Canine leishmaniasis: the key points for qPCR result interpretation

Verónica Martínez1*, Javier Quilez1, Armand Sanchez2, Xavier Roura3, Olga Francino2 and Laura Altet2

Abstract

Background: Diagnosis and follow up of CanL is difficult since the range of clinical signs is varied and seroprevalence is high in endemic areas. The aims of this study were: i) demonstrate the advantages of Leishmania qPCR to diagnose and control CanL and highlight its prognostic value and ii) propose guidelines for tissue selection and infection monitoring.

Findings: This study included 710 dogs living in an endemic area of leishmaniasis. Forty percent (285/710) exhibited clinical signs consistent with CanL. Infection was detected in 36.3% (258/710) of the dogs of which 4.5% (32/710) were detected by qPCR, 16.2% (115/710) detected by ELISA and 15.6% (111/710) tested positive for both tests. Only 17.9% (127/710) of the dogs were classified sick (affected) with CanL.

All symptomatic dogs with medium or high ELISA titers were qPCR-positive in blood samples. All dogs with inconclusive or low ELISA results with high or medium qPCR parasitemia values developed the disease. Seventy one percent of asymptomatic ELISA-positive dogs confirmed by qPCR (medium to high parasitemia) developed the disease. Bone marrow or lymph node aspirate should be selected to ensure the absence of the parasite in asymptomatic dogs: 100-1,000 parasites/ml in bone marrow are detectable in blood, whereas lower parasite loads are usually negative. Almost 10% of negative samples in blood were positive in conjunctival swabs.

Conclusions: Because qPCR allows parasite quantification, it is an effective tool to confirm a diagnosis of CanL in (i) cases of inconclusive ELISA results, (ii) when the dog has not yet seroconverted, or (iii) for treatment monitoring.
Table 1 Categories for cutoff values of qPCR and ELISA

| qPCR result     | Parasites/ml of blood | Parasites/ml of bone marrow | ELISA result | Titer (%) |
|-----------------|-----------------------|-----------------------------|--------------|-----------|
| Negative        | 0                     | 0                           | Negative     | < 20      |
| Low positive    | 0-10                  | 0-100                       | Uncertain*   | 20-35     |
| Medium positive | 10-100                | 100-1,000                   | Low positive | 35-80     |
| High positive   | 100-1,000             | 1,000-10,000                | Medium positive | 80-150 |
| Very high positive | > 1,000               | > 10,000                    | High positive | > 150     |

* *This category was incorporated for the purpose of this study based on previous data (data not shown).*
treatment or only manifested dermatological signs, in which the tissue of choice should be a swab of the dermatological lesion. In dogs with clinical signs, but low or uncertain ELISA titters, the qPCR has a significant prognostic value. In our study, 100% of symptomatic dogs with inconclusive ELISA results (uncertain or low positive), but with patent parasite detection (high or medium parasitemia measured with qPCR) develop the disease (Figure 1).

In dogs without clinical signs, the qPCR also has an important prognostic value since 71.4% of asymptomatic ELISA-positive dogs confirmed by qPCR with medium to high parasitemia values will end up with patent leishmaniasis.

As a conclusion, dogs whose parasitemia range from medium to high or very high positive are sick or eventually will become sick with CanL.

The tissue of choice

Due to the varied tropism that the parasite exhibits regarding different tissues, the quantity of the parasite is different and oscillates among them [7]. It is important that the clinician chooses the most informative one in each case since qPCR can be performed on different tissues such as blood, bone marrow, lymph node, body fluids, histopathology samples or conjunctival swabs.

To ensure the absence of the parasite in dogs without clinical signs, the sample of choice is bone marrow or lymph node aspirate since it is been proved to be the most sensitive ones [17]. Moreover, blood is a valid tissue to perform routine qPCR analysis of CanL to evaluate response to the treatment. In our study, we found that in few samples in which both blood and bone marrow were analyzed, 77.8% (7/9) of the blood samples detected Leishmania while 88.9% (8/9) of the bone marrow did. The difference corresponds to two dogs that were asymptomatic and had very low titers in bone marrow (2 parasites/ml) and negative titers in peripheral blood. Parasite loads equal or greater than 100-1,000 parasites/ml in bone marrow are detectable in blood, whereas lower parasite loads (1-100 parasites/ml in bone marrow) are usually negative in blood, since there is a correlation between both tissues (bone marrow usually being higher than blood). In this way blood is a valid tissue for monitoring treatment efficacy despite that the first qPCR diagnosis has been performed in bone marrow.

Almost 10% (8/83) of samples which were negative in blood were positive in conjunctival swab. This result correlates with other study that reported that 83% of the dogs experimentally infected with L. infantum were already positive by PCR of conjunctival swabs at 6 weeks after infection, whereas only 17% of the buffy coat samples obtained at the same time were found to be positive [15]. Prevalence values increase if the dog shows ocular signs such as conjunctivitis, uveitis, blepharitis or periocular alopecia. The prevalence of ocular lesions in dogs with leishmaniasis range from 16% to 80% according to various studies [18,19]. Therefore, the tissue of choice in cases of ocular signs is conjunctival swab. It could also be used for early diagnosis since sensitivity is superior to serologic testing or parasite culture [15] and samples are obtained in a less invasive manner.

In the same way, lesional swab is recommended when only dermatological lesions are present. Other studies report that 65% of the dogs were found to be PCR positive for skin lesions compatible with Leishmania [15]. All together, we suggest guidelines for the tissue of choice for qPCR given prior clinical signs and ELISA results (Figure 2).

Monitoring CanL

In cases of positive qPCR it is recommended to monitor the parasite load one month after treatment to evaluate its response. If parasite titer decreases, treatment is effective and a new qPCR control is just recommended at the end of treatment (6-12 months). None of the current anti-leishmanial drugs that are being used have proved to induce parasitological remission in dogs since
a small parasite load usually remains in the majority of cases [20,21]. If the parasite load still remains positive, even after treatment, the clinician should evaluate a different treatment for the disease or the possible presence of co-infections or other diseases [22]. qPCR is an effective tool to monitor treatment efficacy especially in those cases were repeated treatments are needed and to detect early possible relapses to avoid clinical disease.

As a preventive measure, annual screening is recommended for all dogs, especially those living in endemic areas.

Conclusions
In conclusion, due to the fact that qPCR allows quantification of parasite load in a precise manner, it could be an effective tool to confirm a diagnosis of canine leishmaniasis mostly (i) in those cases were serology is inconclusive, (ii) in cases where the dog has not yet seroconverted, (iii) for treatment monitoring. Dogs whose parasitemia range from medium to high or very high positive are sick or eventually will become sick of CanL.

Acknowledgements
Thanks to the veterinarians, dog owners and veterinary clinics that willingly provided the samples for this research. Publication of the CBVD6 thematic series has been sponsored by Bayer Animal Health GmbH. This work was founded by the European commission (LUPA, GA-201370).

Authors’ contributions
VMD: Redacted the manuscript. VMD and JQO: collected samples, extracted DNA, performed qPCR, performed phenotypic classification and edited the manuscript. XR, OF, ASB and LA: edited the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 15 January 2011 Accepted: 13 April 2011 Published: 13 April 2011

References
1. Gramiccia M, Gradoni L: The current status of zoonotic leishmaniasis and approaches to disease control. Int J Parasitol 2005, 35:1169-1180.
2. Ferroglio E, Maroli M, Gastaldo S, Mignone W, Rossi L: Canine leishmaniasis, Italy, Emerg Infect Dis 2005, 10:1618.
3. Petersen CA: Leishmaniasis, an Emerging Disease Found in Companion Animals in the United States. Top Companion Anim Med 2009, 24:182-188.
4. Martín-Sánchez J, Acedo C, Muñoz-Pérez M, Pesson B, Maechal O, Morillas-Márquez F: Infection by Leishmania infantum in cats: Epidemiological study in Spain. Vet Parasitol 2007, 145:267-273.
5. Fernández-Bellon H, Solano-Gallego L, Bardagí M, Alberola J, Ramis A, Ferrer L: Immune response to Leishmania infantum in healthy horses in Spain. Vet Parasitol 2006, 135:181-185.
6. Sabrin R, Ferroglio E, Okeaka A, Romano J, Revilla M, Arnal MC, Trinciouglia A, Gortazar C: Characterization of widespread canine leishmaniasis among wild carnivores from Spain. Vet Parasitol 2008, 155:198-203.
7. Solano-Gallego L, Morelli P, Arboix M, Alberola J, Ferrer L: Prevalence of Leishmania infantum infection in dogs living in an area of canine leishmaniasis endemcity using PCR on several tissues and serology. J Clin Microbiol 2001, 39:560-563.
8. Baneth G, Aroch I: Canine leishmaniasis: A diagnostic and clinical challenge. Vet J 2008, 175:14-15.
9. Gomes YM, Paiva Cavalcanti M, Lira RA, Abath FG, Alves LC: Diagnosis of canine visceral leishmaniasis: biotechnological advances. Vet J 2008, 175:45-52.
10. Cortés E, Sanz AJ, Vela C, Ranz AI: Leishmaniosi canina. Diagnóstico serológico de la leishmaniosi: análisis comparativo de ensayos inmunoenzimáticos e IFI. Inf Vet 2005, SEP:28-33.
11. Francino O, Atlet L, Sanchez-Robert E, Rodriguez A, Solano-Gallego L, Alberola J, Ferrer L, Sanchez A, Roure X: Advantages of real-time PCR assay for diagnosis and monitoring of canine leishmaniasis. Vet Parasitol 2006, 137:214-221.
12. Stephan C, Wesseling S, Schink T, Jung K: Comparison of eight computer programs for receiver-operating characteristic analysis. Clin Chem 2003, 49(3):433-439.
13. Schoonjans F, Crotti A, Maroli M, Oliva G, Roura X, Zatelli A, Zini E: Guidelines for diagnosis and clinical classification of leishmaniasis in dogs. J Am Vet Med Assoc 2010, 236:1184-1191.
15. Strauss-Ayali D, Jaffe CL, Burshtain O, Gonen L, Baneth G. Polymerase chain reaction using noninvasively obtained samples, for the detection of Leishmania infantum DNA in dogs. J Infect Dis 2004, 189:1729-1733.

16. Moreno J, Alvar J. Canine leishmaniasis: epidemiological risk and the experimental model. Trends Parasitol 2002, 18:399-405.

17. Moreira MAB, Luvizotto MCR, Garcia JF, Corbett CEP, Laurenti MD. Comparison of parasitological, immunological and molecular methods for the diagnosis of leishmaniasis in dogs with different clinical signs. Vet Parasitol 2007, 145:245-252.

18. Pena MT, Roura X, Davidson MG. Ocular and periocular manifestations of leishmaniasis in dogs: 105 cases (1993-1998). Vet Ophthalmol 2000, 3:35-41.

19. Koutinas AF, Polizopoulou ZS, Sandomichelakis MN, Argyriadis D, Fytianou A, Plevraki KG. Clinical considerations on canine visceral leishmaniasis in Greece: a retrospective study of 158 cases (1989-1996). J Am Anim Hosp Assoc 1999, 35:376-383.

20. Baneth G, Shaw SE. Chemotherapy of canine leishmaniosis. Vet Parasitol 2002, 106:315-324.

21. Noli C, Auxilia ST. Treatment of canine Old World visceral leishmaniasis: a systematic review. Vet Dermatol 2005, 16:213-232.

22. Tabar MD, Francino O, Attet L, Sanchez A, Ferrer L, Roura X. PCR survey of vectorborne pathogens in dogs living in and around Barcelona, an area endemic for leishmaniasis. Vet Rec 2009, 164:112-116.

Cite this article as: Martínez et al.: Canine leishmaniasis: the key points for qPCR result interpretation. Parasites & Vectors 2011 4:57.