Molecular approach for contemporary detection of *Hepatozoon* spp. and *Cytauxzoon* spp. in felids: preliminary data

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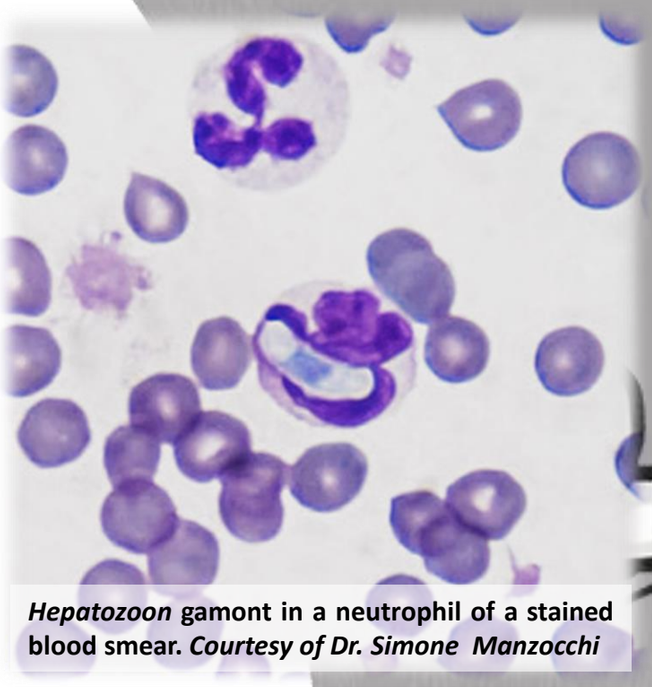
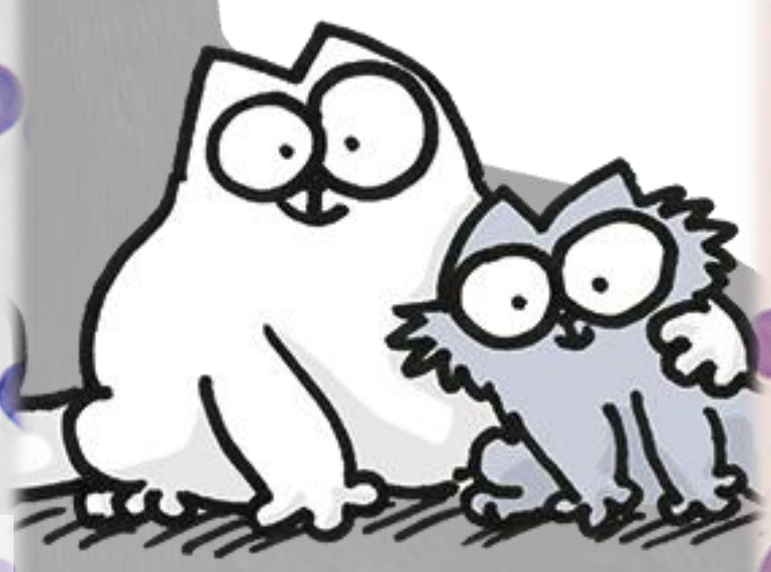
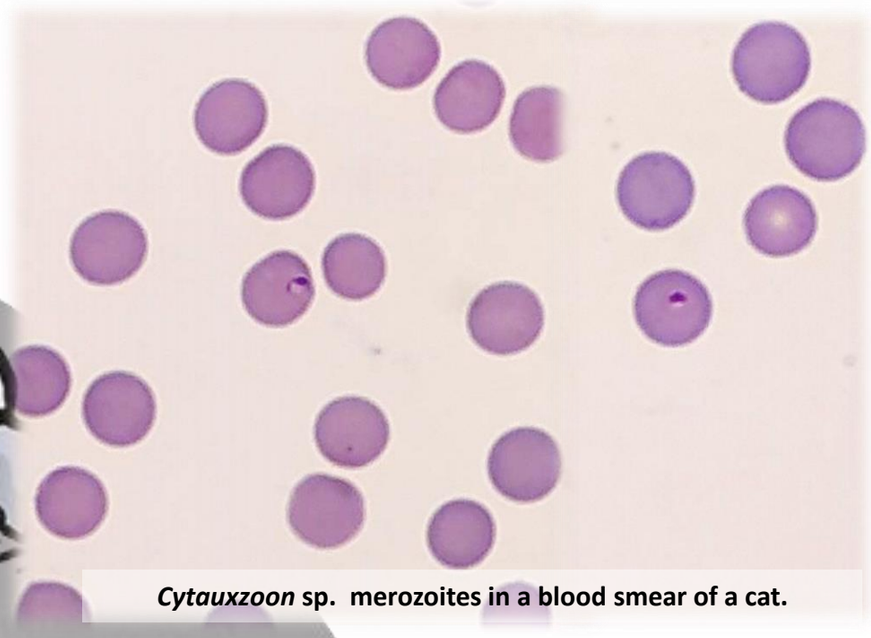
INTRODUCTION

Cytauxzoonosis and hepatozoonosis are emerging vector-borne protozoan diseases affecting both domestic and wild felids. Usually, felids are asymptomatic but with persistent blood parasitaemia suggesting their potential role as reservoir (Díaz-Regañón et al. Parasit Vectors 2017, 10:112).
No molecular procedure detecting both protozoa is nowadays available, thus the aim of this study was to define a rapid molecular assay able to simultaneously detect these protozoa.



Hepatozoon **gamont in a neutrophil of a stained blood smear.** ***Courtesy of Dr. Simone Manzocchi***





Cytauxzoon **sp. merozoites in a blood smear of a cat.**

MATHERIAL AND METHODS

K_3EDTA blood samples of owned and free-ranging cats were included for assay validation. Primers were designed to amplify a **~300 bp region of the 18S-rRNA gene** of Piroplasmida order by SYBR green real time PCR. Results were achieved through the melting curve analysis (Fig. 1). Specificity was verified using different strains of protozoa (ATCC isolates or field samples positive to *Cytauxzoon* sp., *Hepatozoon* spp., *Babesia/Theileria* spp., *Leishmania* spp., *Toxoplasma* spp.) and bacterial isolates. Sensitivity tests are still ongoing.

DISCUSSION AND CONCLUSION

This assay showed high specificity, accuracy and absolute repeatability. No cross-reactivity was observed with bacteria or other protozoa belonging to the same order. Moreover, the latter can be distinguished by melting curve analyses: Babesia spp. showed a melting temperature (Tm) of **76°C**, *Hepatozoon* spp. of **78°C** and *Cytauxzoon* sp. of **81.5°C** (Fig. 1). However, since most of the blood samples/isolates used for validation were not certificated (i.e. due to the lack of ATCC strains) validation tests are ongoing to increase the number of blood samples and strains tested. If preliminary data will be confirmed, this procedure could represent an innovative and useful method, for both screening and diagnostic confirmation of Piroplasmida infections in felids.

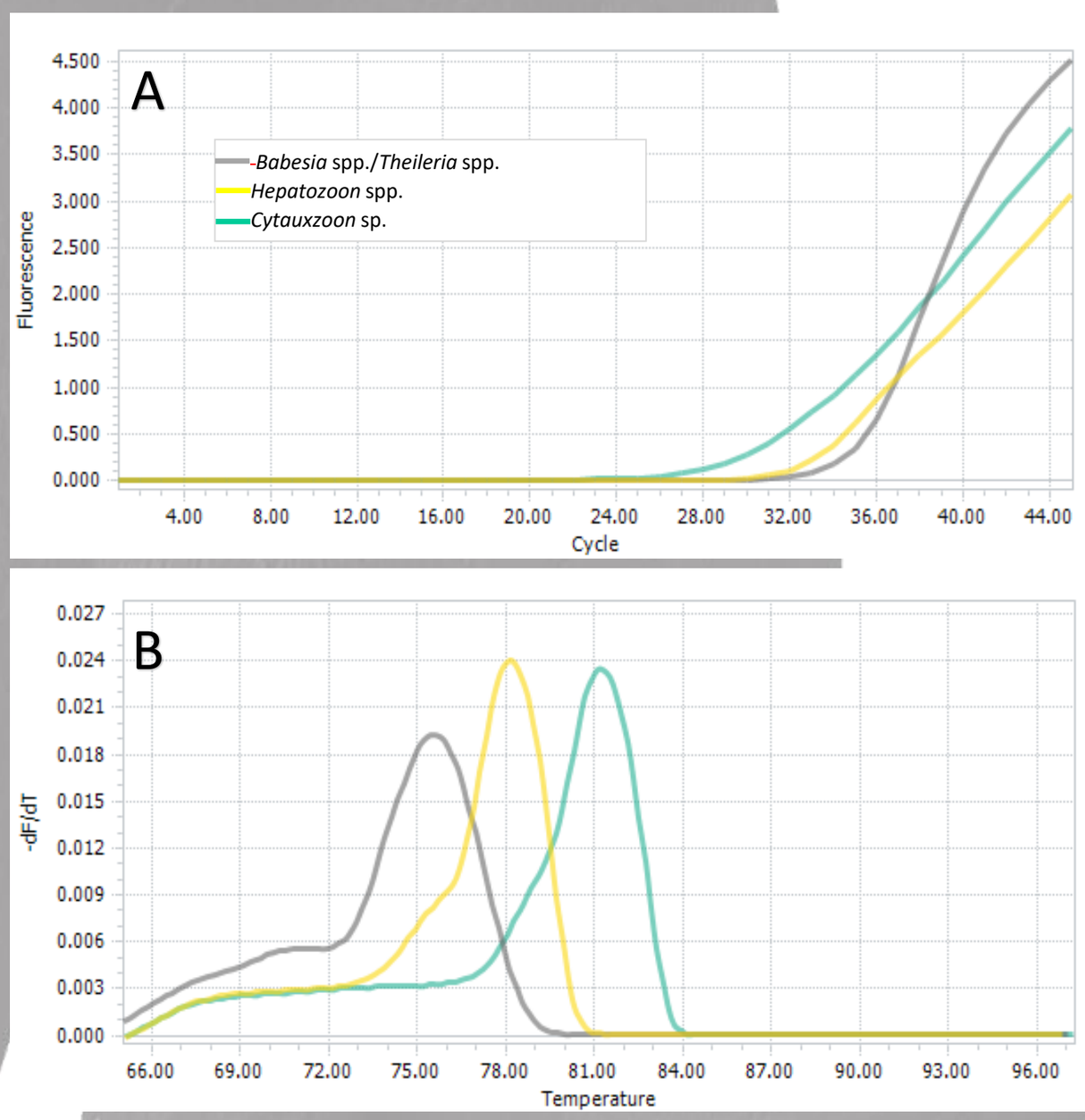


Fig. 1 Amplification curves (**A**) and melting peaks (**B**) of positive controls.