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Detection of *Tritrichomonas foetus* and *Pentatrichomonas hominis* in intestinal tissue specimens of cats by chromogenic in situ hybridization

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ABSTRACT

In this retrospective study 102 cats were analyzed for the presence of trichomonads in intestinal tissue sections using chromogenic in situ hybridization (CISH). Two intestinal trichomonad species are described in cats: *Pentatrichomonas hominis* and *Tritrichomonas foetus*. While *P. hominis* is considered a mere commensal, *T. foetus* has been found to be the causative agent of feline large-bowel diarrhea. For the detection of both agents within intestinal tissue CISH assays using three different probes were performed. In the first CISH run a probe specific for all relevant members of the order Trichomonadida (OT probe) was used. In a second CISH run all positive samples were further examined on three consecutive tissue sections using the OT probe, a probe specific for the family of Tritrichomonadidae (Tritri probe) and a newly designed probe specifically detecting *P. hominis* (Penta hom probe). In total, four of the 102 cats were found to be positive with the OT probe. Thereof, one cat gave a positive reaction with the *P. hominis* probe and three cats were positive with the *T. foetus* probe. All Trichomonas-positive cats were pure-bred and between 8 and 32 weeks of age. In one cat positive for *T. foetus* large amounts of parasites were found in the gut lumen and invading the intestinal mucosa. The species of the detected trichomonads were confirmed by polymerase chain reaction and nucleotide sequencing of a part of the 18S ribosomal RNA gene. In this study, the usefulness of CISH to detect intestinal trichomonads within feline tissue samples was shown. Additionally, the specific detection of *P. hominis* using CISH was established. Generally, it was shown that CISH is well suited for detection and differentiation of trichomonosis in retrospective studies using tissue samples.

1. Introduction

Several trichomonad species of the phylum Parabasala are well-known pathogens in veterinary medicine. In cats two trichomonad species have received scientific attention, *Tritrichomonas foetus* (family Tritrichomonadidae) and *Pentatrichomonas hominis* (family Trichomonadidae) (Cepicka et al., 2010). *P. hominis* (Honigberg et al., 1968) is known to inhabit the digestive tract, mainly the large intestine, of several vertebrates such as humans, dogs, monkeys, guinea pigs and cats (Wenrich, 1944; Jongwutiwes et al., 2000). In former studies *P. hominis* was erroneously considered to be the causative agent of the chronic large-bowel diarrhea in cats (Romatowski, 1996; Gookin et al., 1999; Romatowski, 2000). After experimental induction of transient diarrhea in specific pathogen free cats by *T. foetus* it became unanimously accepted that the disease was due

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to *T. foetus* and not *P. hominis* (Gookin et al., 2001). *T. foetus* is mainly known as the causative agent of bovine trichomonosis (Parsonson et al., 1976), a venereal disease in heifers. It could be demonstrated that cattle are susceptible to infection with *T. foetus* isolated from cats and vice versa causing comparable lesions (Stockdale et al., 2007, 2008). A recent study suggested the recognition of genetically distinct 'cattle genotype' and 'cat genotype' of *T. foetus* (Šlapeta et al., 2010). Furthermore, *T. foetus* was found to be the same species as *Tritrichomonas suis* (Lun et al., 2005), and was shown to be a facultative pathogen in the large intestine of pigs (Mostegl et al., 2011).

In cats, *T. foetus* colonizes the ileum, caecum and colon in close proximity to the mucosal surface (Yaeger and Gookin, 2005). Although the presence of *T. foetus* in the feline reproductive tract is regarded as unlikely (Gray et al., 2010), there is a single report of a natural *T. foetus* infection in the feline uterus (Dahlgren et al., 2007). Cats affected with *T. foetus* were usually less than 12 months of age with only single cases ranging up to 13 years of age (Gookin et al., 1999; Gunn-Moore et al., 2007; Stockdale et al., 2009), lived in multi-cat households, and were predominantly pure-bred cats (Gookin et al., 1999, 2004). Infections of cats with *T. foetus* were first described in the USA (Gookin et al., 1999), but several reports followed from other countries, such as the UK (Mardell and Sparkes, 2006; Gunn-Moore et al., 2007), Germany (Gookin et al., 2003b; Schrey et al., 2009), Switzerland (Frey et al., 2009), the Netherlands (Van Doom et al., 2009), Italy (Holliday et al., 2009), Greece (Xenoulis et al., 2010), Australia (Bissett et al., 2008, 2009; Bell et al., 2010), New Zealand (Kingsbury et al., 2010), and Korea (Lim et al., 2010).

Trichomonads in cats can be diagnosed by examination of fecal smears, after cultivation (Gookin et al., 2003a; Hale et al., 2009), or by species-specific polymerase chain reaction (PCR) assays on fecal samples targeting a part of the 18S ribosomal RNA (rRNA) gene (Gookin et al., 2002, 2007). Another newly described method for diagnosing trichomonads directly within formalin-fixed and paraffin wax-embedded tissue sections is fluorescence in situ hybridization (FISH) specific for a part of the 18S rRNA. With this technique the correlation of the presence of the protozoan organism with tissue lesions can easily be assessed. However, the auto-fluorescence of blood cells, which are within the size range of trichomonads, is the main disadvantage of the FISH technique (Gookin et al., 2010). Chromogenic in situ hybridization (CISH) does not display this disadvantage and has been shown to be a reliable method for detecting trichomonads (Mostegl et al., 2010), and *T. foetus* in particular (Mostegl et al., 2011), within formalin-fixed and paraffin-embedded tissue sections.

In this study, formalin-fixed and paraffin-embedded intestinal tissue sections of 102 cats were examined retrospectively, using three different CISH probes specific for all trichomonads, all members of the family Tritrichomonadidae or *P. hominis* to assess the involved species, the quantity of parasite cells and the associated lesions.

2. Materials and methods

2.1. Cat specimen

In total 102 intestinal formalin-fixed and paraffin wax-embedded tissue sections of cats (55 male, 45 female and 2 of unknown sex) from the archive of the Institute of Pathology and Forensic Veterinary Medicine were used. Included were 96 samples of cats obtained at necropsy and 6 biopsy or organ samples which were examined between 1997 and 2010. All chosen cats suffered from diarrhea and were between 4 weeks and 2 years of age. Represented breeds were European shorthair ($n = 67$), Persian ($n = 7$), European longhair ($n = 4$), Siamese, Maine Coon, British shorthair (each $n = 3$), Ragamuffin, Burmese (each $n = 2$), Exotic shorthair, Bengal, Oriental shorthair, Norwegian Forest Cat, Ragdoll, Abyssinian (each $n = 1$) and 5 cats of unknown breed. All but one tissue sample included small and large intestine, with the exceptional case comprising only small intestinal tissue. At conventional histological examination of the intestine presence of trichomonad-like organisms was registered in only two of the cases (cat 2 and cat 4).

2.2. P. hominis probe design

A CISH oligonucleotide probe for the specific detection of *P. hominis* was designed (Penta hom probe). First, homology studies comprising all 18S rRNA sequences of *P. hominis* available in GenBank were performed using the Sci Ed Central (Scientific & Educational software, Cary NC, USA) software package. A region of complete homology in all *P. hominis* sequences was chosen as probe. The selected Penta hom probe sequence was 5′-GTG AAC GTT GAA ACG TAG GGA CAT TGC TGT CCA ATT CCG-3′. Subsequently, the probe sequence was subjected to the Basic Local Alignment Search Tool (BLAST; www.ncbi.nlm.nih.gov/blast.cgi) to search against the GenBank and exclude unintentional cross-reactivity. The Penta hom probe was synthesized and labeled with digoxigenin (Eurofins MWG Operon, Ebersberg, Germany). Afterwards, it was tested on a formalin-fixed and paraffin-embedded protozoal culture containing *P. hominis*. Negative results were achieved with other protozoal cultures including *Histomonas meleagridis*, *Hypotrichomonas acosta*, *Monocercomonas colubrorum*, *Tetratrichomonas gallinarum*, *Trichomonas gallinae*, *Trichomitus batrachorum*, *Tritrichomonas augusta* and *T. foetus* (Mostegl et al., 2010). To rule out further cross-reactivity the probe was tested on various embedded cultures and tissue samples including several species of other protozoan parasites, fungi, bacteria and viruses as listed in Mostegl et al. (2010).

2.3. Chromogenic in situ hybridization

Two CISH runs were performed on the feline formalin-fixed and paraffin wax-embedded tissue sections including small and large intestine. In the first run an oligonucleotide probe (order Trichomonadida (OT) probe) specific for all known trichomonads (Mostegl et al., 2010) was used. All positive samples were subjected to a second CISH run, per-