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Contribution of real-time PCR on blood for canine leishmaniosis diagnosis

Aïda Bouratbine*[1], Lilia Zribi[1], Ahmed Chakroun[1], Nour el houda Ben Fayala[2], Rania Maatoug[1], Olfa Souissi[1], Gaetano Oliva[2], Karim Aoun[1]

1. Institut Pasteur de Tunis, Laboratoire de Parasitologie LR 20-IPT-06, Tunis, Tunisia. 2. University Federico II, Napoli, Italy. *aida.bouratbine@pasteur.rns.tn

Background

Blood collection in dogs is a non-invasive sampling method that allows performing both serology and PCR. Real-time Polymerase chain reaction (qPCR) targeting kinetoplastic DNA (kDNA) is a sensitive and rapid method which is largely used to detect *Leishmania* infection in diverse hosts and different biological materials. The aim of this study was to assess the accuracy of kDNA qPCR on blood for diagnosis of canine leishmaniosis (CanL).

Materials and Methods

During the period 2019-2023, 175 dog blood samples collected on anti-coagulant tubes were received by the Department of Parasitology, Institut Pasteur of Tunis for CanL diagnosis. Plasma was used to detect anti-*Leishmania* specific antibodies by ELISA (ID Screen® Leishmaniasis Indirect, ID VET). DNA was extracted from the buffy coat; then kDNA qPCR was performed as described by Mary et al using a FAM-TAMRA TaqMan probe [1].

Results

One hundred and eleven samples were positive by ELISA while 64 were negative. Among the 111 seropositive dogs, 45 (40.5%) were also positive by kDNA qPCR (Table 1), the others didn't show any parasitic DNA in their blood. Of the 64 seronegative dogs, only one (1.5%) had a positive kDNA qPCR. The positive percent agreement (PPA) between the 2 tests was fairly low (40.1%). However, PPA varied depending on the antibody level, being higher in dogs with high antibody levels (> 80% by ID vet ELISA) compared to dogs with lower antibody levels (PPA=51% *versus* PPA=13%; p <0.001). The negative percent agreement between the 2 tests was high (98.4%).

Table 1: qPCR results in seropositive and seronegative dogs.

	Seropositive dogs	**Seronegative dogs**
qPCR positive	45 (40.5%)	1 (1.5%)
qPCR negative	66 (59.5%)	63 (98.5%)
Total	111	64

Conclusion

In conclusion, qPCR on blood is not sensitive enough for CanL diagnosis mainly in dogs with low antibody levels. However, it could be useful for monitoring dogs under treatment. Positive kDNA qPCR in seronegative dogs is a rare event and must be confirmed by another technique using a more specific *Leishmania* target.

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Conflict of interest: none declared.

References

1. Mary C, Faraut F, Lascombe L, et al. Quantification of *Leishmania infantum* DNA by a real-time PCR assay with high sensitivity. Journal of Clinical Microbiology. 2004; 42(11): 5249-55