

Development and comparative evaluation of different LAMP and PCR assays for coprological diagnosis of feline tritrichomonosis

Joanna Dąbrowska[1], Jacek Karamon[1], Maciej Kochanowski[1], Bruno Gottstein[2], Tomasz Cencek[1], Caroline F. Frey[2], Norbert Müller[2]

1 Department of Parasitology and Invasive Diseases, National Veterinary Research Institute, Partyzantów Avenue 57, 24-100, Puławy, Poland; 2 Institute of Parasitology, Vetsuisse Faculty, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland

e-mail: joanna.dabrowska@piwet.pulawy.pl

The protozoan parasite *Tritrichomonas foetus* may cause severe diarrhea in cats all over the world. In the present study, we first assessed the suitability of the loop-mediated isothermal amplification (LAMP) methodology in coprological molecular diagnosis of feline tritrichomonosis. For this purpose, we compared a novel LAMP (targeted to the β-tubulin gene, named TF-βtub-LAMP) with a previously described LAMP assay (targeted to the elf 1α 1 gene, named TF-elf1a1-LAMP) that had been successfully applied for diagnosis of the bovine form of tritrichomonosis. Furthermore, we developed a novel real-time PCR (targeted to rDNA, named rt-TF-rDNA-2-PCR) that was assessed regarding its diagnostic performance in relation to previously established conventional (targeted to rDNA, named c-TF-rDNA-PCR) and real-time (targeted to rDNA, named rt-TF-rDNA-1-PCR) PCR assays as well as to the above mentioned LAMP methodology. With all these test systems, comparative investigations including analyses of reference fecal samples spiked with serial dilutions of *in vitro* cultivated T. foetus trophozoites as well as cross-reactivity testings e.g. with other trichomonad species were carried out. Here, the best methodical performance was demonstrated for the two real-time PCRs in that a sensitivity with a detection limit of < 1 trophozoites and a maximal specificity for diagnosis of *Tritrichomonas* sp. was achieved. The other test systems exhibited either an approximately 10-times lower sensitivity (c-TF-rDNA-PCR, TF-βtub-LAMP, TF-elf1a1-LAMP) or a lower specificity (rt-TF-rDNA-1-PCR). Conversely, the diagnostic performance assessed with clinical fecal samples from cats demonstrated identical sensitivities (8 positive testings in 20 clinical samples) for TF-βtub-LAMP, TF-elf1a1-LAMP, and rt-TF-rDNA-2-PCR. Diagnostic sensitivities were significantly higher than those found for rt-TF-rDNA-1-PCR (5 positive testings) and the c-TF-rDNA-PCR (6 positive testings), respectively. Accordingly, our data suggested the rt-TF-rDNA-2-PCR as well as TF-βtub-LAMP, and TF-elf1α1-LAMP to be well suited molecular tools for direct (i.e. without including an *in vitro* cultivation step) coprological diagnosis of tritrichomonosis in cats. Interestingly, relative high (TF-elf1α1-LAMP, 7 positive testings) to at least moderate (TF-βtub-LAMP, 6-7 positive testings) diagnostic sensitivities were also achieved by testing clinical samples by simple visual inspection of colorimetric changes during the LAMP amplification reactions. Accordingly, the LAMP assays described in the present study may serve as practical molecular tools to perform epidemiological studies on feline (and bovine) tritrichomosis under simple laboratory or even field conditions.