Enteric protozoan infections in kittens affected by intestinal disorders: a cross sectional survey in the Umbria region

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Intestinal disorders are a quite frequent occurrence in domestic cats younger than 1 year of age. Aim of the present study was to conduct a cross-sectional survey on the prevalence of enteric protozoan infections among cats clinically affected by intestinal disorders and to give a description of the clinical patterns.

The study involved 92 kittens, less than 12 months old, belonging to cat shelters (n. 37) and private households (n. 55) and showing alterations of the macroscopic characteristics of the stools. Each animal was subjected to clinical examination and data collection. Individual faecal samples were collected during the visits. Each sample was examined by means of a flotation-centrifugation technique, processed for the copro-antigenic identification of *Giardia duodenalis* and *Cryptosporidium* spp. and tested for the detection of *Tritrichomonas foetus* by molecular tools.

The results of the present survey showed that enteric protozoan parasitism is a condition frequently associated with intestinal disorders in cats under 1 year of age. Overall, 21.74% of the animals tested positive for at least one protozoan species. In the infected animals, the following parasites were identified: *G. duodenalis* (14.13%), *Cystoisospora* spp. (10.87%) and *T. foetus* (2.17%). Having an age less than 4 months and living in a shelter resulted prognostic for protozoan infections. Otherwise, clinical signs were not indicative, with the exception of haematochezia and steatorrhoea, for, respectively, *Cystoisospora* spp. and *G. duodenalis* infections. Given the limited number of *T. foetus* infections detected, further studies are necessary to confirm any consideration concerning this pathogen.

Key words - Kittens, enteritis, protozoa.

INTRODUCTION

Intestinal tract disorders associated with infectious agents of viral, bacterial and protozoal origin are a frequent finding in cats under one year of age. The factors that may favour the spread/persistence of the faecal-oral transmission cycle of infectious diarrhoic agents in these subjects are in fact many; among them: the immaturity of the immune system, the incomplete development of the intestinal microbiota, overcrowding, promiscuity, poor hygiene and sanitary conditions (e.g.,

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colonies/shelters/breeding farms), and stress-inducing conditions including changes in habitat and diet.[1,2,3]
The most common intestinal protozoan agents that are the cause of intestinal disorders in young cats include: *Giardia duodenalis*, *Tritrichomonas foetus*, *Cystoisospora* (previously *Isospora*) spp. and *Cryptosporidium* spp.
Giardia duodenalis (Sarcomastigophora, Diplomonadida) is a poly-flagellated protozoan, parasite of humans and of domestic and wild animals, which localises in the proximal small intestine where it adheres to the apical summit of enterocytes, thereby affecting their absorption capacity.[4] The transmission of *G. duodenalis* is ensured by the faecal dissemination of infectious cysts that can survive for months in the external environment. The prevalence of Giardia infection in cats is quite variable; the morbidity is maximal in subjects under one year of age,[5] while it is mostly asymptomatic in adult animals.[6,7] Symptomatology, when present, is characterised by the presence of mucous diarrhoea which tends to become chronic, while systemic clinical signs are rare.

Intestinal disorders are a frequent finding in cats under 1 year of age and protozoan infections are among the various causes.

Tritrichomonas foetus (Sarcomastigophora, Trichomonadida) is a protozoan parasite usually associated with reproductive disorders of the bovine species[8] and only in more recent times has it been counted among the parasitic agents capable of causing large bowel disorders in the domestic cat.[9,10] As for *G. duodenalis*, the cycle of the parasite does not include the presence of resistant cystic forms; however, the existence of pseudo-cystic forms that can justify the high resistance of the parasite in the external environment has been hypothesised.[11,12] Most infected subjects develop chronic or intermittent diarrhoea of the large bowel that can persist for up to 24 months;[13] a small proportion of animals may remain asymptomatic while still shedding the parasite.[14] A significant number of case reports indicates the presence of *T. foetus* in co-infections with other more commonly found enteric protozoa, among which *G. dudoenalis*;[15,16,17] in such cases symptoms appear to be more severe.[18]
Cystoisopora felis and *C. rivolta* (Apicomplexa, Eucoccidida) are protozoan agents responsible for feline coccidiosis, a disease with a cosmopolitan distribution. Infection occurs through ingestion of sporulated oocysts present in the environment but also through predation of small paratenic hosts.[19] Parasites exhibit an elective tropism for the jejunum-ileal tract (occasionally also for the coecal tract in the case of *C. felis*)[20,21] and through their asexual reproduction cycle (schizogonie) they can cause the progressive destruction of the intestinal epithelium. The clinical signs are almost exclusively observed in kittens,[20] in whom the highest level of protozoan pathogens is also found;[22,23] the clinical signs are anorexia, vomiting and diarrhoea, sometimes haemorrhagic.[22,24] Subsequently, cats develop a level of immunity such that in the adult sporadic infections are mild and/or inapparent.
Among the *Cryptosporidium* genus (Apicomplexa, Eucoccidiida), *C. felis* is the cat-adapted species, although cases of infection caused by *C. parvum* and *C. muris* have also been reported.[25,26,27] Similarly to *Cystoisospora*, *Cryptosporidium* is transmitted through the ingestion of infectious oocysts that are present in contaminated food, water and the environment.[28] Although frequently isolated,[23,29,30,31] in most cats *Cryptosporidium* infection has a subclinical course;[32] clinically overt forms, characterised by marked abdominal tenderness and watery diarrhoea, are almost exclusively observed in association with other protozoan pathogens, in particular *G. duodenalis* and *T. foetus*,[27,33,34] or in very young debilitated and/or immunocompromised subjects.[35,36,37]
With the aim of providing support to clinicians in planning diagnostic and prevention strategies against intestinal disorders in young cats, a cross-sectional survey was conducted to estimate the burden of protozoal parasitism associated with enteric syndromes.

MATERIALS AND METHODS

Population sampled

The survey was conducted in the period between August 2014 and January 2015 and involved 92 cats under one year of age, divided into three age groups: ≤4 months (n. 34), 5-8 months (n. 40), 9-12 months (n. 18). The animals were selected within catteries (n. 37) and Veterinary Practices in the Province of Perugia (n. 55). The two enrolment criteria were, respectively: i) the presence of alterations of the physical characteristics of the stools consistent with a faecal score between 4 and 7 with reference to the Purina Fecal Scoring System (Figure 1); ii) the absence of anti-protozoal treatments in the two weeks prior to the sampling.
A physical examination was carried out on each subject with collection of signalment and clinical history data. The clinical record reported the signs resulting from the general examination and from the particular gastro-intestinal examination, including the faecal score between 4 and 7 (Fecal Scoring System Nestlé, Purina).
Individual faecal samples were collected from each animal, placed in refrigerated containers (+ 4 °C) and promptly sent to the laboratory for the execution of specific laboratory tests for detection of, respectively, *G. duodenalis*, *T. foetus*, *Cystoisospora* spp. and *Cryptosporidium* spp.

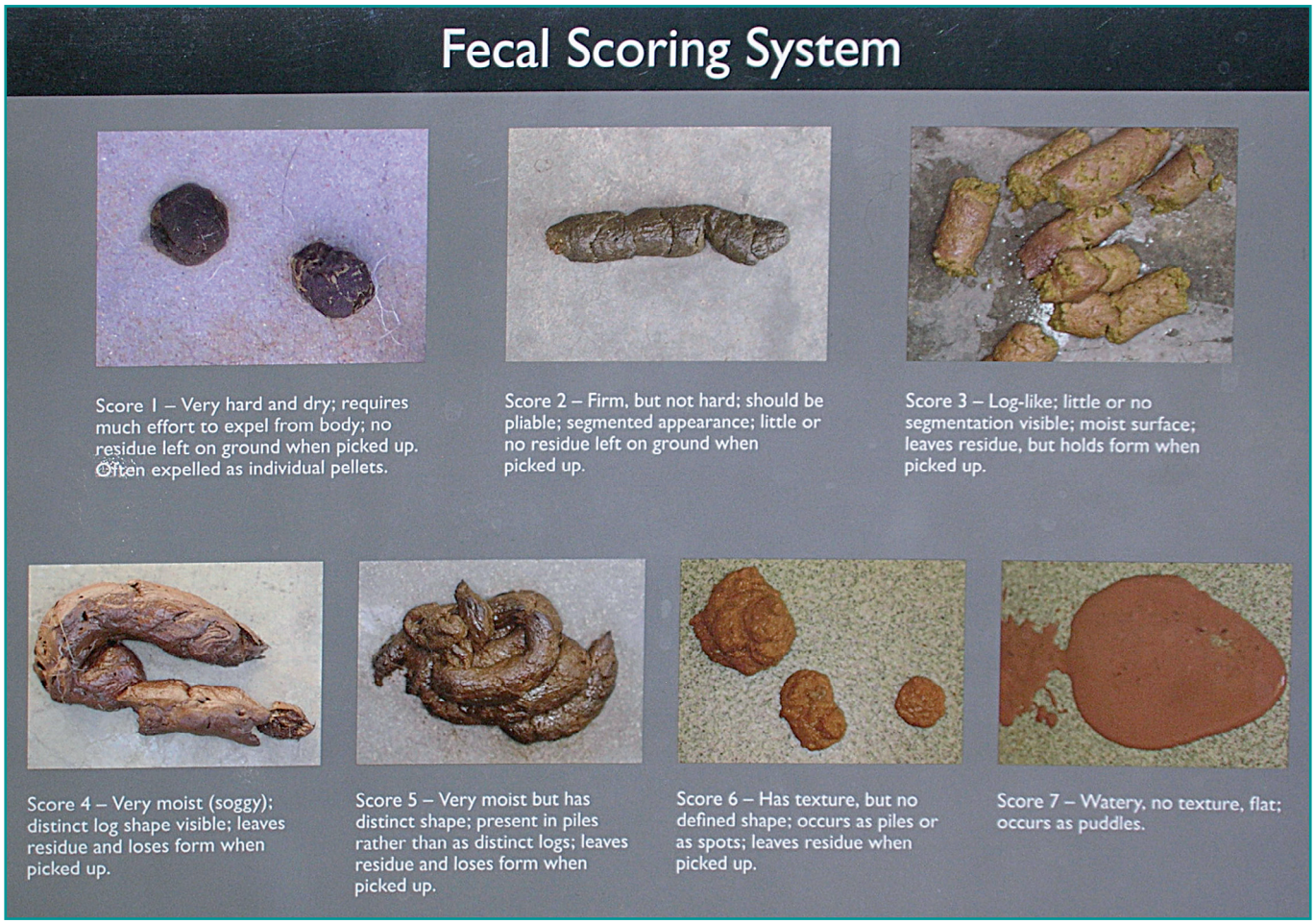

Figure 1 - Nestlé Purina Fecal Scoring System.

Laboratory tests

A small aliquot was taken from each faecal sample for the preparation of faecal smears examined with rapid differential staining (Diff-Quick, Medion Diagnostics, Düdingen, Switzerland) to search for trophozoitic forms of *G. duodenalis* and *T. foetus*.

The faecal samples were then homogenised, filtered and centrifuged. The sediment obtained was then subjected to, respectively, enrichment by flotation, a coproantigen test and the extraction of genomic DNA to be subjected to PCR for the search of *T. foetus*.

Faecal flotation was performed using a 33% zinc sulfate flotation solution (ZnSO4 33%, SW=1200)[38] for the detection of cysts/oocysts of *G. duodenalis*, *Cystoisospora* spp. and *Cryptosporidium* spp.

The coproantigen test for *G. duodenalis* and *Cryptosporidium* spp. was conducted using a Direct Immunofluorescence commercial kit (MERIFLUOR Crypto-Giardia, Meridian® Bioscience, Cincinnati OH, USA), in accordance with the instructions provided by the manufacturer.

The extraction of genomic DNA was obtained from 200 µl of faecal sediment maintained at -18 °C after thawing and resuspension. The QIAamp Fast DNA Stool Mini Kit (QIAGEN®, Valencia, CA) was used for the extraction, following the modified protocol described by Gookin *et al.*[39] Modifications included an incubation with proteinase K for 1 hour at 56 °C, two wash steps with buffer AW1 and an additional centrifugation after the final wash. The concentration and purity of the extracted DNA were evaluated by spectrophotometric analysis (Biophotometer, Eppendorf AG®, Hamburg, Germany).

A population of cats <12 months of age underwent a clinical investigation and the collection of faeces for the detection of *G. duodenalis*, *T. foetus*, *Cystoisospora* spp. and *Cryptosporidum* spp.

The extraction products were subjected to PCR according to the protocol described by Gookin *et al.*,[6] modified. The modifications included the use of a single pair of primers, TFITS-F (5'-CTGCCGTTGGATCAGTTTCG-3') and TFITS-R (5'-GCAATGTGCATTCAAAGATCG-3'), which amplify a fragment of 208 bp included between

the ITS1 region and the 5.8S gene of the *T. fetus* rRNA, and the use of an annealing temperature of 54 °C. All PCR reactions were obtained on a final volume of 50 µl containing appropriate quantities of genomic DNA (50 ng min), 25 µl of NZYTaq 2x Green Master Mix (Nzytech®, Lisbon, Portugal), 2 µl of each primer (10 µM) and sterile distilled H_2O. The target sequence was amplified using the following temperature protocol: initial denaturation step at 95 °C for 5', followed by 50 amplification cycles at 95 °C for 30" (denaturation), 54 °C for 30" (annealing), 72 °C for 30" (extension) and a final extension phase at 72 °C for 5'. DNA from *T. foetus* trophozoites isolated from cat faeces in the Laboratory of Parasitology and Parasitic Diseases of the Veterinary Science Department of the University of Messina was included as positive control.

The samples were tested for the DNA detection of *T. foetus* by means of a PCR protocol.

20 µl aliquots of the amplification product were loaded on 1.2% agarose gel (Agarose E, CONDA SA, Madrid, Spain) containing 5 µl of SafeView (SafeView™ Nucleic Acid Stains, ABM Inc., Canadian) and were analysed by electrophoresis using the loading buffer TBE 5X (0.089M Tris base, 0.089M boric acid (pH 8.3), 2 mM Na_2EDTA, CONDA, SA, Madrid, Spain) and applying a voltage of 120 V for about 45'.

The amplified products were visualised with the transilluminator and the molecular weight of the amplified fragment was estimated by comparison with the 100 bp ladder (100 bp DNA Ladder, Microtech, Naples, Italy) and the positive control. In addition, to confirm the identity of the amplicons obtained the PCR products were sequenced using a capillary sequencer ABI 3730XL 96 at BMR Genomics of Padua (http://www.bmr-genomics.it/); the sequences obtained were compared with the sequences of *T. foetus* deposited in GenBank by BLAST analysis (Basic Local Alignment Search Tool).[40]

Data processing

The prevalence for each protozoal infection and coinfection was defined as the ratio between the number of cats positive for individual protozoan pathogens over the total number of subjects analysed, with the related 95% confidence intervals (CIs), using the Episheet spreadsheet, available at: http://krothman.host.byet2.com/episheet.xls.

A descriptive analysis of the distribution of positivities with respect to the age and origin of the animals was also performed.

RESULTS

The prevalence rates observed for each pathogen of interest are shown in Table 1, as well as those relating to helminth infestations identified with the copro-microscopic investigation. Of the 92 faecal samples analysed, 21.74% (No. 20/92) were positive for at least one protozoan infection, with *G. duodenalis* being the most frequently found pathogen (No. 13/92, 14.13%). *Cystoisospora* spp. and *T. foetus* infections were observed respectively in No. 10 (10.87%) and No. 2 (2.17%) subjects. Five animals presented mixed infections (5.43%, 95% CI 0.8-10.07); of these, two subjects (2.17%) had a co-infection with *G. duodenalis* and *T. foetus*, while 3 (3.26%) were positive for both *G. duodenalis* and *Cystoisospora* spp. Total negativity was found for *Cryptosporidium* spp.

The distribution of positives compared to the age group and origin of the animals is reported in Table 2. In cats coming from catteries the overall prevalence of protozoal infections was equal to 29.73%, against a percentage of positives in household animals of 16.36%. In sheltered cats, *Cystoisospora* spp. was the most frequently found parasite (18.9%), while in household cats the highest degree of positivity was observed for *G. duodenalis* (14.54%). *Tritrichomonas foetus* was only found in household, Maine Coon cats, purchased from cat breeders in Northern Italy.

The overall prevalence rate for protozoan infections as well as the percentage of positives for *G. duodenalis* and *Cystoisospora* spp. infections were maximum in subjects <4 months of age and decreased progressively in the two subsequent age groups (Table 2). *Tritrichomonas foetus* was observed only in subjects between 5 and 8 months of age.

Table 3 shows the distribution of the main clinical signs and the faecal alterations detected in the course of protozoal infections. General symptoms, variously associated one with the other, were observed in 9 patients with giardiasis; the most frequently found symptom was weight loss - from moderate to severe -, present in 7 subjects (53.84%); 2 animals (15.38%) presented dehydration and moderate sensory deprivation. Symptoms related to intestinal abnormalities were observed in 10 subjects; the most frequent of these was bowel loop distension, present in 6 animals (46,15%), in association or not with flatulence, observed in 5 subjects (38.46%).

The results show a reasonable prevalence of protozoal infections among which *G. duodenalis* is the most frequently found pathogen, followed by *Cystoisopora* spp. and *T. foetus*.

With regards to cats with coccidiosis, 3 subjects showed general symptoms such as anorexia, sensory deprivation,

hyperthermia; weight loss was observed in only one animal; on the other hand, all of the animals showed enteric symptoms, such as abdominal tenderness and bowel loop dilation, detected by palpation [evident in 8 (80%) and in 6 (60%) of subjects, respectively].

In 70% of cases the faecal score of animals positive to *G. duodenalis* and of animals positive to *Cystoisospora* spp. was between 6 and 7; in case of giardiasis the presence of mucus and steatorrhea was extremely frequent, while in the presence of coccidian infections there was a prevalence of haematochezia.

In the presence of tritrichomoniasis the only noticeable alteration at the clinical objective examination was weight loss in one of two infected subjects, while dilated bowel loops and a severe proctitis were present in both animals. The faecal score was of 6 and mucus was always present.

DISCUSSION

The current study investigated animals with enteric disorders of an age group (<12 months) traditionally considered highly susceptible to enteric infections of protozoan nature. The prevalence found (21.74%) is similar to the one reported in previous studies[7,8] conducted in random populations of cats of variable age and selected regardless of the presence/absence of symptoms. The prevalence of protozoan infections found in the study was therefore below our expectations. The existance of sub-clinical forms, commonly observed in adult subjects, may perhaps justify the finding of similar prevalence rates, in spite of the diversity of the sample population.

The comparison between parasitic status and signalment-history data allowed to show that the highest positivity rates for protozoan disorders are found in subjects with enteric disorders in the early months of life (<4 months) and coming from multi-cat environments, such as catteries. The data confirm what has been observed in previous studies[41,42,43,44] and underline the impact that overcrowded and/or multi-cat environments have in fostering the spread and perpetuation of the transmission cycle of such pathogens, as well as on the exacerbation of clinical symptoms.

The percentage of positivity for *G. duodenalis* was of 14,13%, in line with the prevalence range reported in trials conducted both in Europe (4.4%-37%)[45] and out-

The overall prevalence rate of protozoal infections (21.74%) is lower than expected.

Table 1 - Prevalence rates for each pathogen considered and co-infection rates

	No. positive cases/total %	(95% CI)
Total protozoan infections	20/92	21.74% (13.73%-30.17%)
Giardia duodenalis	13/92	14.13% (7.01%-21.25%)
Tritrichomonas foetus	2/92	2.17% (0.19%-4.15%)
Cystoisospora spp.	10/92	10.87% (4.5%-17.22%)
Cryptosporidium spp.	0/92	0% (0%)
Total helminth infections		
Toxocara cati	12/92	13.04% (6.16%-19.93%)
Aelurostrongylus abstrusus	8/92	8.69% (2.94%-14.45%)

Table 2 - Distribution of positive cases compared to the age and origin

	Age			Origin	
	< 4 months (No. 34)	**5-8 months** (No. 40)	**9-12 months** (No. 18)	**Shelter** (No. 37)	**Household** (No. 55)
Overall protozoan infections in the population sampled	12 (35.29%)	6 (15%)	2 (11.11%)	11 (29.73%)	9 (16.36%)
Giardia duodenalis	8 (23.52%)	4 (10%)	1 (5.55%)	5 (13.51%)	8 (14.54%)
Tritrichomonas foetus	–	2 (5%)	–	–	2 (3.63%)
Cystoisospora spp.	6 (17.64%)	3 (7.5%)	1 (5.55%)	7 (18.9%)	3 (5.45%)
T. foetus + *G. duodenalis*	–	2 (5%)	–	–	2 (3.63%)
G. duodenalis + *Cystoisospora* spp.	2 (5.88%)	1 (2.5%)	–	1 (2.7%)	2 (3.63%)

Table 3 - Distribution of the sample population and of individual infections compared to the clinical signs and the faecal score

Symptoms of the sampled population (No. 92)	*Giardia duodenalis* (No. 13)	*Cystoisospora* spp. (No. 10)	*Tritrichomonas foetus* (No. 2)
Hyperthermia	—	1 (10%)	—
Sensory deprivation	2 (15.38%)	2 (20%)	—
Dehydration	2 (153.8%)	—	—
Weight loss	7 (53.84%)	1 (10%)	1 (50%)
Postural abnormalities	—	—	—
Anorexia	—	2 (20%)	—
Polyphagia	—	—	—
Tenesmus	—	—	—
Vomiting	1 (7.6%)	1 (10%)	—
Abdominal tenderness	—	8 (80%)	1 (50%)
Dilated bowel loops	6 (46.15%)	6 (60%)	2 (100%)
Halitosis	—	—	—
Flatulence	5 (38.46%)	—	—
Perianal soiling	—	2 (20%)	—
Perianal irritation	2 (15.38%)	—	2 (100%)
Characteristics of the stools			
F.S*. 4**	2 (15.38%)	1 (10%)	—
F.S. 5***	2 (15.38%)	2 (20%)	—
F.S. 6****	5 (38.46%)	3 (30%)	2 (100%)
F.S. 7*****	4 (30.76%)	4 (40%)	—
Mucus	11 (84.61%)	2 (20%)	2 (100%)
Steatorrhea	10 (76.92%)	—	—
Haematochezia	—	7 (70%)	—
Melena	—	—	—

* FS: Faecal score.
** 4: very moist, with distinct log shape; loses form when picked up.
*** 5: very moist, irregularly formed; leaves residue; loses form when picked up.
**** 6: no defined shape.
***** 7: watery.

side of Europe (2.4%-80%).[46] With regard to Italy, with the exception of the study conducted by Papini *et al.* (by using an ELISA copro-antigen assay the study reported a prevalence of 15.8%), the more recent studies reported lower positivity rates, regardless of the diagnostic technique used (copro-antigen detection, biomolecular tests),[15,48,49,50] with rates between 1.3% and 7.5%. Such differences may be dependent on the type of population sampled and on the diagnostic techniques used.

G. duodenalis infection was observed with almost the same frequency in both household and sheltered cats, in contrast with the data referred to protozoal infections as a whole. Given the opportunistic nature of *Giardia,* it can be assumed that stressful events, such as habitat changes associated with the introduction into a new household, together with the consequent dietary changes, may have exacerbated the symptoms in young asymptomatic subjects, many of which, although family-owned, came from a shelter environment.

Cystoisospora spp. was found in 10 cats out of 92 (10.87%). The prevalence is high in comparison with the one reported in recent Italian[48,49] and in-

The prevalence rates of the individual pathogens are in line with reports in the literature. The age and origin of the animals are important diagnostic signalment and history data.

ternational studies[51,52,53] and also in this case it was ascribed to the type of population sampled. The highest rate was observed in cats coming from catteries, in agreement with what reported by other authors[42,43,54,55,56].

The prevalence rate for *T. foetus* was of 2.17%; this is suggestive of a minor role of the parasite in enteric syndromes of the young cat. The diagnostic approach used in the survey has surely allowed an accurare assessment of the status of infection, with no risk of underestimation; the PCR protocol used for the samples is in fact the test with the highest sensitivity and specificity compared to both traditional faecal smear tests and to culture[39,58,60].

The prevalence rate is in line with the one found in Italy by Mancianti *et al.* (2%)[15] by sampling randomly a population of asymptomatic animals; it is instead in disagreement with the markedly higher prevalence rates reported by both Holliday *et al.*[57] and by many international authors.[16,18,58,59,60] Such difference may be attributable to the population studied, which in our case mostly involved crossbred instead of purebred cats, which are apparently more concerned by the parasite.[18,58,59,61] The tendency of the parasite to infect purebred subjects appears to be confirmed also by our results, as both subjects that tested positive for *T. foetus* were Maine Coon cats purchased from cat breeders in Northern Italy; the onset of the enteric symptoms took place a few weeks after introduction into the new household.

The clinical symptoms observed in the single infections were poorly specific and often overlapping, therefore of little diagnostic aid if we exclude the presence of haematochezia associated with *Cystoisospora* spp. and the presence of mucus and steatorrhea in the course of giardiasis. In addition, for T. foetus infections the signs observed could hardly be associated with the specific causal agent, as in both cats a *G. duodenalis* co-infection was present.

In the presence of enteric disorders intestinal protozoa of the cat should be included in the differential diagnosis and searched for with specific diagnostic techniques.

In conclusion, the current survey confirms that the presence of protozoan parasites is an event commonly associated with enteric disorders in cats under one year of age. It is therefore necessary for Veterinarians to: i) collect a precise clinical and environmental history, which is often more diagnostically useful than the existing clinical findings; ii) perform specific tests in order to detect and discriminate between the different pathogens involved, also in view of the different therapeutic protocols to which they respond; iii) set a correct diagnostic algorithm based on techniques that are not routinely used in veterinary practices (e.g., coproantigen detection, PCR), without which a definitive diagnosis is not possible.

KEY POINTS

- Domestic cats can host different species of protozoa responsible for intestinal disorders which in most cases become clinically evident in younger subjects.
- The current study investigated the prevalence of protozoan infections in a population of young cats (<1 year of age) with enteric disorders from the Umbria region.
- The overall prevalence rate of protozoan infections amounted to 21.74%, with higher rates in the lower age group (<4 months) and in animals from shelters.
- The most commonly encountered protozoan was *G. duodenalis* (14.13%), followed by *Cystoisospora* spp. (10.87%) and *T. foetus* (2.17%).
- The prevalence of *T. foetus* is low when compared to that of other protozoa; however, the clinical history confirms some trends already reported in the literature: age, breed and origin (shelter).
- In young cats, enteric protozoa should be included in the differential diagnosis of intestinal disorders and searched for by means of specific methodologies.

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