

Supplementary file 2. Details of microbiological tests and specific kits used

Stool culture and PCR for fecal pathogens: First, a stool culture and multiplex polymerase chain reaction (PCR) were performed with *Allplex™ Gastrointestinal Panel Assays®: Allplex™ GI-Bacteria (I) and Allplex™ GI-Parasite (1)* in both patients and pets. After these, stool samples were again tested for the following pathogens, which were genotyped:

- *Giardia duodenalis* DNA detection was achieved using a real-time PCR (qPCR) method, targeting the gene coding the small subunit ribosomal RNA (*ssu* rRNA) of the parasite (2). The molecular diversity of the parasite at the assemblage level was investigated only in the *Giardia* isolates that tested positive by qPCR and yielded cycle threshold (C_T) values ≤ 34 . To do so, a nested PCR was employed to amplify a fragment of the *ssu* rRNA gene (3,4).
- The presence of *Cryptosporidium* spp. was assessed with a nested-PCR protocol to amplify a fragment of the *ssu* rRNA gene of the parasite (5).
- Identification of *Blastocystis* spp. was achieved by a direct PCR protocol targeting a fragment of the *ssu* rRNA gene of the parasite (6).
- *Enterocytozoon bieneusi* detection was conducted by a nested PCR protocol to amplify a fragment of the internal transcribed spacer (ITS) region as well as portions of the flanking large and small subunit of the ribosomal RNA gene as previously described (7).
- Detection of *Encephalitozoon* genus members (*E. intestinalis*, *E. cuniculus*, *E. hellem*) was conducted with a direct PCR protocol to amplify an *ssu* rRNA gene fragment of the parasite (8).
- PCR assays targeting 2 different and conserved regions of the viral genome for the diagnosis of acute hepatitis E virus (HEV) infection as well as molecular evaluation of HEV and ratHEV were performed (9,10,11).
- Real-time PCR testing for *Toxoplasma gondii* in feces from cats and *Leishmania* spp. in conjunctival swabs from dogs: Total nucleic acid (DNA and RNA) was extracted from 0.2 g feces or 200 μ l swab eluate, respectively, with the "MagMAX™ CORE Nucleic Acid Purification Kit" (ThermoFisher) via the KingFisher Flex platform (ThermoFisher), according to the manufacturer's instructions. Total nucleic acid was eluted in 200 μ l (feces) or 100 μ l (swab) of elution buffer (ThermoFisher), and 5 μ l was amplified in subsequent single plex real-time PCR reactions. Real-time PCR was performed at IDEXX, Vet Med Labor GmbH, employing the LightCycler 480 system (Roche) with proprietary forward and reverse primers and hydrolysis probes. The target gene for *Toxoplasma gondii* detection was the internal transcribed spacer 1 (ITS-1) gene (L49390), and for *Leishmania* spp. detection it was the major surface glycoprotein gene, GP63 (Y08156).
- For the detection helminths, a multiplex fluorescence immunoassay method detecting antigens of Ancylostomatidae, Ascarididae, Trichuridae, *Dipylidium caninum*, and *Giardia* were performed, only in feline and canine feces (Idexx Fecal Dx®)(12,13).

PCR assays performed in blood: PCR assays for the diagnosis of acute hepatitis E virus (HEV) infection, as well as molecular evaluation of HEV and ratHEV, were performed on patients' and pets' blood (9,10,11). In dogs and cats, a PCR for detecting *Leishmania* spp. was also performed (Idexx).

Serology: Several serological tests were performed in patients and pets, which are described in *Table S3*.

Nasopharyngeal and rectal swabs: In both patients and pets, we performed nasopharyngeal swab cultures for the detection of *Staphylococcus aureus* and *S. pseudintermedius* colonization. In addition, rectal swabs were performed for the detection of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* and the carbapenem-resistant bacteria *Enterobacteriaceae*.

TABLE S1. Polymerase chain reaction protocols used for the molecular identification and/or characterization of protist pathogens in the human and animal fecal samples investigated in this study

Temperature and time								
Target organism	Locus	Initial denaturation	Denaturation	Annealing	Extension	No. cycles	Final extension	Reference
<i>Giardia duodenalis</i>	<i>ssu</i> rRNA	95 °C 15 min	95 °C 15 s	60 °C 1 min	72 °C 30 s	45	–	Verweij et al., 2003 (2)
	<i>ssu</i> rRNA	95 °C 2 min	95 °C 45 s	58/55 °C 30 s	72 °C 45 s	35	72 °C 4 min	Appelbee et al., 2003 (3)
<i>Cryptosporidium</i> spp.	<i>ssu</i> rRNA	94 °C 3 min	94 °C 40 s	50 °C 40 s	72 °C 1 min	35	72 °C 10 min	Tiangtip and Jongwutiwes, 2002 (5)
<i>Blastocystis</i> spp.	<i>ssu</i> rRNA	95 °C 3 min	94 °C 1 min	59 °C 1 min	72 °C 1 min	30	72 °C 2 min	Scicluna et al., 2006 (6)
<i>Enterocytozoon bienewisi</i>	ITS	94 °C 3 min	94 °C 30 s	57/55 °C 30 s	72 °C 40 s	35	72 °C 10 min	Buckholt et al., 2002 (7)
<i>Encephalitozoon</i> spp.	<i>ssu</i> rRNA	94 °C 10 min	94 °C 30 s	60 °C 30 s	72 °C 30 s	35	72 °C 10 min	Fedorko et al., 1995 (8)

TABLE S2. Oligonucleotides used for the molecular identification and/or characterization of the protist pathogens in animal fecal samples investigated in this study

Target organism	Locus	Oligonucleotide	Sequence (5'–3')	Generated amplicon (bp)	Reference
<i>Giardia duodenalis</i>	<i>ssu</i> rRNA	Probe	FAM–CCC GCGCGGTCCCTGCTAG–BHQ1	62	Verweij et al., 2003 (2)
		Gd-80F	GACGGCTCAGGACAACGGTT		
	<i>ssu</i> rRNA	Gd-127R	TTGCCAGCGGTGTCCG		
		Gia2029	AAGTGTGGTGCAGACGGACTC	300	Appelbee et al; 2003 (3)
		Gia2150c	CTGCTGCCGTCCTTGGATGT		
	RH11	CATCCGGTCGATCCTGCC		Hopkins et al., 1997 (4)	
	RH4	AGTCGAACCCTGATTCTCCGCCAGG			
<i>Cryptosporidium</i> spp.	<i>ssu</i> rRNA	CR-P1	CAGGGAGGTAGTGACAAGAA	587	Tiangtip and Jongwutiwes, 2002 (5)
		CR-P2	TCAGCCTTGC GACCATACTC		
		CR-P3	ATTGGAGGGCAAGTCTGGTG		
		CPB-DIAGR	TAAGGTGCTGAAGGAGTAAGG		
<i>Blastocystis</i> spp.	<i>ssu</i> rRNA	BhRDr	GAGCTTTTAACTGCAACAACG	600	Scicluna et al., 2006 (6)
		RD5	ATCTGGTTGATCCTGCCAGT		
<i>Enterocytozoon bienewisi</i>	ITS	EBITS3	GGTCATAGGGATGAAGAG	390	Buckholt et al., 2002 (7)
		EBITS4	TTCGAGTTCTTTCGCGCTC		
		EBITS1	GCTCTGAATATCTATGGCT		
		EBITS2.4	ATCGCCGACGGATCCAAGTG		
<i>Encephalitozoon</i> spp.	<i>ssu</i> rRNA	MICROF	CACCAGGTTGATTCTGCCTGA	250–270	Fedorko et al., 1995 (8)
		MICROR	CCTCTCCGGAACCAAACCCTG		

ITS: internal transcribed spacer; *ssu* rRNA: small subunit ribosomal RNA

TABLE S3. Serological tests performed in patients and pets

PETS' SEROLOGY			
		Method	Kit
Dog	<i>Leishmania</i> (Ab)	ELISA	CIVTEST Leishmania, Hipra
	<i>Ehrlichia canis</i> (Ab)	ELISA	VetLine Ehrlichia, NovaTec Immunodiagnostica
	<i>Borrelia burgdorferi</i> (Ab)	ELISA	SNAP 4DX Plus IDEXX
	<i>Rickettsia</i> spp. (Ab)	IFI	MegaFLUO Rickettsia, Megacor
	<i>Babesia canis</i> (Ab)	ELISA	BABESIA-ELISA DOG, AFOSA
	<i>Anaplasma</i> spp. (Ab)	ELISA	SNAP 4DX Plus IDEXX
	<i>Leptospira</i> (Ab)	MAT	MAT was performed according to OIE standards (Office International des Epizooties OIE, 2008)
Cat	<i>Leishmania</i> (Ab)	IFI	MegaFLUO LEISH kit, Megacor
	<i>Leptospira</i> (Ab)	MAT	MAT was performed according to OIE standards (Office International des Epizooties OIE, 2008)
	<i>Toxoplasma</i> IgM (Ab)	IFI	Anti-Toxoplasma gondii IIFT Dog (IgM), Euroimmun
	<i>Toxoplasma</i> IgG (Ab)	IFI	Anti-Toxoplasma gondii IIFT Dog (IgG), Euroimmun
PATIENTS' SEROLOGY			
		Method	Kit
Patients owning dogs	<i>Toxocara canis</i> (IgG)	ELISA	NovaLisa Toxocara canis IgG (Novatec)
	<i>Strongyloides</i> (IgG)	ELISA	NovaLisa Strongyloides IgG (Novatec)
	Hepatitis E virus	ELISA	Wantai Diagnostic IgG kit (Beijing, China)
Patients owning cats	<i>Bartonella henselae</i> (IgG)	CLIA	Bartonella henselae Virclia IgG MONOTEST (Vircell)
	<i>Toxoplasma gondii</i> (IgG)	CLIA	<i>Toxoplasma gondii</i> Virclia IgG MONOTEST (Vircell)
	Hepatitis E virus	ELISA	Wantai Diagnostic IgG kit (Beijing, China)

Ab: antibodies; CLIA: chemiluminescence immunoassay; ELISA: enzyme-linked immunosorbent assay; IFI: indirect immunofluorescence; MAT: microagglutination test; Ag: antigen.

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