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Short communication

Fulminant *Tritrichomonas foetus* ‘feline genotype’ infection in a 3-month old kitten associated with viral co-infection

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ABSTRACT

Tritrichomonas foetus is a flagellate protist which commonly causes a waxing and waning large bowel diarrhoea in young cats. We report severe *T. foetus* infection of the colon, cecum and ileum with concurrent feline enteric coronavirus (FCoV) and feline panleukopenia virus (FPV) in a 3-month-old Bengal kitten with an 8-day history of vomiting, diarrhoea, failure to thrive and coughing. Protozoa filling the lumen and crypts and occasional invading into lamina propria were identified within the affected colon and confirmed by PCR as *T. foetus* ‘feline genotype’. Assessment of faeces by PCR revealed concurrent infection with FCoV and FPV. It is possible that immunosuppression by FPV played a role in the unprecedented *T. foetus* infection intensity observed histologically. Studies during and after resolution of FPV infection, will be critical to determine if *T. foetus* co-infection affects long-term prognosis of FPV survivors.

1. Introduction

Tritrichomonas foetus ‘feline genotype’ causes waxing and waning large bowel diarrhoea, predominantly in multi-cat households (Gookin et al., 2017; Yao and Koster, 2015). Several studies have described co-infection with *T. foetus* and various pathogens, including *Giardia*, *Toxocara*, *Eucoleus*, *Cryptosporidium*, *Cystoisospora*, feline enteric coronavirus (FCoV) and *Clostridium perfringens* (Bell et al., 2010; Gookin et al., 2001, 2004; Kuehner et al., 2011; Paris et al., 2014; Profizi et al., 2013). So far, only *Cryptosporidium* sp. has been demonstrated to be associated with more severe clinical disease (Bell et al., 2010; Gookin et al., 2001, 2004). Gookin et al. (2001) examined the relationship between *Cryptosporidium* sp. and *T. foetus* and found diarrheal duration and severity was greater in cats with co-infection of *Cryptosporidium* sp. Detection of *T. foetus* was also noted more commonly co-associated with feline coronavirus and *C. perfringens* than as a singular pathogen in a retrospective study of 1088 cats within the United Kingdom (Paris et al., 2014).

The aim of this report was to describe co-infection of *T. foetus*, feline panleukopenia virus (FPV) and FCoV virus in an owned Bengal cat, which together caused necrotising enteritis, marked protozoal colitis and lymphoid depletion. The severe *T. foetus* infection was unprecedented and presumed to be a consequence of viral immunosuppression, resulting in euthanasia.

2. Material and methods

2.1. Case presentation

A three-month-old female Bengal kitten (*Felis catus* x *Prionailurus bengalensis bengalensis*) presented to the University of Sydney Veterinary Teaching Hospital following an 8-day history of vomiting, diarrhoea, coughing and failure to thrive. Euthanasia was elected by the registered veterinarian due to progressive clinical deterioration and poor prognosis. Prior to euthanasia, deep pharyngeal swab and conjunctival swab were collected for further testing. Faecal sample was collected postmortem.

2.2. Pathology

The carcass was submitted to the Veterinary Pathology Diagnostic Services of the University of Sydney for postmortem and histopathological examination. A complete postmortem examination was performed and samples were collected and fixed in 10% neutral buffered formalin and processed for histopathology. Sections (4 µm) were stained with hematoxylin and eosin (H&E), Periodic acid-Schiff stain (PAS), Giemsa stain and Grocott’s methenamine silver stain.

2.3. Molecular diagnostics

A faecal sample was submitted for the IDEXX Feline Diarrhoea

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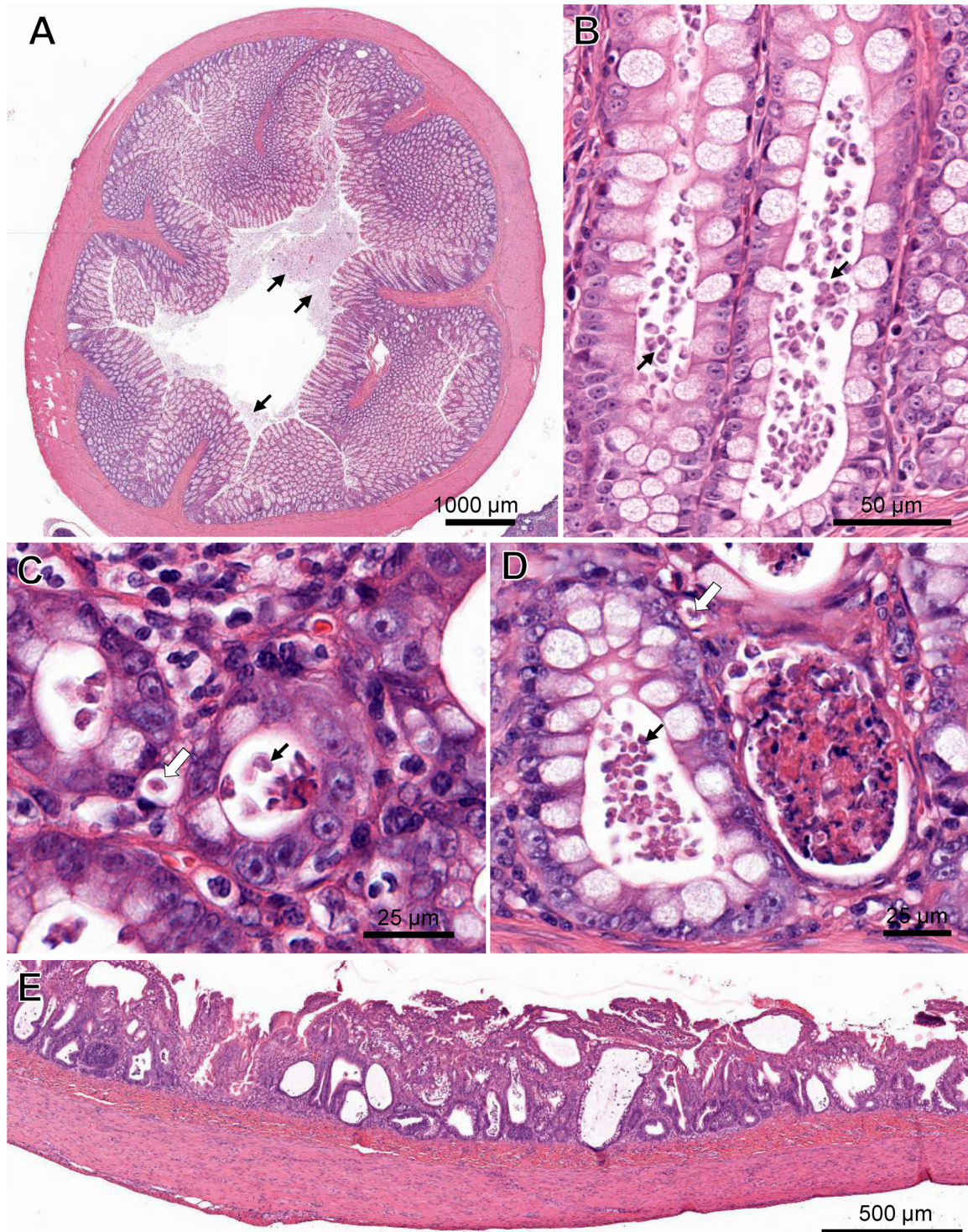


Fig. 1. Gastrointestinal pathology in a cat co-infected with *Trichostrongylus foetus*, feline enteric coronavirus (FCoV) and feline panleukopenia virus (FPV). (A) Cross-section of the colon with marked protozoal (*T. foetus*) luminal infiltrate (arrows). (B) Numerous teardrop-shaped *T. foetus* overlying the mucosal luminal surface of a crypt of Lieberkühn in the colon. (C) *T. foetus* (arrow) in the cross-section of the crypts of the colon. (D) Crypt abscesses containing cellular and karyorrhectic debris and *T. foetus* (arrows) in the colon. Note the invading *T. foetus* in the lamina propria (arrow outlines). (E) Severe attenuation of the superficial epithelium of the small intestine of the cat PCR positive for FCoV and FPV. H&E. (Virtual Slide for Virtual Microscopy: [VM05606](#)).

RealPCR Panel (IDEXX, Sydney, Australia) targeting *Clostridium perfringens* enterotoxin A gene, *Cryptosporidium* spp., feline coronavirus (FCoV), feline panleukopenia virus, *Giardia* spp., *Salmonella* spp., *Toxoplasma gondii* and *T. foetus*.

Pathogens known to contribute to respiratory disease (*Bordetella bronchiseptica*, *Chlamydomphila felis*, feline calicivirus, feline herpesvirus type 1 (FHV-1), H1N1 influenza virus and *Mycoplasma felis*) were

targeted using the IDEXX Feline Upper Respiratory Disease (URD) RealPCR Panel (IDEXX, Sydney, Australia) via deep pharyngeal and conjunctival swab.

Determination of *T. foetus* genotype was evaluated by PCR amplification of the ITS rDNA region using primers TFR3/TFR4 as previously described (Felleisen, 1997; Šlapeta et al., 2010, 2012). All PCRs were run with a negative control of sterile PCR-grade water. PCR product

was directly and bidirectionally sequenced at MacroGen Inc. (Seoul, Korea) and assembled sequence using CLC Main Workbench 6.9.1 (CLC bio, a QIAGEN Company, Denmark) and deposited in GenBank (National Center for Biotechnology Information, NCBI) under the Accession Numbers: [MH882783](#).

3. Results

3.1. Marked necrotising to lymphoplasmacytic *Tritrichomonas foetus*-associated colitis

Multiple sections (n = 4) from the caecum and colon were examined in transverse and longitudinal sections. All sections demonstrated numerous 5 by 7 µm teardrop-shaped flagellated protozoa filling the intestinal lumen and crypts (Fig. 1A and B). Glands within the colonic mucosa were frequently ectatic and contained myriad protozoa (Fig. 1A–D). The colonic lamina propria, and to a lesser extent, the submucosa, were expanded by many lymphocytes, plasma cells and macrophages, and lesser numbers of neutrophils. According to the lesion scoring system described by Yaeger and Gookin (2005) for *T. foetus*-positive cats, this cat had a severe grade of microabscesses in crypts and a mild lymphoplasmacytic and moderate neutrophilic lamina propria infiltrate. Adjacent to some of the microabscesses were noticeable invading *T. foetus* trophozoites in the lamina propria (Fig. 1C and D). Additional colonic lesions were considered mild to moderate in grade.

3.2. Marked necrotising feline panleukopenia virus (FPV)-associated enteritis

Numerous sections (n = 14, proximal duodenum to distal ileum) from small intestines showed marked villous blunting and fusion, crypt loss and distortion with numerous crypt abscesses and marked infiltration of the lamina propria with many lymphocytes and plasma cells, and fewer macrophages and neutrophils admixed with cellular and karyorrhectic debris (necrosis), clear space (oedema) and extravasated erythrocytes (haemorrhage) (Fig. 1E). Small numbers of *T. foetus* trophozoites were also present in distal ileum crypts. Mesenteric lymph nodes had significant lymphoid depletion, follicular hyalinosis and marked sinusoidal histiocytosis.

3.3. Molecular confirmation of *Tritrichomonas foetus* infection

The faecal sample was positive for FPV, FCoV, and *T. foetus* using a PCR panel. The Feline URD Panel was negative.

To molecularly characterise the genotype of *T. foetus* real-time PCR positive, ITS rDNA (297 nt, excluding amplification primers) was amplified and sequenced. The obtained ITS rDNA was 100% identical with *T. foetus* ‘feline genotype’ (JX187001).

4. Discussion

This case report supports previous data that co-infection has direct implications on host-viral and host-bacterial interactions and pathogenesis of *T. foetus* in cats (Gookin et al., 2001). The findings described in the small intestine and mesenteric lymph nodes were consistent with late-stage FPV infection (Balboni et al., 2014). FPV infects rapidly dividing cells, including lymphoid tissue and proliferating cells of the bone marrow resulting in cell lysis and functional immunosuppression which predisposes these animals to other infectious agents (Truyen et al., 2009). FCoV causes a mild diarrhoea and coinfects cats with *T. foetus* (Paris et al., 2014; Vogel et al., 2010). FCoV may have contributed to the increased *T. foetus* burden, however, its role was likely minor in the face of severe feline parvoviral enteritis. It is likely the host response to *T. foetus* was compromised, leading to increased numbers of *T. foetus*. Bovine *T. foetus* infections elicit a parasite-specific antibody

response to adhesin and contact-dependent cytotoxicity, and these mechanisms may be hindered in immunosuppressed animals (Tolbert and Gookin, 2016; Voyich et al., 2001). Fluctuations in intestinal microbiota in the weaned kitten, influenced by co-infection of enteric pathogens, may have contributed to the trichomonad disease burden (Gookin et al., 2017; Šlapeta et al., 2015).

Co-infections with FPV or FCoV may influence prognosis of trichomoniasis in cats. Many cats infected with *T. foetus* undergo spontaneous resolution of their diarrhoea and become ‘asymptomatic carriers’, shedding low levels of the organism without clinically apparent diarrhoea (Gookin et al., 2017). It is thought trichomoniasis resolves within 2 years in healthy cats, but in cats compromised with concurrent viral infection e.g. FPV, the time period until resolution is unknown (Gookin et al., 2017). Co-infection with other diarrheic agents such as FPV in this case may serve to increase the trichomonad disease burden. This has the potential to create an environment whereby a known reservoir of infection is shedding large amounts of the infectious agent in question. *T. foetus* is a protozoan known to persist in the environment for long periods of time (Hale et al., 2009; Van der Saag et al., 2011). Increased environmental *T. foetus* contamination creates a long-term persistent problem.

Future prospective studies undertaking ancillary diagnostic testing in cases of FPV, during and after resolution of FPV infection, will be critical to determine if *T. foetus* co-infection is significant and affects long-term prognosis of FPV survivors.

Conflict of interest

There is no conflict of interest to be declared.

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