Veterinary Parasitology 175 (2011) 160–164

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

Morphological and molecular characterisation of a mixed *Cryptosporidium muris*/*Cryptosporidium felis* infection in a cat

Louise FitzGerald[a], Mark Bennett[a], Josephine Ng[a], Philip Nicholls[a], Fleur James[b], Aileen Elliot[a], Mike Slaven[a], Una Ryan[a,*]

[a] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch Drive, Murdoch, WA, 6150, Australia
[b] School of Veterinary Clinical Sciences, Murdoch University, Murdoch, WA, 6150, Australia

ARTICLE INFO

Article history:
Received 1 July 2010
Received in revised form
24 September 2010
Accepted 4 October 2010

Keywords:
Cryptosporidium muris
Cryptosporidium felis
Cat
Mixed infection
Morphology
Genotyping

ABSTRACT

To date *Cryptosporidium muris* has been identified by microscopy and genotyping in cats in two studies. We report morphological and genetic evidence of a mixed *C. muris* and *C. felis* infection in a cat and provide the first histological, immunohistochemical, *in situ* hybridisation and genetic confirmation of a *C. muris* infection in the stomach of a cat. The cat suffered persistent diarrhoea after the initial consultation, which remained unresolved, despite several medical interventions. Further studies are required to determine the range, prevalence and clinical impact of *Cryptosporidium* species infecting cats.

1. Introduction

Cryptosporidium is a genus of protozoan parasites whose members can cause diarrhoea in many hosts including humans and domestic animals. Currently 23 species of *Cryptosporidium* are accepted as valid including *Cryptosporidium muris*, which infects rodents as its primary host and *Cryptosporidium felis* in cats (Xiao, 2010; Fayer et al., in press).

Cryptosporidium spp. infection is relatively common in cats and epidemiological surveys conducted worldwide have reported that the prevalence in cats ranges from 0 to 29% (Lucio-Forster et al., in press). This apparent variation in the rate of infection might be due, in part, to the method of detection (e.g. concentration of oocysts and direct light microscopy versus microscopy of stained smears or PCR), as well as the population being sampled (animal age differences, owned animals, stray populations, shelter animals) and symptomatic versus asymptomatic animals (Lucio-Forster et al., in press).

Genetic characterisation of oocysts recovered from faecal samples of cats have identified *C. felis* (Ballweber et al., 2009; Palmer et al., 2008; Huber et al., 2007; Thomaz et al., 2007; Fayer et al., 2006; Santín et al., 2006; Morgan et al., 1998; Sargent et al., 1998; Gasser et al., 2001; Ryan et al., 2003; Hajdusek et al., 2004) and *C. muris* in two studies (Santín et al., 2006; Pavlasek and Ryan, 2007). The identification of *C. muris* in cats in the latter two studies was based on genotyping of oocysts recovered from faeces. No histological studies were conducted and therefore it was not possible to determine if the cats were actually infected with *C. muris* or were merely acting as mechanical vectors. In the present study, we report on genetic, morphological and histological characterisation a mixed *C. muris*/*C. felis* infection in a cat.

* Corresponding author. Tel.: +61 8 9360 2482.
E-mail address: Una.Ryan@Murdoch.edu.au (U. Ryan).

0304-4017/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2010.10.003

2. Materials, methods and results

In 2008, a 2-year old male neutered domestic long haired cat presented for investigation of chronic diarrhoea. The clinical signs were characteristic of small bowel diarrhoea with an increased frequency of defecation. Appetite was normal and weight loss and vomiting were not features of his initial clinical presentation. Physical examination at the time of initial presentation was unremarkable. Screening haematology, biochemistry and urinalysis identified a mild increase in creatine kinase activity (413 U/L; reference range 50–100 U/L). Fasting feline trypsin-like immunoreactivity was normal (30 μg/L; control reference 12–82 μg/L). The cat tested negative for feline leukaemia virus and feline immunodeficiency virus (Simplify, AGEN Biomedical; Brisbane, Australia). Initial symptomatic therapy consisted of cobalamin (Vitamin B12, Troy, Australia) at 200 mg/kg by subcutaneous injection weekly for 6 treatments and dietary modification to increase the content of soluble fibre, however there was little response to these interventions. Further symptomatic therapy was trialled, including metronidazole (Flagyl, Sanofi Aventis, Spain) at 9.4 mg/kg every 12 h for 10 days and fenbendazole (Panacur 100, Virbac Animal Health, Australia) at 50 mg/kg once daily per os for 5 days.

The cat represented 13 months later with continuing diarrhoea and he had also begun to vomit most days. An abdominal ultrasound was performed and identified mild mesenteric lymphadenomegaly, mildly irregular splenomegaly and normal gastrointestinal wall thickness and layering. Fine needle aspirate cytology of the mesenteric lymph nodes and spleen identified mild reactivity in both locations. Gastroduodenoscopy showed that there were areas of marked gastric mucosal oedema, however the duodenal mucosa was unremarkable. Mucosal pinch biopsies were collected from the stomach and duodenum. The cat was prescribed empirical amoxycillin-clavulanate (Clavulox; Pfizer, Australia) at 13.9 mg/kg every 12 h per os and a novel protein diet trial whilst results were pending.

Faecal samples were collected and examined using malachite green staining as previously described (Elliot et al., 1999). Parasites were examined with the aid of an ocular micrometer in a Zeiss Axioskop microscope at 1000× magnification and this revealed the presence of two different sized *Cryptosporidium* sp. oocysts; large oocysts which resembled *C. muris* in size and shape (8.0 μm × 5.8 μm, mean width/length ratio 1.4, $n = 30$) and smaller oocysts which resembled *C. felis* in size and shape (4.6 μm × 4.0 μm width/length ratio 1.15, $n = 20$) (Fig. 1).

Endoscopic biopsy specimens from the stomach and duodenum were fixed in 10% neutral buffered formalin for 24 h, then processed routinely and embedded in paraffin. Histological sections were cut at 5 μm and stained with hematoxylin and eosin. Microscopic examination of the stomach biopsies revealed the presence of abundant *Cryptosporidium* sp. organisms within the gastric pits and within the lumina of fundic glands. The affected glands were frequently dilated and filled with numerous *Cryptosporidium* spp. organisms (Fig. 2A and B). There was a mild increase in fibrous tissue within some areas of the lamina propria, leading to mild separation of glands accompanied by a mild,

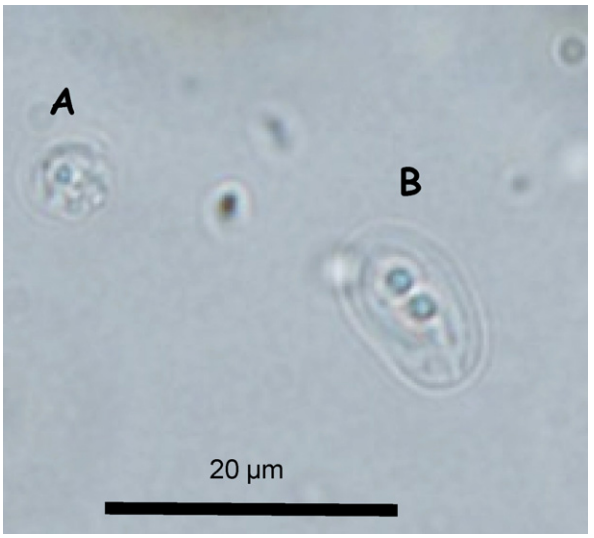

Fig. 1. Malachite green stained wet mount of cat faecal sample showing (A) *C. felis*-like oocysts and (B) *C. muris*-like oocysts.

multifocal lymphoplasmacytic and neutrophilic inflammatory cell infiltrate. In the duodenum, the *Cryptosporidium* sp. stages were closely associated with the apical surface of enterocytes (Fig. 2C). There was a mild, patchy increase in lymphocytes and plasma cells in the lamina propria along with low numbers of scattered neutrophils and a mild, multifocal increase in intraepithelial lymphocytes.

Approximately 1 μg of purified PCR product DNA (~500 bp) from the *C. muris* 18S rRNA gene, from a rodent-derived *C. muris* isolate, was labelled with digoxigenin to produce DNA probes for *in situ* hybridisation using the DIG-Nick Translation Mix, according to the manufacturer's instructions (Roche Diagnostics). The digoxigenin-labelled DNA was added to a probe cocktail mixture consisting of 50% formamide, 10% dextran sulfate and 2× SSC buffer. Sections were deparaffinised, rehydrated, probed, washed, developed, counter-stained and mounted as previously described (Bennett et al., 2008). An irrelevant DNA probe for bandicoot papillomatosis carcinomatosis virus type 1 was used as a negative control.

For immunohistochemistry, histological sections were dewaxed in xylene and rehydrated through graded ethanols to water. Endogenous peroxidase activity was then blocked using 3% hydrogen peroxide. The primary antibody, mouse anti-*Cryptosporidium* (Serotec MCA-2571), was diluted 1:200 with antibody diluent (Dako-Cytomation) and applied to tissue sections for 30 min. Following thorough rinsing with phosphate buffered saline (PBS), primary antibody binding was detected using a horseradish peroxidase-labeled streptavidin biotin system (LSAB – Dako) according to the manufacturer's instructions. Slides were rinsed in tap water and the slide was counterstained lightly with Harris' hematoxylin. Omission of the primary antibody was used as a negative control.

In situ hybridisation and immunohistochemical experiments confirmed that the organisms deep within the lumina of scattered gastric glands (Fig. 3a–c) were indeed members of the genus *Cryptosporidium*. Immunohisto-