion animals in the United States. *Top. Companion Anim. Med.*, **24** (4), 182–188.

18. Aoun K. & Bouratbine A. (2014). – Cutaneous leishmaniasis in North Africa: a review. *Parasite*, **21** (14), 9 pp. doi:10.1051/parasite/2014014.

19. Bouratbine A., Aoun K., Ghrab J., Harrat Z., Ezzedini M.S. & Etlijani S. (2005). – Spread of *Leishmania killicki* to central and south-west Tunisia. *Parasite*, **12** (1), 59–63.

20. Maia-Elkhoury A.N.S., Alves W.A., Sousa-Gomes M.L., de Sena J.M. & de Luna E.A. (2008). – Visceral leishmaniasis in Brazil: trends and challenges. *Cad. Saúde Pública*, **24** (12), 2941–2947.

21. Maia C. & Campino L. (2011). – Can domestic cats be considered reservoir hosts of zoonotic leishmaniasis? *Trends Parasitol.*, **27** (8), 341–344.

22. Ciaramella P., Oliva G., Luna R.D., Gradoni L., Ambrosio R., Cortese L., Scalone A. & Persechino A. (1997). – A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. *Vet. Rec.*, **141** (21), 539–543.

23. Greene C.E. (2012). – Infectious diseases of the dog and cat, 4th Ed. Saunders, St Louis, Missouri, 1376 pp.

24. Rallis T., Day M.J., Saridomichelakis M.N., Adamama-Moraitou K.K., Papazoglou L., Fytianou A. & Koutinas A.F. (2005). – Chronic hepatitis associated with canine leishmaniosis (*Leishmania infantum*): a clinicopathological study of 26 cases. *J. Comp. Pathol.*, **132** (2–3), 145–152. doi:10.1016/j.jcpa.2004.09.004.

25. Baneth G., Koutinas A.F., Solano-Gallego L., Bourdeau P. & Ferrer L. (2008). – Canine leishmaniosis – new concepts and insights on an expanding zoonosis: part one. *Trends Parasitol.*, **24** (7), 324–330.

26. Ferrer L., Rabanal R., Fondevila D., Ramos J.A. & Domingo M. (1988). – Skin lesions in canine leishmaniasis. *J. Small Anim. Pract.*, **29** (6), 381–388.

27. Martinetti L. (2013). – Dépistage, traitement et prévention de la leishmaniose canine en Corse : enquête auprès des vétérinaires praticiens de l'île. Thesis in veterinary medicine. École Nationale Vétérinaire de Toulouse, 99 pp.

28. Koutinas A.F., Carlotti D.N., Koutinas C., Papadogiannakis E.I., Spanakos G.K. & Saridomichelakis M.N. (2010). – Claw histopathology and parasitic load in natural cases of canine leishmaniosis associated with *Leishmania infantum*. *Vet. Dermatol.*, **21** (6), 572–577.

29. Papadogiannakis E.I., Koutinas A.F., Saridomichelakis M.N., Vlemmas J., Lekkas S., Karameris A. & Fytianou A. (2005). – Cellular immunophenotyping of exfoliative dermatitis in canine leishmaniosis (*Leishmania infantum*). *Vet. Immunol. Immunopathol.*, **104** (3–4), 227–237.

30. Blavier A., Keroack S., Denerolle P., Goy-Thollot I., Chabanne L., Cadoré J.L. & Bourdoiseau G. (2001). – Atypical forms of canine leishmaniosis. *Vet. J.*, **162** (2), 108–120.

31. Vidor E., Dereure J., Pratlong F., Dubreui N., Bissuel G., Moreau Y. & Rioux J. (1991). – Le chancre d'inoculation dans la leishmaniose canine à *Leishmania infantum*. Étude d'une cohorte en région cévenole. *Prat. Méd. Chir. Anim. Compagnie*, **26**, 133–137.

32. Solano-Gallego L., Koutinas A., Miró G., Cardoso L., Pennisi M.G., Ferrer L., Bourdeau P., Oliva G. & Baneth G. (2009). – Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniasis. *Vet. Parasitol.*, **165** (1–2), 1–18.

33. Amara A. (2003). – Manifestations oculaires chez les chiens leishmaniens. *Point Vét.*, **34**, 50–55.

34. Gomes Y.M., Paiva Cavalcanti M., Lira R.A., Abath F.G.C. & Alves L.C. (2008). – Diagnosis of canine visceral leishmaniasis: biotechnological advances. *Vet. J.*, **175** (1), 45–52.

35. Ravel S., Cuny G., Reynes J. & Veas F. (1995). – A highly sensitive and rapid procedure for direct PCR detection of *Leishmania infantum* within human peripheral blood mononuclear cells. *Acta Trop.*, **59** (3), 187–196.

36. Gomes A.H.S., Ferreira I.M.R., Lima M.L.S.R., Cunha E.A., Garcia A.S., Araújo M.F.L. & Pereira-Chioccola V.L. (2007). – PCR identification of *Leishmania* in diagnosis and control of canine leishmaniasis. *Vet. Parasitol.*, **144** (3–4), 234–241.

37. Chaouch M., Mhadhbi M., Adams E.R., Schoone G.J., Limam S., Gharbi Z., Darghouth M.A., Guizani I. & Benabderrazak S. (2013). – Development and evaluation of a loop-mediated isothermal amplification assay for rapid detection of *Leishmania infantum* in canine leishmaniasis based on cysteine protease B genes. *Vet. Parasitol.*, **198** (1–2), 78–84.

38. Adams E.R., Schoone G.J., Ageed A.F., Safi S.E. & Schallig H.D.F.H. (2010). – Development of a reverse transcriptase loop-mediated isothermal amplification (LAMP) assay for the sensitive detection of *Leishmania* parasites in clinical samples. *Am. J. Trop. Med. Hyg.*, **82** (4), 591–596.

39. Castro-Júnior J.G., Freire M.L., Campos S.P.S., Scopel K.K.G., Porrozzi R., Da Silva E.D., Colombo F.A., da Silveira R. de C.V., Marques M.J. & Coimbra E.S. (2014). – Evidence of *Leishmania* (*Leishmania*) *infantum* infection in dogs from Juiz de Fora, Minas Gerais State, Brazil, based on immunochromatographic dual-path platform (DPP®) and PCR assays. *Rev. Inst. Med. Trop. S. Paulo*, **56** (3), 225–229.

40. Benzouine B. (2000). – Anatomie et histologie du tube digestif du chien. Application à l'étude des lésions digestives chez les chiens leishmaniens. Thesis in veterinary medicine. École Nationale de Médecine Vétérinaire de Sidi Thabet, Tunisia.

41. Ferrer L. (1991). – Leishmaniasis. *In* Proc. of the 16th World Small Animal Veterinary Association Congress, 2–5 October, Vienna, 52–54.

42. Keenan C.M., Hendricks L.D., Lightner L., Webster H.K. & Johnson A.J. (1984). – Visceral leishmaniasis in the German shepherd dog. I. Infection, clinical disease, and clinical pathology. *Vet. Pathol.*, **21** (1), 74–79.

43. Amara A., Mrad I., Melki M., Ben M'rad M. & Rejeb A. (2009). – Étude histologique des lésions testiculaires chez les chiens leishmaniens. *Rev. Méd. Vét.*, **160** (1), 54–60.

44. Otranto D. & Dantas-Torres F. (2013). – The prevention of canine leishmaniasis and its impact on public health. *Trends Parasitol.*, **29** (7), 339–345.

45. World Health Organization (WHO) (2015). – Status of endemicity of visceral leishmaniasis, worldwide, 2002. WHO, Geneva. Available at: http://gamapserver.who.int/mapLibrary/Files/Maps/Leishmaniasis_VL_2013.png (accessed on 7 January 2015).

46. Rodríguez-Cortés A., Ojeda A., Todolí F. & Alberola J. (2013). – Performance of commercially available serological diagnostic tests to detect *Leishmania infantum* infection on experimentally infected dogs. *Vet. Parasitol.*, **191** (3–4), 363–366.

47. Marty P., Pomares C., Michel G., Delaunay P., Ferrua B. & Rosenthal E. (2011). – Leishmaniose viscérale méditerranienne. *Bull. Acad. Nat. Méd.*, **195**, 181–188.

48. Andrade H.M., Toledo V.P.C.P., Pinheiro M.B., Guimarães T.M.P.D., Oliveira N.C., Castro J.A., Silva R.N., Amorim A.C., Brandão R.M.S.S., Yoko M., Silva A.S., Dumont K., Ribeiro M.L. Jr, Bartchewsky W. & Monte S.J.H. (2011). – Evaluation of miltefosine for the treatment of dogs naturally infected with *L. infantum* (=*L. chagasi*) in Brazil. *Vet. Parasitol.*, **181** (2–4), 83–90. doi:10.1016/j.vetpar.2011.05.009.

49. Athanasiou L.V., Saridomichelakis M.N., Kontos V.I., Spanakos G. & Rallis T.S. (2013). – Treatment of canine leishmaniosis with aminosidine at an optimized dosage regimen: a pilot open clinical trial. *Vet. Parasitol.*, **192** (1–3), 91–97.

50. Noli C. & Saridomichelakis M.N. (2014). – An update on the diagnosis and treatment of canine leishmaniosis caused by *Leishmania infantum* (syn. *L. chagasi*). *Vet. J.*, **202** (3), 425–435.

51. Bianciardi P., Brovida C., Valente M., Aresu L., Cavicchioli L., Vischer C., Giroud L. & Castagnaro M. (2009). – Administration of miltefosine and meglumine in healthy dogs: clinicopathological evaluation of the impact on the kidneys. *Toxicol. Pathol.*, **37** (6), 770–775.

52. Solano-Gallego L., Miró G., Koutinas A., Cardoso L., Pennisi M.G., Ferrer L., Bourdeau P., Oliva G. & Baneth G. (2011). – LeishVet guidelines for the practical management of canine leishmaniosis. *Parasit. Vectors*, **4**, 86.

53. Torres M., Bardagí M., Roura X., Zanna G., Ravera I. & Ferrer L. (2011). – Long term follow-up of dogs diagnosed with leishmaniosis (clinical stage II) and treated with meglumine antimoniate and allopurinol. *Vet. J.*, **188** (3), 346–351.

54. Farca A.M., Miniscalco B., Badino P., Odore R., Monticelli P., Trisciuoglio A. & Ferroglio E. (2012). – Canine leishmaniosis. *In vitro* efficacy of miltefosine and marbofloxacin alone or in combination with allopurinol against clinical strains of *Leishmania infantum*. *Parasitol. Res.*, **110** (6), 2509–2513.

55. Wylie C.E., Carbonell-Antoñanzas M., Aiassa E., Dhollander S., Zagmutt F.J., Brodbelt D.C. & Solano-Gallego L. (2014). – A systematic review of the efficacy of prophylactic control measures for naturally-occurring canine leishmaniosis. Part I: vaccinations. *Prev. Vet. Med.*, **117** (1), 7–18.

56. Oliva G., Nieto J., Manzillo V.F., Cappiello S., Fiorentino E., Di Muccio T., Scalone A., Moreno J., Chicharro C., Carrillo E., Butaud T., Guegand L., Martin V., Cuisinier A.M., McGahie D., Gueguen S., Cañavate C. & Gradoni L. (2014). – A randomised, double-blind, controlled efficacy trial of the LiESP/QA-21 vaccine in naïve dogs exposed to two *Leishmania infantum* transmission seasons. *PLoS Negl. Trop. Dis.*, **8** (10), e3213. doi:10.1371/journal.pntd.0003213.

57. Passantino A., Russo M. & Coluccio P. (2010). – Canine leishmaniosis and euthanasia in Italy: a critical legal–ethical analysis. *Rev. Sci. Tech. Off. Int. Epiz.*, **29** (3), 537–548.

58. Courtenay O., Quinnell R.J., Garcez L.M., Shaw J.J. & Dye C. (2002). – Infectiousness in a cohort of Brazilian dogs: why culling fails to control visceral leishmaniasis in areas of high transmission. *J. Infect. Dis.*, **186** (9), 1314–1320.

59. Manzillo V.F., Oliva G., Pagano A., Manna L., Maroli M. & Gradoni L. (2006). – Deltamethrin-impregnated collars for the control of canine leishmaniasis: evaluation of the protective effect and influence on the clinical outcome of *Leishmania* infection in kennelled stray dogs. *Vet. Parasitol.*, **142** (1–2), 142–145.