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A NEW QPCR APPROACH FOR THE SIMULTANEOUS DETECTION OF *CYTAUXZOON* SPP. AND *HEPATOZOON* SPP. IN FELIDS

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INTRODUCTION: *Cytauxzoon* spp. and *Hepatozoon* spp. are protozoa responsible of cytauxzoonosis and hepatozoonosis in a wide range of mammals worldwide. Nevertheless, they are still little studied in felids. Molecular assays reported in literature (usually conventional PCR protocols and among them nested-PCRs) are often time- and cost-consuming with different sensitivity/specificity. Real-time quantitative polymerase chain reactions (qPCRs) to detect some piroplasms' species such as *Theileria anulata* in cattle and buffalo (Ros-García et al., 2012. Parasit Vectors, 5:171; Kundave et al., 2014. Trop Biomed, 31:728-35), *Theileria equii* and *Babesia caballi* in horses (Lobanov et al., 2018. Parasit Vectors, 11:125) are reported. Since qPCR protocols targeting simultaneously *Hepatozoon* and *Cytauxzoon* have never been set up, the aim of this study was to develop a new qPCR assay to quickly screen a large number of samples.

MATERIALS AND METHODS: Primers designed by Tabar et al., 2008 (Vet Parasitol, 151: 332-6) were used to amplify a 373 bp region of 18S-rRNA gene of the order Piroplasmida by SYBR green qPCR. Standard curves and limit of detection of the assay were determined by using 5 (i.e. 1, 10, 10^2, 10^3, 10^4 copies/µl) fold dilution series of DNA of *Babesia microti* ATCC isolate, and the specificity tested on a panel of different species of protozoa (ATCC isolates of *B. microti* and *Toxoplasma gondii*, sequenced field samples of *Cytauxzoon europaeus*, *Hepatozoon felis*, *Hepatozoon silvestris*, *Babesia venatorum*, *Babesia caballi*, *Babesia bigemina*, *Leishmania infantum*).
The assay was tested on experimental samples, i.e. whole blood from 206 owned/stray cats and 12 captive exotic felids (i.e. tiger, lion, leopard, caracal), and organs and blood clots of 19 wild cats. Each assay was performed in duplicate. Results were achieved through the melting curve temperature (Tm) analysis.

RESULTS AND CONCLUSIONS: This assay showed high specificity for piroplasms and high sensitivity (limit < than 10 copies/µl). Based on Tm is possible to quickly distinguish *Cytauxzoon* spp. infection from *Hepatozoon* spp. as the results of species-specific temperature peak (i.e. 81°C *C. europaeus*, 78°C *H. felis*, 78.5°C *H. silvestris*).
In addition, the qPCR was able to detect and differentiate some other piroplasms such as *T. gondii* (75°C), *B. venatorum* (79°C), *B. caballi* (80°C), *B. bigemina* (80.5°C), and *B. microti* (81°C). The limit of the study is represented by the same Tm of *C. europaeus* and *B. microti*. This case unavoidably requires a further step of sequencing for the distinction.
Overall, 12 cats were positive to *H. felis*, 19 to *H. silvestris* and 6 to *C. europaeus*, 1 tiger to *H. felis* and 1 to *H. silvestris*, 6 wild cats to *H. felis*, 2 to *H. silvestris* and 3 to *C. europaeus*. All confirmed by conventional PCR and subsequent sequencing. This procedure could represent a useful method to confirm *Cytauxzoon* spp. and *Hepatozoon* spp. infection in felids, to evaluate other potential piroplasms infection, and to quickly screen a large number of samples.