A retrospective molecular study of some intestinal protozoa in healthy pet cats from Italy.

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Abstract

Domestic cats are hosts of several intestinal protozoan parasites that can be responsible for enteric disease. The purpose of the current study was to determine the prevalence by PCR technique of *Tritrichomonas foetus, Toxoplasma gondii*, *Giardia duodenalis* and *Cryptosporidium* spp and to genotype some of them in faeces of 146 privately owned cats, without a history of diarrhea within the previous 3 months. PCR assays resulted positive in 32 (22.9%) feline stools. Three animals (2%) scored positive for *T. foetus* and *Cryptosporidium* DNAs, respectively, 15 specimens (10.3%) resulted positive for *T. gondii* and 11 (7.5%) for *G. duodenalis*. Coinfections were never observed.

The specimens positive for *T. gondii* gave hints for clonal genotype I (N. 7), genotype II (N. 1) and genotype III (N. 7), respectively. The isolates of *G. duodenalis* were referable to assemblage F (N. 9) and to assemblage C (N. 2). Results of typing analysis allowed the identification of *C. felis*, in all cases. In conclusion the results obtained in

the present survey would add some information to the epidemiology of these protozoa, considering that they can occur in healthy pet cats, also.

Key wors: Feline, *Tritrichomonas foetus, Toxoplasma gondii*, *Giardia duodenalis*, *Cryptosporidium* PCR.

Domestic cats are hosts of several intestinal protozoan parasites that can be responsible for enteric disease. Some of them, such as *Toxoplasma gondii*, are zoonotic and can represent a public health hazard, some species of *Cryptosporidium* and assemblages of *Giardia intestinalis* seem to be involved in human infection[1] while *Tritrichomonas foetus* is a flagellate responsible for feline intestinal tritrichomoniasis[2] and does not affect humans.

Cats are the only domestic felids shedding *T. gondii* oocysts, with prevalences of 0.1%-0.4% in European countries[3].

Giardia duodenalis and *Cryptosporidium* spp. can infect cats worldwide. Cats are the specific hosts of *Cryptosporidium felis*. *Cryptosporidium parvum* and *Cryptosporidium muris* have also been detected in naturally infected cats, probably due to the broader host range of these species. The published studies of this infection in cats worldwide refer prevalence rates ranging from 0.6% to 15.4% [1]. The health status of investigated

38 animals, (normal or diarrheic), different age groups, and diagnostic techniques used
39 probably contributed to the variations in infection rates in different studies.
40 The overall prevalence of *Giardia* infection in feline is approximately 4% [4].
41 Assemblages A to F were all detected in cats in a multi country study [5], although
42 assemblage A and the cat-specific assemblage F were the dominant ones. This finding
43 was corroborated also by other studies [6,7].
44 *Tr. foetus*, is reported worldwide in cats with diarrhea, mostly affecting young animals
45 and/or kept in crowded environments, with prevalence more than 30% [8].
46 The purpose of the current study was to determine the prevalence by PCR technique and
47 to genotype some protozoan intestinal parasites in privately owned cats, without a
48 history of diarrhea within the previous 3 months.
49 One hundred forty six stool samples were collected immediately after shedding from
50 cats living in Tuscany (provinces of Pisa and Florence) and Liguria (province of
51 Genoa). All the samples were obtained from privately owned, healthy European short
52 hair cats, of both genders, aged between 1-3 years, referred to private veterinary clinics
53 for neutering. Inclusion criteria were animals allowed to roam outdoor, without diarrhea
54 from 3 months, not administered any drug in the previous 30 days.
55

56 Faecal specimens were submitted to copromicroscopic examination. Samples were
57 processed by a conventional flotation method using 262 mg/ml $ZnCl_2$ and 275 mg/ml

NaCl, as described by Schares et al. (2005) [9]. Copromicroscopical examination was carried out at 400X magnification.

Parasite DNA was extracted by fecal samples using a ZR Fecal DNA MiniPrep™ (Zymo Research Corp.).

All primers and restriction enzymes were provided by Eurofins MGW (M-Medical, Milano, Italy).

DNA from *Tr. foetus* was detected by using a single-tube nested PCR, as described by Gookin et al. (2002) [10].

Nested PCR (nPCR) for *T. gondii* DNA was performed following the procedure reported by Jones et al. (2000) [11], using two pairs of oligonucleotide primers to amplify regions of the B1 gene. Genotypes were determined via multiplex multilocus PCR-RFLP for 12 genetic markers (SAG1, 3'-SAG2, 5'-SAG2, alt.SAG2, SAG3, BTUB, GRA6, C22-8,C29-2, L358, PK1, and Apico) [12].

G. duodenalis was detected amplifying gdh gene and genotyping was performed by PCR-RFLP using restriction enzymes *Nla*IV and *Rsa*I, [13].

Cryptosporidium spp DNA was detected by nPCR and genotyped with PCR-RFLP performed by restriction enzymes *Ssp*I and *Vsp*I [14].

Furthermore, to avoid any misidentification PCR products of *Tr. foetus, G. duodenalis* and *Cryptosporidium* spp were purified using QIAquick® PCR purification kit (Qiagen, Milan, Italy) according to the manufacturer's instructions and then sequenced. All

78 sequencing procedure was performed by a commercial laboratory (BMR-Genomics,
79 Padova, Italy).
80 The sequences were assembled and corrected by visual analysis of the electropherogram
81 using Bioedit v.7.0.2 (Hall 1999) and compared with those available in GenBank using
82 the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).
83 Copromicroscopic examination was negative for intestinal protozoa considered in the
84 present report in all examined samples.
85 PCR assays resulted positive in 32 (22.9%) feline stools. Three animals (2%) scored
86 positive for *Tr. foetus* and *Cryptosporidium* DNAs, respectively, 15 specimens (10.3%)
87 resulted positive for *T. gondii* and 11 (7.5%) for *G. duodenalis.* Coinfections were
88 never observed.
89 Results of typing analysis allowed the identification of *C. felis*, in all cases. The
90 specimens positive for *T. gondii* were not fully genotyped at all 12 loci, but hints for
91 clonal genotype I (N. 7), genotype II (N. 1) and genotype III (N. 7), respectively were
92 obtained. More detailed results are showed in Table. Nucleic acids of *G. duodenalis*
93 were referable to assemblage F (N. 9) and to assemblage C (N. 2), respectively.
94 The results obtained from sequenced DNAs confirmed the identification obtained by
95 PCR in all specimens.
96 The prevalence registered for *Tr. foetus* (2%) is lower when compared to other results
97 available. However to the best of our knowledge any survey has been carried out in

98 healthy pet cats, since data from literature deal with diarrheic cats or healthy animals
99 living in communities [8]. This parasite is considered significantly associated to diarrhea,
100 even if a recent report [16] referred the possibility to isolate this agent from clinically
101 normal cats, in agreement to the present survey.
102 Fifteen cats scored positive for *T. gondii* DNA, with an overall prevalence of 10.3%.
103 This finding is in agreement with a previous report carried out on colony stray cats from
104 Tuscany [17].
105 The present results cannot be compared to any other similar study. Data from European
106 literature are referred to PCR carried out on oocysts revealed by copromicroscopy.
107 Clonal type II, with the Apico I allele was the most recurrent genotype both in Germany
108 and in Switzerland [3,18]. Furthermore in Germany a clonal genotype III and 10 mixed
109 genotypes were recovered [18]. These finding would not completely agree with our
110 results: in the present survey all apparently clonal and not mixed genotypes were
111 identified, and genotypes suggestive of I and III were prevalent. As far as we know this
112 is the first report of genotyping of *T. gondii* DNA in feline faeces in Italy. In this
113 country genotyping was carried out on *T. gondii* DNA from both domestic and sylvatic
114 animals, indicating the occurrence of strictly clonal genotypes in goats [19] and feline, in
115 respect to mixed types detected in free ranging waterfowl [20] and foxes [21]. These results
116 would be in agreement with the hypothesis of 2 distinct cycles of *T. gondii* in domestic
117 and sylvatic environments [22].

Eleven cats scored positive for *G. duodenalis* DNA, with an overall prevalence of 7.5%, in agreement with Paoletti et al. (2011) [23]. Genotyping yielded 9 assemblages F, the most frequent in feline host and 2 C, the canine assemblage. Recover of canine assemblages in feline feces has been previously reported [1,13,24] and it could be due to occurrence of *Giardia* cysts on feline coats, ingested during grooming. These assemblages have never been demonstrated in humans with *G. duodenalis* infection in USA [1] and even if in one study, assemblage F was reported in people, this finding remains to be confirmed at additional loci [25]. *C. felis* was the sole *Cryptosporidium* species isolated in the present survey, from 3 animals, with a prevalence of 2%. This finding confirms the result of a previous report in cats from the same area, when 1/273 was proven to excrete DNA referable to *C. felis* [26]. Despite the very close association between people and cats *C. felis* infections are infrequently reported in humans [27].

In conclusion the results obtained in the present survey would add some information to the epidemiology of these parasite protozoan, considering that these parasites occur in healthy pet cats, also.

Conflict of interest.

The Authors declare that there is no conflict of interest.

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Examined loci												
Isolate	**SAG1**	**3'SAG2**	**5'SAG2**	**SAG2 new**	**SAG3**	**BTUB**	**C22-8**	**C29-2**	**GRA6**	**L358**	**PK1**	**Apico**
1	**III**	**III**	**III**		**III**	**III**	**III**	**III**	**III**			**III**
2		**I**	**I**	**I**			**I**	**I**	**I**		**I**	
3	**III**	**III**	**III**	**III**	**III**			**III**		**III**	**III**	
4	**I**	**I**	**I**	**I**		**I**	**I**	**I**	**I**		**I**	**I**
5	**III**	**III**	**III**	**III**	**III**			**III**		**III**		
6	**I**	**I**	**I**	**I**		**I**	**I**				**I**	**I**
7	**I**	**I**		**I**		**I**		**I**				
8	**III**	**III**		**III**	**III**			**III**	**III**		**III**	**III**
9	**II**	**II**	**II**	**II**				**II**			**II**	**II**
10	**I**	**I**										
11	**III**	**III**	**III**	**III**	**III**	**III**				**III**		**III**
12	**III**	**III**		**III**	**III**		**III**	**III**	**III**			
13	**I**	**I**		**I**		**I**				**I**		**I**
14	**I**	**I**	**I**	**I**		**I**	**I**					
15	**III**	**III**	**III**		**III**		**III**	**III**		**III**		**III**

Table – Genotyping results of *Toxoplasma gondii* DNAs isolated from feline feces