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Intestinal parasite infections in dogs affected by multicentric lymphoma and undergoing chemotherapy

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ABSTRACT

Prevalence and species composition of intestinal parasites were evaluated in dogs affected by high-grade multicentric lymphoma and undergoing chemotherapy and in control healthy dogs. Obtained data were statistically analyzed. The overall prevalence of intestinal parasite infections was 33.3%. In lymphoma dogs, the prevalence of protozoa infections (46.7%) was significantly higher ($p < 0.05$) than that of helminth infections (6.7%) and *Giardia duodenalis*, *Cryptosporidium* spp., *Neospora caninum*, *Cystoisospora ohioensis*-complex, *Entamoeba* sp. and *Spirocerca lupi* were identified. In the control group, only 3/15 dogs (20%) were found positive and no statistically significant differences emerged regarding helminth (hookworms and *Toxocara canis*) and protozoa (*G. duodenalis*) infections. Results from this study may suggest a potential higher prevalence of opportunistic intestinal protozoa, including some potentially zoonotic species, in dogs affected by high-grade multicentric lymphoma, emphasizing the need to monitor lymphoma-affected dogs for these protozoa, especially those undergoing chemotherapy.

1. Introduction

The worldwide incidence of cancer has increased in the last 10 years in both humans and companion animals [1,2]. In Italy, the number of canine cancer patients is growing, with an estimated incidence rate ranging from 142.8/100,000 to 800/100,000 dogs [2–4]. Among dog cancer diseases, lymphoma is one of the most common, accounting for up to 24% of all reported neoplasms, and is the most common hematopoietic tumor [5,6]. The annual incidence has been estimated between 13 and 24 cases per 100,000 dogs. As in human medicine, the majority of canine lymphomas (60%–80%) arise from malignant B cells [3,7]. Its prognosis appears to be variable, with a survival time ranging from four weeks to several years, depending on the severity of the different lymphoma types and sub-types, the World Health Organization (WHO) score of lymphoma clinical stage, the treatment administered, and the presence of concurrent diseases [5,6]. In dogs, chemotherapy is the treatment of choice for hematological malignancies and solid metastatic tumors, both with palliative and curative intents [8]. Alkylating agents, steroids, antitumor antibiotics and anti-microtubule compounds are the most common used drug classes in veterinary medicine for the treatment of lymphomas. They may be administered as a single agent, or combination drug therapy to reduce the development of drug resistance [8,9]. The dosage of these cytotoxic agents is based on the concept of maximum tolerated dose. Therefore, some side effects are expected and accepted in treated dogs during chemotherapy administration [10]. The most frequent complications of anticancer drugs administration include myelosuppression and gastrointestinal signs, with risk of neutropenic septic episodes that need to be treated aggressively [8].

In human medicine, opportunistic pathogens are the most common causes of life-threatening infections in immunocompromised individuals and may lead to severe or fatal outcomes [11]. Human patients whose immune system has been impaired by drugs for cancer treatment or bone marrow transplantation are highly susceptible to various pathogens, including bacteria, viruses and parasites [12]. Among these pathogens, endoparasites are one of the major causes of uncontrolled debilitating clinical signs, especially in immunocompromised children [13]. Among human intestinal parasites, the nematode species *Strongyloides stercoralis* and some protozoa, as *Cryptosporidium* spp., *Cystoisospora belli* and *Blastocystis hominis*, stand out for their opportunist character and frequency in human cancer patients [11,14–16].

In veterinary medicine, only few studies are available about internal parasites in lymphoma or immunocompromised canine and feline

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patients, and most of them deal with a single parasite species [17–22]. Considering this lack of data, the aim of this study was to evaluate the prevalence and species composition of fecal parasite infections in dogs diagnosed with lymphoma and undergoing chemotherapy. In addition, differences before and during the treatment with chemotherapeutic drugs were also evaluated.

2. Method and materials

2.1. Animals

The study population comprised client-owned dogs affected by lymphoma and undergoing chemotherapeutic treatment (study group), referred to the Veterinary Teaching Hospital "Mario Modenato" of the Department of Veterinary Sciences of Pisa University (Italy) between January 2014 and June 2016. Healthy age-matched client-owned dogs not affected by lymphoma, living in the same geographical area and presented in the same period for routine vaccination, were used as control group. Inclusion criteria for dogs in the study group were a diagnosis of lymphoma (obtained by physical examination, lymph node cytology and immunophenotyping, hemato-biochemical analysis, bone marrow cytology, abdomen ultrasound and thoracic x-ray), the absence of concurrent diseases, the agreement of dog owners to the administration of a chemotherapeutic treatment and available fecal samples for parasitological analysis. Clinical stage was assessed according to the WHO criteria. A chemotherapy induction protocol with vincristine (0.75 mg/m2 intravenously once a week), cyclophosphamide (50 mg/m2 per os 3–4 days/week), and prednisone (40 mg/m2 per os daily for the first week, then tapered to 5 mg/m2 orally daily in the further weeks) was administered for eight weeks. In the case of a complete remission, a maintenance protocol including melphalan (0.1 mg/Kg per os) and chlorambucil (0.1 mg/Kg per os), administered alternatively twice a week, associated with vincristine (0.75 mg/m2 IV once a month) was used.

For control dogs, the inclusion criteria were the absence of clinical signs of disease in the past two months and available fecal samples for parasitological analysis. Control dogs were defined healthy based on owner questioning and on the results of complete physical examination.

None of the dogs included in the study showed any gastrointestinal signs in the past two months, and none received antiparasitic drugs within at least the previous two months before fecal sample collection. A complete blood count and serum biochemistry profiles were conducted both for dogs with lymphoma and for healthy control dogs. Except for a single dog whose owner decided not to treat the animal, all dogs that in the present study scored positive for endoparasites at the fecal examination were treated with previously recommended antiparasitic protocols.

This study was approved by the University of Pisa Institutional Animal Experimental Use Committee (0009069/2014). Written informed consent was also obtained from all owners of enrolled dogs.

2.2. Parasitological analysis

From all examined dogs, individual fecal samples were collected and examined for intestinal parasites. More precisely, from dogs of the study group fecal samples were collected at presentation and at different time points during the administration of the chemotherapeutic protocol, i.e. during the induction phase, at the end of the induction phase and in the maintenance phase, when possible. In the case of control dogs, fecal samples were collected at the time of presentation. Fresh fecal samples were examined soon after collection or they were stored at 4 °C and examined within 24 h.

All fecal samples were qualitatively screened macroscopically to detect the presence of proglottids, nematodes and fragments of parasites. Furthermore, fecal samples were examined microscopically by flotation test (at least 2 g of feces) with a low-density solution (saturated NaCl solution, specific gravity 1.2) and a high-density solution (saturated zinc chloride solution, specific gravity 1.56), to evaluate the presence of helminth eggs and protozoal (oo)cysts [23]. A commercial rapid immunoassay (RIDAQUICK® *Cryptosporidium/Giardia* Combi cassettes, R-Biopharm Italia srl) was used to detect *Giardia* and *Cryptosporidium* fecal antigens [24]. In a dog found positive for *Neospora caninum*-like unsporulated oocysts at coprological examination, serological testing for *N. caninum* antibodies were performed by using 12-well slides (Fullerton Lab, Fullerton CA., USA), as previously described [22,25]. Furthermore, an aliquot (200 µl) of the fecal sample was extracted using the ZR faecal DNA miniprep (Zymoresearch, USA), and further analyzed by PCR with species-specific primer pairs (Np6+/Np21+ that amplify a 337 bp fragment of the Nc 5 region) [22,26]. Finally, for the isolation of nematode larvae from fecal samples, the Baermann technique was used [24].

2.3. Statistical analysis

Data were analyzed using the software XLSTAT. The prevalence of isolated parasites was calculated as the number of positive animals/total number of examined animals. Data were preliminarily analyzed using a $\chi 2$ test with the Yates correction or the Fisher exact test, when appropriate. Multivariate and univariate analyses deliberately were not performed because of the small sample size. Values of $p < 0.05$ were considered statistically significant.

3. Results

Fifteen dogs in the study group and 15 dogs in the control group met the inclusion criteria. Dogs by breed included crossbreds (12), Dobermann (3), Labrador retriever (2), Jack Russel terrier (2), and one dog for each of the following breed: Italian hound, Golden retriever, Belgian shepherd, Dachshund, Beagle, Airedale terrier, Brittany spaniel, Chihuahua, English setter, Basset hound and French bulldog (Table 1).

High-grade multicentric lymphomas were diagnosed in all dogs of the study group. In 12 of these 15 dogs, B-cell lymphoma was diagnosed, while the remaining three dogs were found affected by T-cell lymphoma. Regarding lymphoma sub-types, in nine dogs a centroblastic lymphoma and in further three dogs a pleomorphic lymphoma was diagnosed. In the remaining three dogs the lymphoma sub-type remained undetermined. The WHO clinical stage was III, IV and V in the case of eight, three and four dogs, respectively (Table 1).

The overall prevalence of intestinal parasite infections was 33.3% (10/30 dogs) (Table 2). Out of 30 examined dogs, six animals (20%) were infected by a single parasite species, while four dogs (13.3%) were found infected by multiple species. However, the difference between single and multiple infections was not statistically significant. The overall prevalence of protozoan infections was higher (26.7%; 8/30) than that of helminth infections (10%; 3/30), but also in this case no significant differences were found at statistical analysis. *Giardia duodenalis* (6/30; 20%), *Cryptosporidium* spp. (2/30; 6.7%), *Cystoisospora ohioensis*-complex (2/30; 6.7%), *Entamoeba* sp. (1/30; 3.3%), *N. caninum* (1/30; 3.3%), *Spirocerca lupi* (1/30; 3.3%), *Toxocara canis* (1/30; 3.3%) and hookworms (*Ancylostoma/Uncinaria*) (1/30; 3.3%), were the intestinal parasite species identified in this study (Table 2). In the single dog scored positive for *N. caninum*-like oocysts at coprological examination, the infection was confirmed both by serological and molecular analysis [22].

Among dogs affected by lymphomas, the overall prevalence of parasite infections was 46.7% (7/15) (Table 2). In this dog group, the overall prevalence of protozoa infections resulted significantly higher ($p < 0.05$) than that of helminth infections (1/15; 6.7%) at statistical analysis. More precisely, protozoan infections were diagnosed in all positive dogs (7/15; 46.7%), while only a single dog (1/7; 14.3%) concurrently infected by *Entamoeba* sp., was found positive for the nematode species *S. lupi*. Among lymphoma patients that scored

Table 1
Lymphoma type and sub-type, WHO clinical stage, identified gastrointestinal parasites and number of analysed fecal samples/dog in 15 dogs affected by multicentric high-grade lymphoma.

	Patient dog age, sex, breed	Lymphoma sub-type, immunophenotyped, clinical stage	Parasites	Total number of analysed fecal samples
1	7 years, male Belgian shepherd	Centroblastic B-cell Stage III	M: Negative	1
2	10 years, female Doberman	B-cell † Stage IV	B: Negative A: Negative	2
3	8 years, female, Crossbred	Centroblastic B-cell Stage III	M: *Entamoeba* sp., *Spirocerca lupi*	1
4	6 years, male, Jack Russel Terrier	Centroblastic B-cell Stage V	B: Negative A: *Giardia duodenalis*, *Cryptosporidium* spp.	3
5	9 years, female, Labrador Retriever	Centroblastic B-cell Stage III	B: *Giardia duodenalis* A: *Cryptosporidium* spp., *Cystoisospora ohiensis*-like	2
6	6 years, female Italian Hound	Centroblastic B-cell Stage III	B: Negative A: *Giardia duodenalis*	2
7	8 years, male, Doberman	Centroblastic B-cell Stage III	M: *Giardia duodenalis*	1
8	9 years, female, Crossbred	Centroblastic B-cell Stage III	B: Negative A: Negative	2
9	10 years, male, Dachshund	Pleomorphic T-cell Stage III	B: *Cystoisospora ohiensis*-like	1
10	7 years, male, Jack Russel Terrier	Centroblastic B-cell Stage V	A: Negative	1
11	8 years, male, Crossbred	Pleomorphic T-cell Stage V	B: Negative I: *Neospora caninum* M: *Neospora caninum*, *Giardia duodenalis*	3
12	8 years, female, Beagle	Centroblastic B-cell Stage IV	B: Negative A: Negative	2
13	9 years, male, Labrador Retriever	B-cell † Stage IV	B: Negative A: Negative M: Negative	3
14	12 years, male, Golden Retriever	Pleomorphic T-cell Stage III	A: Negative	1
15	12 years, female, Airedale Terrier	B-cell † Stage IV	A: Negative	1

B: fecal analysis performed before chemotherapy; **I**: fecal analysis performed during the induction chemotherapy phase; **A**: fecal analysis performed soon after the induction chemotherapy phase; **M**: fecal analysis performed during the maintenance chemotherapy phase; †: sub-type not determined.

Table 2
Prevalence (%) and species composition of gastrointestinal parasites identified in a group of 15 dogs affected by high-grade multicentric lymphoma (LD), both before and during chemotherapy, and in a control group (CD) of 15 dogs not affected by lymphoma.

Parasite	Prevalence (%) LD N = 15	CD N = 15	Overall N = 30	*P* value
Giardia duodenalis	33.3	6.7	20	0.168
***Cryptosporidium* sp.**	13.3	0	6.7	0.482
***Cystoisospora ohioensis*-complex**	13.3	0	6.7	0.482
***Entamoeba* sp.**	6.7	0	3.3	1
Neospora caninum	6.7	0	3.3	1
Toxocara canis	0	6.7	3.3	1
Spirocerca lupi	6.7	0	3.3	1
Hookworms	0	6.7	3.3	1
Protozoa	46.7	6.7	26.7	0.0352*
Helminths	6.7	13.3	10.0	1
Single infection	20.0	20	20.0	0.080
Multiple infection	26.7	0	13.3	0.096
Total parasite infections	46.7	20.0	33.3	0.245

* *P* value < 0.05.

positive for parasites, the most common identified protozoan species was *G. duodenalis* (5/7; 71.4%), followed by *Cryptosporidium* spp. (2/7; 28.6%), *C ohioensis*-complex (2/7; 28.6%), *Entamoeba* sp. (1/7; 14.3%), and *N. caninum* (1/7; 14.3%). However, among dogs affected by lymphoma with available fecal analysis at presentation (9) (Table 1), only two dogs (22.2%; 2/9) scored positive for protozoa, while none of these dogs was found positive for nematodes. More specifically, *G. duodenalis* was identified in the first dog and *C. ohioensis*-complex in the second dog (Table 1). During chemotherapy, nine out of 15 dogs (60%) scored negative for parasites, while six (40%) were found positive at fecal analysis, but no statistical differences regarding the prevalence of intestinal parasites were found before and during the chemotherapy treatment. However, in dogs of the study group scoring positive for parasites while receiving chemotherapy, the prevalence of protozoa infections was significantly higher ($p < 0.05$) than that of helminth infections. More specifically, among dogs found positive during chemotherapy administration identified parasites included *G. duodenalis* (4/6; 66.7%), *Cryptosporidium* spp. (2/6; 33.3%), *C. ohioensis*-complex (1/6; 16.7%), *N. caninum* (1/6; 16.7%), *Entamoeba* sp. (1/6; 16.7%) and *S. lupi* (1/6; 16.7%) (Table 1). Among these parasites, the protozoan species *Cryptosporidium* spp., *Entamoeba* sp. and *N. caninum*, and the nematode *S. lupi* were identified only among dogs receiving chemotherapy. Multiple infections were diagnosed in three out of the six (50%) dogs found positive while receiving chemotherapy and in one out of ten (10%) dogs soon after the induction chemotherapy phase (Table 1).

Among healthy dogs, three out of 15 animals (20%) were found positive at fecal analysis and the prevalence of helminth infections was higher than that of protozoan infections (Table 2). More specifically, hookworms (*Ancylostoma/Uncinaria*; 1/15; 6.7%), *T. canis* (1/15; 6.7%) and *G. duodenalis* (1/15; 6.7%) were identified in this dog group.

From the comparison of healthy dogs and dogs diagnosed with lymphoma, the overall prevalence of endoparasites was not statistically different between the two groups (Table 2). Conversely, the overall prevalence of protozoa infection was statistically higher ($p < 0.05$) in lymphoma dogs compared with control dogs. However, regarding the specific prevalence of *G. duodenalis*, no significant differences were found between the control group (1/15; 6.7%) and the study group (5/15; 33.3%) and, within this latter group, neither before (1/15; 6.7%) nor during (5/15; 33.3%) the chemotherapy (Table 2).

4. Discussion

This case-control study investigated the prevalence and species composition of intestinal parasites in dogs affected by lymphoma before and during chemotherapy treatment administration, compared to clinically healthy dogs. Both healthy and lymphoma diseased dogs were found infected by protozoa and nematode species. A higher overall prevalence of intestinal parasites, but statistically insignificant, was observed in lymphoma-affected dogs. However, the prevalence of protozoa infections was higher in lymphoma patients than in control dogs. Furthermore, in dogs with lymphoma the prevalence of protozoa infections was significantly higher than that of helminth infections, both before and during chemotherapy. In addition, the protozoan species *Cryptosporidium* spp., *N caninum* and *Entamoeba* sp. were identified only among dogs receiving chemotherapy.

In the case of *Cryptosporidium* spp., no dogs with cancer were found positive at the time of diagnosis. Furthermore, this protozoan was not identified in healthy dogs from the control group, albeit all dogs were client-owned dogs living in the same geographical area. Moreover, the overall prevalence of *Cryptosporidium* found in the present study, i.e. overall 13.3% in dogs affected by lymphoma during the chemotherapy treatment, was higher than that previously reported in dogs in Italy, ranging from 0.2% to 3.3% [24]. This observation agrees with previous descriptions in human medicine [11] according to which *Cryptosporidium* is one of the most common parasites reported in human cancer and immunocompromised patients, which are considered particularly susceptible to *Cryptosporidium* spp. infections [11]. In immunocompromised and cancer humans, especially those undergoing chemotherapy, the reported prevalence of cryptosporidiosis is in fact higher than that found in healthy human populations [27]. *Cryptosporidium* has also been reported to be significantly associated with human colorectal cancer, also before chemotherapy administration [28]. This latter finding, coupled with results from some studies showing an association between intestinal cancer development and *Cryptosporidium parvum* experimental infections in mice, have led to suspicion of a primary role of this protozoan species in this type of cancer [28,29]. However, the International Agency for Research on Cancer does not include *Cryptosporidium* among the biological agents considered as carcinogenic in humans [30], while it is frequently found in people undergoing immunosuppressive treatment or in patients with Human Immunodeficiency Virus infection [31]. In a study designed to evaluate the prevalence of *Cryptosporidium* infection in children with cancer undergoing chemotherapy, Berahmat et al. [11] observed that the prevalence of *Cryptosporidium* infections was higher among children treated with chemotherapy for more than a year. However, in the study of Berahmat et al. [11] no information was given nor regarding the tumor type neither concerning the treatment administered.

Cryptosporidium is a protozoan genus that includes several species, some of which infect both humans and animals [32]. Infection may occur after the ingestion of oocysts found in the environment, food or water [33]. In dogs, most infections are asymptomatic and most infected dogs have normal stools [34]. However, severe clinical illness has also been reported, particularly in puppies and in immunocompromised animals [32]. Moreover, *Cryptosporidium* oocysts are found more often in dogs with diarrhea [32]. In our study, gastrointestinal signs at the time of fecal collection were not always recorded. Therefore, further studies are needed to investigate eventual relations between cryptosporidiosis and acute-onset diarrhea in dogs affected by lymphoma and receiving chemotherapeutic drugs.

The immune response related to protection against cryptosporidiosis involves both humoral and cellular factors, T-lymphocytes being the most important component in controlling the infection [30]. Indeed, in a previous study *C. parvum* and *Cystoisospora belli* were found exclusively in immunocompromised human patients with low CD4 T-cell count [35]. *C. belli* is a human protozoan species that is also considered an opportunistic parasite in immunosuppressed patients [36]. In dogs, *Cystoisospora* spp. infection and disease are prevalent in animals younger than six months of age, particularly in the post-weaning phase, while among adult dogs the infection is less frequent and usually asymptomatic [37]. Considering that in the present study *C. ohioensis*-complex protozoa were identified in adult dogs affected by high-grade multicentric lymphoma, it would be interesting to further investigate the spread of *Cystoisospora* in this dog population, since data on this topic are lacking.

In this study, *N. caninum* was a one more protozoan species identified only in a diseased dog treated with chemotherapy. *N. caninum* can infect a wide range of animals as intermediate hosts, but its life cycle includes only dogs and other wild canids as definitive hosts [22]. In previous reports, clinical neosporosis has been described in dogs treated with cyclosporine, prednisolone or azathioprine [19,20], showing that these immunosuppressive drugs may predispose infected dogs to the disease caused by this parasite [20]. Thus, results from our study confirm previous reports [19,20,22] about a higher risk of oocyst shedding and clinical neosporosis in adult dogs undergoing immunosuppressive treatment.

One dog with lymphoma and undergoing chemotherapy was found positive for *Entamoeba*, a genus that includes a group of both pathogenic and non-pathogenic intestinal protozoan species of humans and other mammals all over the world, with overall prevalence among dogs ranging from < 1% up to 15.5% [38,39]. Amebic dysentery is caused by the pathogenic species *Entamoeba histolytica*, and the ingestion of contaminated food and water represents the source of infection [38]. Higher rates of infection have been found among young dogs, suggesting a deficient role of immunity [38]. Moreover, *Entamoeba* protozoa have been recently reported in human patients undergoing hematopoietic stem-cell transplantations and in renal transplant recipients [39–41]. Therefore, the excretion of *Entamoeba* cysts in the dog in our study could be related to immunosuppression. The important role of dogs as sources of *Entamoeba* infection for owners has been previously investigated [39,42,43]. However, advanced molecular and serological methods are required to achieve a definitive diagnosis for *Entamoeba* protozoa at the species level, but most of the available veterinary studies do not differentiate pathogenic from non-pathogenic species, these latter including *Entamoeba dispar* and *Entamoeba moshkovskii* [38]. In our study, diagnosis of *Entamoeba* infection was based only on fecal microscopic examination, with no antigenic or molecular techniques performed. Therefore, it is not possible to conclude if cysts found in this dog belong to the pathogenic species, presenting a risk factor for human infection.

In the study herein discussed, *G. duodenalis* was the most common endoparasite identified among dogs affected by lymphoma. *G. duodenalis* is a cosmopolitan potentially zoonotic protozoan species, whose prevalence among client-owned dogs in Italy is about 7–29% [37,44,45]. This parasite is described as including eight major genetic assemblages (A-H) [46]. Assemblages A and B are considered of public health interest as they are pathogenic to humans but can also infect several mammals, including dogs. Conversely, assemblages C and D are considered specific for dogs and only sporadically found in humans [46]. When compared to *Cryptosporidium* spp., *G. duodenalis* appears to be more frequently identified among immunocompetent dogs [47]. In accordance with these previous findings, in the present study *G. duodenalis* was identified in both healthy dogs and dogs with cancer, among these latter ones including animals examined both before and during the chemotherapeutic treatment. However, while the prevalence of this protozoan found among healthy dogs and dogs affected by high-grade multicentric lymphoma examined before chemotherapy was in line with previous data reported in Italy [37,44,45], and Europe [48], in lymphoma patients examined during the treatment period the prevalence of *G. duodenalis* (33.3%) was higher than that previously reported. Therefore, these results suggest that chemotherapy may predispose dogs to *G. duodenalis* infections. Considering the lack of data about the prevalence of this protozoan in canine patients undergoing

chemotherapy, further studies are needed to confirm our results.

Concerning helminths, only a single lymphoma patient receiving chemotherapy scored positive for a nematode species, specifically *S. lupi*. Canine spirocercosis is a neglected and underestimated nematode infection. Natural reservoirs are represented by wild foxes and wolves, and few records have been reported in dogs from Italy [49,50]. Interestingly, this nematode parasite infecting the canine esophagus and stomach has been described as the causative agent of esophageal and gastric sarcoma, primary pulmonary sarcoma and osteosarcoma in dogs [51]. The presence of a *S. lupi* infection recorded in the canine population herein examined may be related to the high density of foxes and wolves in the examined geographical area, increasing the risk of environmental fecal contamination and of infection for pets [52].

In our study, the very low prevalence of helminth infections among dogs affected by lymphoma agrees with previous studies in human medicine, in which only the nematode *S. stercoralis* is frequently associated with chemotherapy administration in human patients with cancer [14,53]. This is not surprising, since *S. stercoralis* infection is considered more likely associated with immunosuppressive status and younger age, both in human and canine patients [53–55].

Finally, results obtained in healthy dogs in this study agree with previous data concerning intestinal parasites reported in different dog populations in Europe [24,37]. More precisely, the prevalence rate, the species composition, and the higher frequency of intestinal helminth species compared to protozoan species found in healthy dogs in this study, agree with previous data, since hookworms, *T. canis* and *G. duodenalis* are included among the most frequent canine intestinal parasite species [24,34,37]. Therefore, the validity as control group of healthy dogs enrolled in our study was confirmed.

This study has several limitations. Firstly, because of the low number of animals included, our results need to be confirmed by examining a larger number of dogs with high-grade multicentric lymphoma and receiving chemotherapy. Furthermore, for some animals with lymphoma fecal samples were not available at the time of presentation, and it was not possible to perform fecal analysis before the chemotherapy treatment. Therefore, it is conceivable that in these dogs the prevalence of intestinal parasites found during chemotherapy administration could be overestimate. Moreover, dogs of the control group were not repeatedly examined, as the dogs in the cancer group. Finally, the clinical significance of identified endoparasites was not herein assessed, and further studies are required to fully evaluate the clinical impact of intestinal parasites among dogs affected by lymphoma, both receiving or not chemotherapy.

5. Conclusions

In conclusion, this is the first study aimed at evaluating the prevalence and species composition of gastrointestinal parasites in cancer-affected dogs. Our results suggest that in dogs affected by high-grade multicentric lymphoma, chemotherapy could be associated with a high prevalence of opportunistic internal parasite infections, encouraging more in-depth studies. Particularly, in this study dogs treated with drugs commonly used in the management of lymphoma scored positive mainly for protozoa, including *G. duodenalis*, *Cryptosporidium* spp., *C. ohioensis*-complex, *Entamoeba* spp. and *N. caninum*. Moreover, some protozoan species identified in this study in dogs affected by lymphoma, such as *G. duodenalis* and *Cryptosporidium* spp., are potentially zoonotic species and may represent a potential risk for human health. Further studies are therefore needed to verify the diffusion of zoonotic *G. duodenalis* and *Cryptosporidium* genotypes in canine populations affected by lymphoma. However, results from this study suggest the need to screen all dogs diagnosed with lymphoma for intestinal protozoa species, and especially high-grade multicentric lymphoma dogs undergoing chemotherapy.

In a recent study [56], significant differences were found in the fecal microbial communities of dogs with multicentric lymphoma compared to healthy dogs, with a decrease of bacterial families that are believed to be associated with canine gut health. This event, as well as possible immunosuppression caused by chemotherapy and cancer [8–10], may represent potential factors concurring to explain findings from the present study. Nevertheless, the low number of examined animals in our study do not permit to extract definitive conclusions. Thus, further and larger cohort studies are needed to confirm these results, as well as to investigate potential risk factors associated with internal parasite infections, high-grade multicentric lymphoma and chemotherapy in dogs.

Authors contribution

Stefania Perrucci conceived and designed the study; all authors contributed to the acquisition of data; Stefania Perrucci, Mario Cervone and Alessandra Gavazza analysed and interpreted the results; Stefania Perrucci and Mario Cervone wrote the manuscript and Alessandra Gavazza critically revised it.

Ethical approval

This study was approved by the University of Pisa Institutional Animal Experimental Use Committee (n. 0009069/2014). Written informed consent was also obtained from owners of all enrolled dogs.

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Declarations of interest

None.

Data statement

All relevant data are within the manuscript.

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