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Faculdade de Medicina Veterinária

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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Abstract

Gastrointestinal parasite risk in dog parks in the Lisbon area

Dog parks may pose a risk for the transmission of parasitic zoonotic agents via the faeces and soil contact. This is the first study to investigate gastrointestinal infections in park-attending dogs in Lisbon.

The research was carried out under the frame of a field study including both parasitological and a survey approach. 369 faecal and 18 soil samples were collected from three dog parks in the Lisbon area and analysed for parasite eggs. 102 questionnaires were filled.

The overall prevalence for positive faecal samples was 33%. Ancylostomatidae represent 17%, *Cryptosporidium* spp. 12%, *Giardia* spp. 11%, *Toxascaris leonina* and *Cystoisospora* spp. 1% each, *Toxocara* spp. 0.5% and *Sarcocystis* spp. 0.3%.

From soil samples, 28% were contaminated with only Ancylostomatidae eggs.

In the last 12 months 94% of the dogs were observed by a veterinarian. 90% were dewormed in the previous six months, from which 28% at least four times a year. Additionally, 26% of the dogs share the house with at least one dog, 50% visit the park daily, and 75% were always allowed to be off-leash. Also, 1% was fed with raw meat. Despite 94% of the owner's claimed faecal collection of their pets, it was common to see 10-20 faecal samples on the environment of every dog space on sampling days. Regarding the pet-owner relationship, 76% of the dogs were allowed to lick their owners' faces, 82% to be in their bedroom and 43% to sleep in their bed.

Approximately one third of faecal samples of dogs in canine parks was infected with gastrointestinal parasites, some with potential zoonotic risk. Less than a quarter of the dogs were dewormed following the recommended schedule (at least 4 times a year). The majority of the owners have close physical contact with their dogs, increasing the transmission risk of zoonoses. Public awareness about potential risks and preventive procedures is therefore advised.

Key words: dog, dog-attending parks, gastrointestinal parasites, zoonoses, environmental contamination, One Health

Resumo

Risco parasitário gastrointestinal em parques caninos na área de Lisboa

Os parques caninos podem representar um risco para a transmissão de agentes parasitários zoonóticos através do contato com fezes e solo. Este é o primeiro estudo a investigar infeções gastrointestinais em cães que frequentam parques caninos em Lisboa.

O estudo foi realizado sob a estrutura de um estudo de campo, incluindo tanto uma abordagem parasitológica como um questionário. 369 amostras fecais e 18 amostras de solo foram recolhidos de três parques caninos na área da Grande Lisboa e analisadas para ovos de parasitas. 102 inquéritos foram preenchidos.

A prevalência global de amostras fecais positivas foi de 33%. A presença de Ancylostomatidae representa 17%, *Cryptosporidium* spp. 12%, *Giardia* spp. 11%, *Toxascaris leonina* e *Cystoisospora* spp. 1% cada, *Toxocara* spp. 0.5% e *Sarcocystis* spp. 0.3%.

Das amostras de solo, 28% estavam contaminadas apenas com ovos de Ancylostomatidae.

Nos últimos 12 meses 94% dos cães foram observados por um veterinário. 90% foram desparasitados nos seis meses anteriores, dos quais 28% pelo menos quatro vezes por ano. Além disso, 26% dos cães partilha a casa com, pelo menos um cão, 50% visita o parque diariamente, e 75% foi sempre autorizado a estar solto. Além disso, 1% era alimentado com comida crua. Apesar de 94% dos proprietários alegar a colheita das fezes dos seus animais de estimação, era comum ver 10-20 amostras fecais em cada espaço canino nos dias de amostragem. Tendo em conta o relacionamento do animal de estimação com o dono, 76% dos cães eram autorizados a lamber as caras dos donos, 82% a estar no seu quarto e 43% a dormir na sua cama.

Aproximadamente um terço das amostras fecais de cães de parques caninos estava infectado com parasitas gastrointestinais, alguns com potencial risco zoonótico. Menos de um quarto dos cães eram desparasitados seguindo o esquema recomendado (pelo menos 4 vezes por ano). A maioria dos proprietários tem contato físico com os seus cães, aumentando o risco de transmissão de zoonoses. A sensibilização do público sobre os riscos potenciais e os procedimentos de prevenção é, portanto, aconselhável.

Palavras-chave: cães, parques caninos, parasitas gastrointestinais, zoonoses, contaminação ambiental, Uma Saúde

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List of Abbreviations and Symbols

% – Percentage
% w/v – Percent weight per volume
& – And
+ – Plus
< – Less than
= – Equal to
± – Plus or minus
® – Registered trademark
µm – Micrometer
AKN – American Kennel Club
ANOVA – Analysis of variance
CAPC – Companion Animal Parasite Council
CI95% – Confidence Interval of 95 per cent
CLM – Cutaneous Larva Migrans
cm – Centimetre
CSF – Centrifugal Sedimentation/Flotation
DNA – Deoxyribonucleic acid
dpi – Days post infection
e.g. – *exempli gratia*
ELISA – Enzyme-Linked Immunosorbent Assay
EM – *Echinococcus multilocularis*
epg – Eggs per gram of faeces
ESBL – Extended-Spectrum Beta-Lactamases
ESCCAP – European Scientific Counsel Companion Animal Parasites
FET – Fisher's exact test
FVM - UL – Faculty of Veterinary Medicine - University of Lisbon
g – Gram
h – Hour
IFA – Immunofluorescent Antibody
IM – Intramuscular
L1 – First-stage larva
L3 – Third-stage larva
L4 – Fourth-stage larva
LPPD – Laboratory of Parasitology and Parasitic Diseases
m^2 – Square meters
mg/kg – Milligram per kilogram

ml – Millilitre

mm – Millimetre

MRSA – Methicillin-resistant *Staphylococcus aureus*

n – Total sample number

n.d. – No date

ºC – Centigrade degrees

OIE – World Organisation for Animal Health

OLM – Ocular Larva Migrans

p – p-value

PCR – Polymerase Chain Reaction

RIVM – National Institute for Public Health and the Environment

rpm – Revolutions per minute

SC – Subcutaneous

sp. – Specie

spp. – Species

™ – Trademark

VLM – Visceral Larva Migrans

WHO – World Health Organization

κ – Cohen's kappa coefficient

χ^2 – Pearson's chi-squared test

I - Introduction

In today's society, pets play an important role and the human-animal bond provides substantial positive benefits. Besides being a source of companionship, support, and entertainment, dogs facilitate social interactions between people. Pets are able to promote their owners physical health by reducing blood pressure and/or heart rate, being moderators of stress, decreasing medication and numbers of visits to physicians, reducing the chances of developing chronic conditions (such as cardiovascular disease) and improving survival time after a heart attack. Psychological well-being is also improved: levels of stress and anxiety, loneliness, and depression are reduced, and feelings of autonomy, competence, and self-esteem are enhanced. Companion animals may have the ability to detect some physical alterations in humans such as cancer, seizures, and hypoglycaemia and can also be used with therapeutic intent to physical problems (Macpherson, 2005; Wells, 2009).

Despite all the positive effects that pets can have in people's lives, this increase in the human-animal bond also has the potential to compromise human health, by causing allergies, biting and spreading zoonoses (Wells, 2009). Dogs are associated to the transmission of more than 60 zoonotic infections (Macpherson, 2005) and among these, gastrointestinal parasitosis can become a serious concern. Small children, pregnant women and immunocompromised people have a higher risk of acquiring parasitic zoonoses (Robertson, Irwin, Lymbery, & Thompson, 2000).

A dog-attending park is a fenced area where dogs can exercise and play off-leash. The ideal dog park should have, at least, an area of about 4050 m^2 surrounded by a 120-180 cm high fence and a double-gated entry. The property should have an adequate drainage as well as a regular maintenance and cleaning of the grounds. Some of them may contain agility equipment and they should include amenities for dog owners, such as shade, benches and a suitable water source (for both dogs and owners). Cleaning supplies, including covered garbage cans and waste bags must be available. Signs which clearly specify the rules and requirements for using the dog park should be placed in the park (American Kennel Club [AKC], n.d.).

Dog parks fulfil the desire of dog owners to spend quality time with their dogs. These parks may pose a risk for the transmission of parasitic zoonotic agents among dogs, humans and wildlife. However, in Lisbon, few studies investigating gastrointestinal infections in urban dogs have been performed and none in park-attending dogs. Information regarding pet-owner relationship, dog-walking habits, husbandry practices and history of deworming care in this dog population are also currently unavailable.

In this thesis, it will be addressed the gastrointestinal parasites that infect dogs, with more relevance to those which have be found in the laboratory work (nematodes and protozoa), that may have a zoonotic potential and is therefore, of major importance either in animal or in public health, having always in mind, the importance of the concept “One World, One Health”. The main gastrointestinal parasites of dogs, as well as their zoonotic potential, are listed in Table 1.

Table 1 - Zoonotic potential of the main gastrointestinal parasites of dogs (adapted from Foreyt, 2001; Nunes, 2014)

	Parasite	**Zoonotic Potential**
Cestodes	*Taenia* spp.	Coenurosis
	Echinococcus spp.	Hydatid disease or Cystic echinococcosis Alveolar echinococcosis
	Dipylidium sp.	Dipylidiosis
Nematodes	*Toxocara* spp.	Visceral larva migrans Ocular larva migrans Covert toxocarosis
	Ancylostoma spp.	Cutaneous larva migrans Eosinophilic enteritis
	Uncinaria sp.	Cutaneous larva migrans
	Strongyloides sp.	Strongyloidiosis Cutaneous larva migrans
	Trichuris sp.	Trichuriosis (rare) Visceral larva migrans (rare)
Protozoa	*Giardia* sp.	Giardiosis
	Cryptosporidium spp.	Cryptosporidiosis

II - Training Period Activities

In the last year of her Integrated Masters in Veterinary Medicine, the author took a training period of six months at the Veterinary Hospital, Faculty of Veterinary Medicine, University of Lisbon (FVM-UL), Portugal from September 2nd 2013 to February 28th 2014. In this period of training, the author was guided by Dr. Ana Reisinho. The author also took a training period of a total of three months in the Netherlands, under the LLP/Erasmus Program, between March 10th and June 06th 2014. Six weeks were at the Parasitology Department of the Utrecht University under supervision of Dr. Rolf Nijsse. Two weeks at the National Institute for Public Health and the Environment (RIVM), Centre for Zoonoses and Environmental Microbiology, Zoonotic and Foodborne Parasitology, supervised by Prof Doctor Joke van der Giessen. Finally, in the last five weeks the author had a training period at the Institute for Risk Assessment Sciences, Division of Veterinary Public Health, Utrecht University, under the supervision of Prof. Doctor Sara Burt. The overall supervisor of this training period in the Netherlands was Prof. Doctor Paul Overgaauw and it was co-supervised by Prof. Doctor Luís Manuel Madeira de Carvalho (FVM-UL). Lastly, the author took a training period of five months in the Laboratory of Parasitology and Parasitic Diseases (LPPD) at FVM-UL, from October 6th 2014 to February 20th 2015. In total the author had about 2500 hours for her training period.

During the first part of the training period, with a duration of about 1300 hours, held in Lisbon, the author followed the routine activities of the Small Animal Hospital, gathering knowledge and practice in internal medicine (which included specialized consultations: neurology, ophthalmology, dermatology, orthopaedics, oncology, endocrinology, cardiology and exotic animals), intensive care unit, surgeries and diagnostic imaging (X-rays, computerized tomography scans and ultrasonography).

In the Netherlands, the number of hours of practical training was 520, divided in three projects. In the first six weeks in Utrecht University, the student was introduced to the general techniques and projects done in the Parasitology Department and was able to perform some soil examination from the city of Assen, in search for *Toxocara* spp. eggs contamination (Figure 1). It was possible to follow and also to practice techniques of egg isolation in faeces and McMaster technique. Regarding an ongoing investigation about *Strongylus* spp. and Cyathostominae in horses, Baermann technique, Polymerase Chain Reaction (PCR), reverse line blot and *in vitro* exposure to ivermectin were demonstrated.

Figure 1 – Embryonated *Toxocara* spp. egg found in a sandpit sample from Assen (original)

At the RIVM the student got acquainted with the ongoing public health projects about diagnostics, surveillance and epidemiology of parasitic zoonoses relevant for the Netherlands: *Echinococcus multilocularis* and *E. granulosus*, *Trichinella spiralis*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Giardia* spp. and *Baylisascaris procyonis*. The author also extracted data about treatment protocols against *Echinococcus multilocularis* for dogs and cats from a variety of articles previously selected and did a small presentation about it (Annex 1).

In the last five weeks, the author was introduced to a project about the role of vermin as a reservoir for antimicrobial resistant bacteria, collecting and analysing samples in the laboratory. This assignment was looking for Extended-Spectrum Beta-Lactamases (ESBL) producing *Escherichia coli* and *Klebsiella* spp., Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Salmonella* spp. in mainly, flies, mice and birds.

During the period in the Parasitology Laboratory at FVM-UL, in which it was spent 720 hours, the author developed the practical part of this work. Samples were collected, processed using the Centrifuge/Sedimentation Flotation (CSF) technique and faecal smears stained by the modified Ziehl Neelsen technique were observed in the optic microscope.

The development of this work was accepted to be presented as posters to the 25th International Conference of the World Association for the Advancement of Veterinary Parasitology in Liverpool, United Kingdom (August, 2015) and to the II Congreso Ibero-Americano de Epidemiología y Salud Pública, in Santiago de Compostela, Spain (September, 2015) (Annex 2).

III - Literature review

1. - Cestodes

Tapeworms have acoelomate, flattened, segmented bodies, without digestive tract and both sexes represented in the same individual. Each adult worm is composed by a scolex (with holdfast organs to attach to the intestinal wall), a neck and strobila (composed of proglottids). Each proglottid contains one or two sets of reproductive organs and as they move away from the neck, maturation occurs with gravid proglottids at the end of the body (Ballweber, 2001; Bowman, 2014). Cestodes of veterinary importance are divided among two groups: Diphyllobothriidea and Cyclophyllidea, and families Taeniidae and Dipylidiidae are included in the last group (Bowman, 2014). In this latter order, gravid proglottids pass with faeces and rupture releasing eggs, each of which consists of an oncosphere or hexacanth embryo (with six hooks) within (Ballweber, 2001; Bowman, 2014).

1.1 - *Taenia* spp.

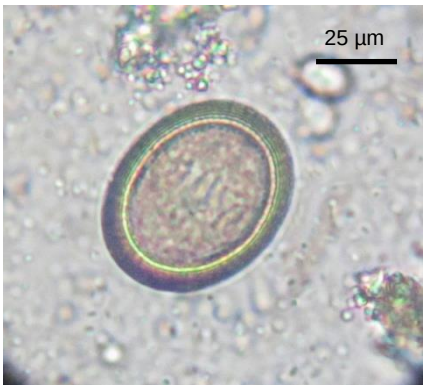
The genus *Taenia* belongs to the Phylum Platyhelminthes, Class Cestoda, Subclass Eucestoda, Order Cyclophyllidea, Family Taeniidae (Kassai, 1999). It has a worldwide distribution (Ballweber, 2001), and the species that have dogs as a definitive host are described in Table 2.

Table 2 - Some species within *Taenia* spp. that have dogs as a definitive host (adapted from Ballweber, 2001)

Species	**Intermediate Host**	**Metacestode**	
		Name	**Localization**
Taenia pisiformis	Lagomorphs	*Cysticercus pisiformis*	Abdominal cavity, liver
Taenia ovis	Sheep, goats	*Cysticercus ovis*	Skeletal, cardiac muscles
Taenia hydatigena	Ruminants	*Cysticercus tenuicollis*	Abdominal cavity, liver
Taenia multiceps	Sheep, cattle, humans	*Coenurus cerebralis*	Nervous tissue
Taenia serialis	Rabbits, rarely cats, humans	*Coenurus serialis*	Musculature, subcutis

With an indirect life cycle, metacestodes can be found in the intermediate host and adults worms in the small intestine of dogs (Ballweber, 2001). These become infected by ingestion of tissues or viscera from infected intermediate hosts and shed infectious eggs. Prepatent period ranges from about four to ten weeks, depending on the specie (European Scientific Counsel Companion Animal Parasites [ESCCAP], 2010). Generally, non-pathogenic infections occur in the definitive host. The routine diagnosis, in dogs, is performed through coprological methods to detect the parasite's eggs. These are brown, slightly oval, measuring up to 49 µm (depending on species), with a radial striated shell (Figure 2) (Ballweber, 2001). Treatment of dogs should be made in areas of high prevalence at least each 6 weeks with praziquantel or epsiprantel (ESCCAP, 2010). Humans can be infected by the larval form of *T. multiceps* and *T. serialis* (Ballweber, 2001; Katagiri & Oliveira-Siqueira, 2007).

Figure 2 – Taeniidae egg with the hexacanth embryo (courtesy of Lídia Gomes)



1.2 - *Echinococcus* spp.

Echinococcus spp., also belong to the Family Taeniidae (Ballweber, 2001) and some species within this genus are described in Table 3.

Table 3 - Some species within *Echinococcus* spp. that have dogs as a definitive host (adapted from Ballweber, 2001)

Species	Definitive Host	Intermediate Host	Metacestode		Geographic Distribution
			Type	Localization	
Echinococcus granulosus	Dog	Ruminants, macropods, horses, pigs, humans	Hydatid cyst	Primarily liver, lungs	America, Europe, Africa, the Middle East, Australia, and New Zealand
Echinococcus multilocularis	Wild canids Dog	Rodents, horses, pigs, humans	Hydatid cyst	Primarily liver, lungs	North-central Europe, Japan, North America

These tapeworms inhabit the small intestine and are very small, measuring 2 to 8 mm; the strobila is composed by four or five segments with the gravid proglottid being the last one (Bowman, 2014). The scolex has four suckers and a rostellum armed with two rows of hooks (Ballweber, 2001).

It has an indirect life cycle; infective eggs are passed in the faeces of the definitive host, which are ingested by intermediate hosts. The parasite migrates to liver, lungs or brain and slowly develops into hydatid cysts (Robertson & Thompson, 2002). *E. granulosus* produces unilocular cysts and *E. multilocularis* produces a multilocular, alveolar cyst (Ballweber, 2001). Canids become infected by ingestion of the metacestode from infected intermediate hosts (Robertson & Thompson, 2002). The prepatent period is approximately seven weeks for *E. granulosus* and four weeks for *E. multilocularis* (Ballweber, 2001).

Usually it is non-pathogenic for the definitive host, and clinical signs usually appear in older intermediate hosts, being well tolerated by these (Ballweber, 2001).

The routine diagnosis, in dogs, is performed through coprological methods to detect eggs that cannot be differentiated from those of *Taenia* spp. (Ballweber, 2001). In Portugal, the prevalence of Taeniidae eggs ranges from 0.2% to 10.8% (samples collected from public places, national park, farm and hunting dogs and kennels) (Crespo, Rosa, & Silva, 2006; Maurício, Rosa, & Crespo, 2006; Gravata, Rosa, & Crespo, 2007; Silva, 2010; Lebre, 2011; Crespo, Fradinho, & Rosa, 2013; Mateus, Castro, Ribeiro, & Vieira-Pinto, 2014; Santos, 2014; Leal, 2015). Coproantigen tests are not commercially available and PCR are only performed in specialized laboratories (ESCCAP, 2010).

For the treatment, praziquantel and epsiprantel can be used, and in endemic areas of *E. granulosus*, high-risk dogs should be treated at least every six weeks, and of *E. multilocularis*, dogs that have access to rodents should be treated every four weeks. Dogs should be prevented from having access to raw offal and carcasses (ESCCAP, 2010).

Humans can become infected with ingestion of eggs of *E. granulosus* (hydatid disease or cystic echinococcosis) and *E. multilocularis* (alveolar echinococcosis) (ESCCAP, 2010). The infection is usually asymptomatic in early stages and after many years clinical signs are liver, lungs and brain associated. After rupture of cysts, anaphylaxis may occur (Ballweber, 2001). Because the cyst of *E. multilocularis* can proliferate, metastasize and invade host organs it has a greater zoonotic impact (Robertson & Thompson, 2002).

Risk factors for human transmission include: limited education, poor hygiene, home slaughter, lack of control of stray populations, dog ownership, and absence of any anthelmintic treatment (Macpherson, 2005; Deplazes, van Knapen, Schweiger, & Overgaauw, 2011). Wildlife populations should be taken into account since rabies vaccination has resulted in an increase in the fox population in central Europe (Robertson & Thompson, 2002). This disease is still part of the list of mandatory notifiable diseases in Portugal and OIE (Direção-Geral de Alimentação e Veterinária [DGAV], 2015).

1.3 - *Dipylidium caninum*

Dipylidium caninum belongs to the Phylum Platyhelminthes, Class Cestoda, Subclass Eucestoda, Order Cyclophyllidea, Family Dipylidiidae (Kassai, 1999). It has a worldwide distribution, and is the most common tapeworm found in the small intestine of dogs and cats (Urquhart, Armour, Duncan, Dunn, & Jennings, 1996). Adults can measure up to 50 cm in length and the scolex has four suckers and a retractable rostellum armed with three rows of hooks. Proglottids are longer than wide and have two sets of reproductive organs and two lateral genital pores (Figure 3a) (Ballweber, 2001).

In Portugal, the prevalence of *D. caninum* ranges from 0.3% to 2.0% (samples collected from public places, kennels, farm and hunting dogs) (Crespo *et al.*, 2006; Maurício *et al.*, 2006; Gravata *et al.*, 2007; Lebre, 2011; Crespo *et al.*, 2013; Mateus *et al.*, 2014). Nevertheless, coprological examination is not very reliable for these parasites and these results are therefore strongly underestimated (Beugnet *et al.*, 2014).

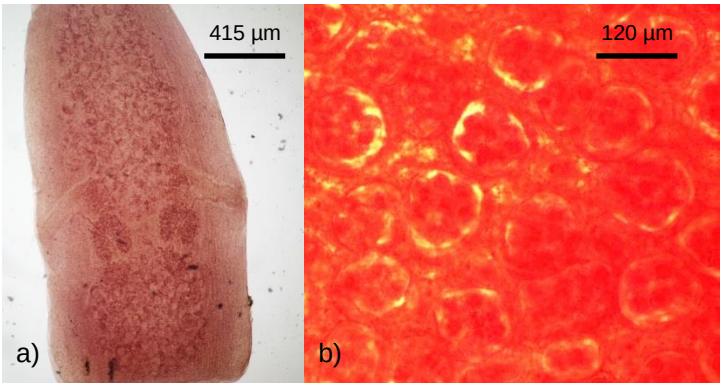
It has an indirect life cycle, where gravid proglottids are passed in the faeces of dogs, rupture and eggs are released, and are ingested by intermediate hosts, fleas (*Ctenocephalids canis*, *C. felis*, *Pulex irritans*), and lice (*Trichodectes canis*). Infection occurs upon ingestion of cysticercoids forms from these (adult) arthropods (Robertson & Thompson, 2002). The prepatent period is between two to three weeks (Ballweber, 2001).

Usually infection is non-pathogenic, however, as proglottids migrate, they can cause pruritis in the perianal area. Diagnosis can be made by observation of gravid proglottids in faeces or by detection of 120 µm x 200 µm egg packets (each with 5 to 30 eggs that measure 50 µm) on faecal flotation (Figure 3b) (Urquhart *et al.*, 1996; Ballweber, 2001). Coprological examination of the faeces for *Dipylidium* sp. is, however, not very reliable since the proglottids are mainly shedded independently from the defecation (Beugnet *et al.*, 2014).

For the treatment, praziquantel and epsiprantel can be used and control of fleas and lice should be performed (Ballweber, 2001).

Human infection occurs mainly in children through accidental ingestion of infected intermediate hosts. Usually the infection is asymptomatic, however, abdominal discomfort, diarrhoea, and pruritus may be present (Robertson & Thompson, 2002).

Figure 3 – *Dipylidium caninum*: a) proglottid with two lateral genital pores; b) egg packets within the proglottid (specimens courtesy of LPPD FVM-UL; originals)



2 - Nematodes

Nematodes have a cylindrical, non-segmented filiform body with a large body cavity (pseudocoelom) (Bowman, 2014). The body is covered with a cuticle that may form specialized structures, such as cervical alae (Ballweber, 2001), cervical papillae, cephalic vesicles (Urquhart *et al.*, 1996), and copulatory bursa or spicules in males (Bowman, 2014). There is a sexual dimorphism with males being smaller than females (Ballweber, 2001).
Nematodes are divided into two classes: the Secernentea and the Adenophorea. For this study, the Adenophorea contains *Trichuris* sp., while the Secernentea contains the remainder of the parasitic nematodes approached (Ascarididae, Ancylostomatidae, and *Strongyloides* sp.) (Ballweber, 2001).

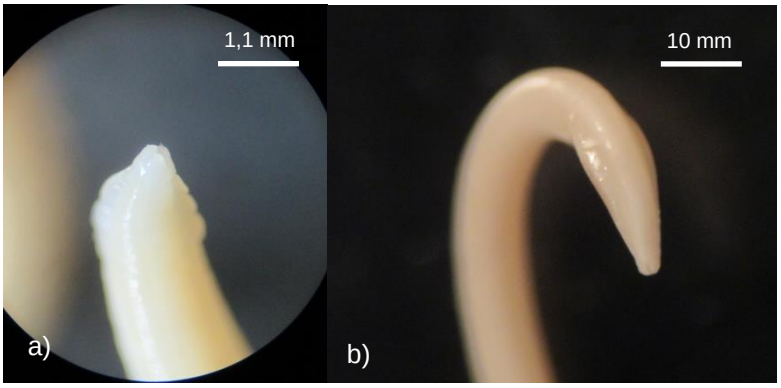
2.1 - *Toxocara canis*

2.1.1 - Etiology

Toxocara canis belongs to the Phylum Nemathelminthes, Class Nematoda, Subclass Secernentea, Order Ascaridida, Family Ascarididae (Ballweber, 2001). With a worldwide distribution, this nematode is also known as “the dog roundworm” (Zajac & Conboy, 2012) and infects the small intestine of canids (Ballweber, 2001; Alho, Seixas, Rafael, & Madeira de Carvalho, 2010). *Toxocara canis* adults length ranges from 7 to 18 cm (Foreyt, 2001), and are cream coloured. These worms have three prominent lips surrounding the mouth opening (Figure 4a) (Elsheikha & Khan, 2011), a glandular esophageal bulb and cervical alae (Bowman, 2014). Males have a small terminal appendix with two spicules (Alho *et al.*, 2010). The posterior end of males is curved ventrally (Elsheikha & Khan, 2011).

T. cati is smaller and infects the small intestine of felids (Ballweber, 2001; Alho *et al.*, 2010). It can be differentiated from *T. canis* by the cervical alae in the anterior end being arrow-shaped (Figure 4b) (Bowman, 2014).

Figure 4 – *Toxocara* spp. anterior ends: a) *T. canis* with three prominent lips; b) *T. cati* with arrow-shaped cervical alae (specimens courtesy of LPPD FVM-UL; originals)



In Europe, the prevalence for *T. canis* in dogs from different epidemiological environments (pet, shelter, stray, and rural dogs) ranges from 3.5% to 34% and for *T. cati* in cats from 8% to 76% (Overgaauw & van Knapen, 2013).

In Portugal, infection rates range from 1.1% to 18% for *T. canis* in dogs (samples collected from public places, pets and kennels) (Guerra, 2012) and lower than 10.4% for *T. cati* in stray cats. Regarding wild carnivores, the prevalence for *T. canis* for Iberian wolf is from 7.3% to 11.8%, and for red fox is from 15% to less than 40%. Iberian Lynx has prevalence for *T. cati* of less than 3% (Guerra *et al.*, 2012). All over the world, 10-30% of soil from parks, sandpits and others are contaminated with *Toxocara* eggs. *T. canis* eggs were found mostly in public parks and *T. cati* eggs in sand-boxes (Deplazes *et al.*, 2011). Soil contamination is higher in urban rather than rural areas because of a higher density of domestic carnivores (Okulewicz, Perec-Matysiak, Buńkowska, & Hildebrand, 2012). In Lisbon, Portugal, 50% of 5 investigated public parks and 85.7% of 10 playgrounds were contaminated with *Toxocara* spp. eggs (Otero *et al.*, 2014).

2.1.2 - Life Cycle

The life cycle of the *Toxocara* spp. is direct, unless there are paratenic hosts, in which case the cycle is indirect. Infected animals with adult worms in the small intestine shed unembryonated eggs in their faeces into the environment. These eggs have an embryonation process which leads to the development of an infective *Toxocara* larva (L3) (Ballweber, 2001). Under optimal conditions (temperatures between 25ºC and 30ºC and relative humidity of 85-95%), it takes nine to fifteen days for the eggs to become infective (Schnieder, Laabs,

& Welz, 2011), but normally the time needed for this process is between three to six weeks to several months, depending on soil type and environmental conditions. The infective stage in the egg can remain viable for at least one year (Overgaauw, 1997b).

Following ingestion of embryonated eggs of *Toxocara*, these hatch in the duodenum and release larvae. These larvae penetrate the mucosa of the small intestine and enter the circulatory system into the liver, via the portal circulation. Those larvae that cannot continue the migration, because they are trapped in capillaries, remain in the liver and encapsulation occurs (Schnieder *et al.*, 2011). Most of the larvae exit this organ, continuing to migrate to the heart and reaching the lung through the pulmonary artery. The larvae can follow two different routes from the lung. They can penetrate the alveoli wall and migrate to the pharynx via bronchioles and trachea (Schnieder *et al.*, 2011; Strube, Heuer, & Janecek, 2013). The larvae are swallowed into the digestive tract, mature into adults in the intestine and these start to eliminate eggs in the faeces (Alho *et al.*, 2010) within four to five weeks (Overgaauw & van Knapen, 2013). This phenomenon occurs mainly in puppies younger than six weeks old (Baños, Baños, & Pelays, 1999). The other route from the lung is re-entering the circulatory system, after penetration of the alveoli wall, and distributing to the somatic tissue (Schnieder *et al.*, 2011; Strube *et al.*, 2013) (skeletal muscle, kidneys, uterus, mammary glands, liver, lungs, brain, heart) (Urquhart *et al.*, 1996; Alho *et al.*, 2010), where larvae will encyst (Bowman, 2014) persisting for long periods (Overgaauw, 1997b). This occurs mostly in dogs older than six weeks old (Baños *et al.*, 1999). The different routes that the larvae perform depend on the age and immune status of the host and the infection dose (a large number of eggs is less likely to produce a patent infection) (Schnieder *et al.*, 2011; Overgaauw & van Knapen, 2013; Strube *et al.*, 2013).

Somatic migration progressively increases from two months of age (Overgaauw, 1997b), and, therefore, the development of larvae into adult worms decreases. This event is called age resistance and takes into account the development of immune competence and the acquired immunity (specific against third stage larvae only) (Schnieder *et al.*, 2011).

Besides infection by ingesting embryonated eggs, there are other ways of transmission of this parasite, such as transplacental and transmammary routes (Okulewicz *et al.*, 2012). This occurs when there is infection of the animals during pregnancy (Schnieder *et al.*, 2011; Strube *et al.*, 2013) or when somatic larvae are reactivated and migrate to the placenta and mammary gland of bitches during pregnancy (Overgaauw, 1997b; Overgaauw & van Knapen, 2013).

Transplacental, intrauterine or prenatal transmission (Schnieder *et al.*, 2011) occurs from day 42 of the gestation period. This route of infection is the most important mode of transmission in puppies, with almost 100% of infection *in utero* (Overgaauw & van Knapen, 2013). A bitch previously infected and harbouring somatic larvae can infect her offspring for three

consecutive pregnancies. The reactivation of larvae during pregnancy seems to be related to the changing hormone status and prolactin may be a trigger. The larvae reach the placenta via the circulatory system (Schnieder *et al.*, 2011) and migrate to the liver of the foetus, where they remain until birth. *Post-partum* migration continues immediately, larvae pass to the lungs, go through tracheal migration and reach the intestine. The duration of prepatency is not unanimous, varying from 16 days (Overgaauw & van Knapen, 2013) up to 46 days after birth (Schnieder *et al.*, 2011). Reactivated larvae may also complete the normal migration into the small intestine of the bitch and mature (Ballweber, 2001).

Puppies also become infected through ingestion of milk containing larvae. This route of transmission, also known as transmammary or lactogenic transmission, has little importance in dogs (Strube *et al.*, 2013). The infection starts immediately after parturition and the peak occurs in the second week of lactation (Schnieder *et al.*, 2011). The larvae can pass in the milk for at least 38 days *post-partum* (Overgaauw & van Knapen, 2013) and the prepatent period is 21 days after birth (Ballweber, 2001). In this route of transmission, larvae develop directly into adults in the intestine of puppies, without having tracheal migration (Deplazes *et al.*, 2011). When the infection occurs during the pregnancy, the larvae migrate directly to the mammary glands. It is less frequent that reactivated somatic larvae migrate to the mammary glands (Strube *et al.*, 2013). A nursing bitch may acquire a patent *Toxocara* infection by ingestion of juvenile intestinal larvae (L4) expelled in the faeces of the infected puppies that develop directly into adult without having tracheal migration. Accidental ingestion of eggs shed in the faeces of the offspring, passing through the intestinal tract can reappear in the bitch faeces (Overgaauw, 1997b; Schnieder *et al.*, 2011). Larvae reactivated by immunosuppression may also complete the normal migration into the small intestine of the bitch and mature (Schnieder *et al.*, 2011).

Definitive hosts can also become infected by ingesting paratenic hosts, such as rodents, birds, sheep, pigs, primates, humans and earthworms (Strube *et al.*, 2013; Bowman, 2014). Paratenic hosts need to have ingested embryonated eggs that are hatched in the intestine and undergo somatic migration to the liver, lungs, heart, kidneys, muscles, and mostly to the brain of the host (Okulewicz *et al.*, 2012) where they encyst (Lee, Schantz, Kazacos, Montgomery, & Bowman, 2010). As encapsulated larvae, they remain viable for up to ten years (Strube *et al.*, 2013). By ingesting infected paratenic hosts, larvae develop in the small intestine of definitive hosts into egg-laying adult worms (Overgaauw & van Knapen, 2013). The prepatent period is 21 days (Ballweber, 2001).

Toxocara spp. have a widespread distribution and wide ranging prevalence due to: the high fecundity of female worms (producing about 200.000 eggs per day) (Strube *et al.*, 2013), high resistance of the eggs to environmental conditions, and somatic encystment of infective larvae (Urquhart *et al.*, 1996), not only in the definitive host, as well as in paratenic hosts, that contribute to infection in stray cats and dogs and fox populations (Strube *et al.*, 2013).

The severity of the disease depends on the age of the host, and number, location and development stage of the parasite (Katagiri & Oliveira-Siqueira, 2007). Usually, this parasite does not cause signs of disease in older animals. Puppies are the most affected by this infection (Zajac & Conboy, 2012) and clinical signs may include vomiting, diarrhoea, distended abdomen and stunted growth rate (Elsheikha & Khan, 2011). Larval migration can cause respiratory signs such as cough, elevated respiratory rate, nasal discharge (Katagiri & Oliveira-Siqueira, 2007), pneumonia (Ballweber, 2001), and liver damage (Foreyt, 2001). Obstruction or perforation of the intestine wall, (Elsheikha & Khan, 2011) and obstruction of the common bile or pancreatic ducts can occur, and can lead to death (Bowman, 2014).

2.1.3 - Diagnosis

Toxocara spp. infections are usually detected by simple or centrifugal faecal flotation, where eggs appear with a subspherical shape and a thick mamillated, rough, pitted shell wall surface (Elsheikha & Khan, 2011; Zajac & Conboy, 2012). A dark, single cell fills the interior of the egg. *T. canis* eggs measure 75 µm x 85-90 µm and *T. cati* eggs are slightly smaller (65 µm x 75 µm) (Zajac & Conboy, 2012). Differentiation of these two egg species is difficult based only on morphological identification on microscopy, although under light and scanning electron microscopic observations, there can be some distinction (but a not clear-cut one) of these two species eggs by their characteristic surface topography (Uga *et al.*, 2000). PCR techniques allow species identification, but are not part of the routine diagnostic procedure adopted by laboratories (Overgaauw & van Knapen, 2013).

Although intermittent shedding of helminth eggs can occur (leading to an underestimation of the infection), this phenomenon has never been described for *T. canis* infections in adult dogs (Nijsse, Mughini-Gras, Wagenaar, & Ploeger, 2014). Coprophagy can be responsible for an overestimation of the infection, since helminth eggs from other dogs, or even helminth eggs and oocysts of parasites that do not infect dogs as a final host, are passed throughout the intestine (Overgaauw & van Knapen, 2013; Nijsse *et al.*, 2014).

2.1.4 - Treatment and Control

Due to the possibility of transplacental transmission, all puppies should be assumed to be infected at birth (Bowman, 2014). They should be dewormed at 2, 4, 6 and 8 weeks of age (ESCCAP, 2010) (due to the possibility of transmammary transmission) and then monthly until 6 months of age. Nursing bitches should be treated concurrently (Overgaauw & van Knapen, 2013).

Deworming bitches before mating and two weeks before anticipate whelping date, with or without an extended daily treatment with fenbendazole from the last third of gestation and the first stage of lactation is still performed. However, it is not generally advised since anthelmintics are not highly effective against somatic larvae and do not prevent prenatal transmission (Overgaauw & van Knapen, 2013).

The general treatment frequency advised for adult dogs is four times per year, depending on lifestyle and infection risks for each animal (ESCCAP, 2010).

The appropriate treatment of roundworms usually consists in the administration of an anthelmintic such as mebendazole, fenbendazole, febantel, pyrantel embonate, nitroscanate, emodepside, milbemycin oxime, moxidectine and selamectin. Praziquantel in combination with fenbendazole, febantel, pyrantel embonate, and milbemycin oxime is also approved (Alho *et al.*, 2010; Companion Animal Parasite Council [CAPC], 2013).

Treatment based on the results of periodic faecal examination can also be performed (Overgaauw & van Knapen, 2013).

Since *Toxocara* spp. eggs are very resistant to environment adversity and remain infective for years (Bowman, 2014), it is important to prevent initial contamination of the environment (Overgaauw & van Knapen, 2013). Regular deworming and periodic faecal examination of pets (Elsheikha & Khan, 2011), strategic anthelminthic treatment on puppies and nursing bitches, not allowing pets to defecate in public places, picking-up the faeces of the dogs (Overgaauw & van Knapen, 2013), adoption of appropriate hygiene procedures (Robertson & Thompson, 2002) and cultivate awareness among the public (Lee *et al.*, 2010) are measures that can be taken to minimise the risk of environmental contamination. Free-roaming animal population should be controlled and dogs and cats should be prevented access to public places (mainly children's playgrounds) (Overgaauw & van Knapen, 2013).

2.1.5 - Zoonotic Risk

Toxocara canis is recognized to have a highly significance to public health, however, human infections with *T. cati* have been underestimated (Fisher, 2003).

Human infection occurs by ingestion of embryonated eggs from contaminated soil originated from faeces of infected animals (Elsheikha & Khan, 2011), unwashed hands, raw vegetables and ingestion of larvae present in under-cooked meat or organs from paratenic hosts. Although direct contact with infected animals is reported as an important source of *Toxocara* eggs for infection, it is usually not considered a risk. The eggs present in the fur need to embryonate several weeks before becoming infective, and will be inactivated in most cases by drying and UV-light, the ingestion of a sufficient number of eggs is unlikely due to the adhesion to the fur and it would be necessary to ingest a large amount of hair to become infected because of the high percentage of non-viable eggs (Overgaauw & van Knapen, 2013).

Children, usually younger than 3 years (Bowman, 2014), are the most affected by this disease because of greater exposure to contaminated soil and the practice of pica or geophagia (Robertson & Thompson, 2002). Low socioeconomic level, poor environment hygiene and wet and warm climates have been associated with an increased rate of the disease (Fillaux & Magnaval, 2013). Regular removal of faeces (at least once a week) and not using them to fertilize vegetable gardens can minimise environmental contamination (Robertson & Thompson, 2002; Bowman, 2014).

After ingestion of embryonated eggs, they hatch and, because humans act as an paratenic host (Overgaauw & van Knapen, 2013), larvae migrate to different tissues inducing inflammatory responses which lead to a disease known as Visceral Larva Migrans (VLM) (Ballweber, 2001; Elsheikha & Khan, 2011). Nodules containing larvae occur principally in the liver, lungs, kidneys, and brain (Bowman, 2014). Formation of granulomas leads to larval death (Ballweber, 2001). Although most *Toxocara* infections remain unapparent (Strube *et al.*, 2013); clinical signs of VLM include fever, eosinophilia, hepatomegaly and respiratory signs such as coughing, asthma, and pneumonitis (Robertson & Thompson, 2002; Elsheikha & Khan, 2011). Larvae can also invade the eye leading to Ocular Larva Migrans (OLM) and causing generally, a unilateral granulomatous retinitis (which can be mistaken for a retinoblastoma) (Robertson & Thompson, 2002). Besides the tissue invaded, the severity of problems caused depends on the number of migrating larvae and the age of the host (Strube *et al.*, 2013). Covert toxocarosis is another syndrome where clinical symptoms are non-specific (Overgaauw, 1997a), such as chronic weakness, digestive pain, various allergic signs, diffuse myalgias and cough (Fillaux & Magnaval, 2013). Neurological and atopic symptoms have also been described associated to toxocarosis (Overgaauw & van Knapen, 2013).

2.2 - *Toxascaris leonina*

2.2.1 – Etiology

Toxascaris leonina, like *Toxocara* spp., belongs to the Family Ascarididae (Ballweber, 2001) and is also known as “roundworm” (Zajac & Conboy, 2012). With a worldwide distribution, although more prevalent in cooler climates (Bowman, 2014), it can infect the small intestine of dogs and cats (Ballweber, 2001). Adults length ranges up to 10 cm (Bowman, 2014) and can be differentiated from *Toxocara* spp. based on cervical alae (*T. leonina* ‘s resembles a spear), and male tail (does not have terminal appendix) (Okulewicz *et al.*, 2012).
T. leonina is less frequent than the other carnivore ascarids (Baños *et al.*, 1999) and in Portugal, its prevalence ranges from 0.5% to 5.1% (faecal samples collected from public places, national parks, pets and kennels) (Gravata *et al.*, 2007; Silva, 2010; Ferreira *et al.*, 2011; Lebre, 2011; Mateus *et al.*, 2014; Neves, Lobo, Simões, & Cardoso, 2014).

2.2.2 - Life Cycle

The life cycle of *T. leonina* is direct, unless there are paratenic host, in which case the cycle is indirect. Eggs are passed in faeces and infective L3 develops within the eggs in about a week (Ballweber, 2001). When the transmission occurs through ingestion of eggs containing infective L3, the larva invades the mucosa of the small intestine where it develops and molts to L4, returning to the lumen to mature to adults (Ballweber, 2001; Bowman, 2014). When paratenic host ingest infective eggs, larvae hatch and invade other tissues, encyst and remain arrested in the infective stage (Bowman, 2014). By ingesting these paratenic hosts (major route of infection) (Ballweber, 2001), larvae are released in the digestive system and develops into adults in the intestine of the definitive host (Elsheikha & Khan, 2011). The prepatent period can be from seven to eleven weeks, when eggs are ingested, or it can be shortened by two weeks when paratenic hosts are ingested (Ballweber, 2001).

Usually, this parasite does not cause signs of disease, however they can be associated with diarrhoea, distended abdomen and stunted growth rate (Ballweber, 2001; Elsheikha & Khan, 2011).

2.2.3 - Diagnosis

Infections with *T. leonina* are usually detected by faecal flotation, where eggs are subspherical, the internal ovum, which does not fill the egg, appears light in colour, the outer shell is smooth (which differentiate from *Toxocara* spp.), and measure 75–85 µm × 60–75 µm (Ballweber, 2001; Elsheikha & Khan, 2011).

2.2.4 - Treatment and Control

Treatment can be accomplished with regular anthelminthic drugs containing: pyrantel embonate, fenbendazole, febantel (Ballweber, 2001), mebendazole, (Foreyt, 2001), nitroscanate, emodepside, moxidectin or milbemycin oxime 3 to 4 times a year (Alho *et al.*, 2010).

2.2.5 - Zoonotic Risk

Despite the fact that cases of VLM from *T. leonina* have been reported, the zoonotic risk is of less importance than from *T. canis* (Elsheikha & Khan, 2011).
The zoonotic potential of *T. leonina* is considered as absent or as very limited, because somatic migration in the definitive hosts does not occur as part of the normal life cycle, and larvae are not vertically transmitted (Overgaauw & van Knapen, 2000).

2.3 - *Ancylostoma* spp. and *Uncinaria* sp.

2.3.1 - Etiology

Ancylostoma spp. and *Uncinaria* sp. belong to the Phylum Nemathelminthes, Class Nematoda, Subclass Secernentea, Order Strongylida, Family Ancylostomatidae (Ballweber, 2001). *Ancylostoma* spp. have a worldwide distribution, but is more frequent in the tropics and subtropics. *U. stenocephala* is more prevalent in temperate and sub-arctic areas (Urquhart *et al.*, 1996). Some of the species with most reliable background information are listed in Table 4.

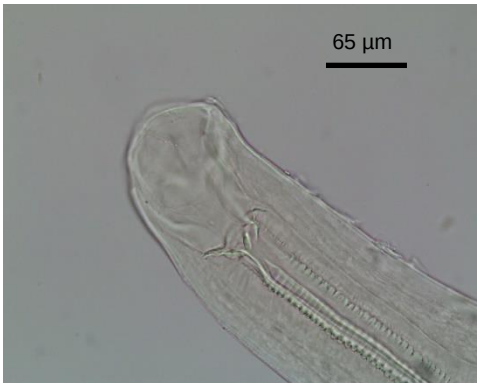
Table 4 - Some species within the family Ancylostomatidae (adapted from Bowman, Montgomery, Zajac, Eberhard, & Kazacos, 2010)

Species	**Definitive Host**	**Geographical Distribution**
Ancylostoma braziliense	Domestic and wild canids Cat	Africa, America, Asia, Australia
Ancylostoma caninum	Dog and fox	Tropics and warm temperate areas Central and southern Europe
Ancylostoma ceylanicum	Dog and Cat	Australia, Asia, South America
Uncinaria stenocephala	Domestic and wild canids Rarely cat	Colder climates America, Asia, Europe, Oceania

Since in Europe only *Ancylostoma caninum* and *Uncinaria stenocephala* are relevant, the following review sections will focus on these two only.

The members of this family are present in the small intestine and have the anterior end bent dorsally resulting in a “hook”, giving these parasites the name of “hookworms” (Figure 5) (Ballweber, 2001). *A. caninum* adults’ length ranges from 9-12 mm (males) to 15-18 mm (females), and from 4-5 mm (males) to 7-12 mm (females) in *U. stenocephala* (Elsheikha & Khan, 2011). Members of the genus *Ancylostoma* have ventral teeth in the buccal capsule, whose number can help differentiating from each other (Prociv & Croese, 1996; Ballweber, 2001). *A. caninum* has three teeth along the anterior margin of each denticular plate (Prociv & Croese, 1996) as *U. stenocephala* has cutting plates instead of teeth in the buccal cavity (Ballweber, 2001).

Figure 5 – Anterior end of *Ancylostoma* sp. (specimen courtesy of LPPD FVM-UL; original)



In Portugal, the prevalence of Ancylostomatidae ranges from 0.8% to 70.3% (faecal samples collected from public places, national park, pets, farm and hunting dogs and kennels) (Crespo *et al.*, 2006; Maurício *et al.*, 2006; Gravata *et al.*, 2007; Silva, 2010; Ferreira *et al.*, 2011; Lebre, 2011; Rosa, Nunes, Costa, Crespo, & Almeida, 2011; Frias, 2012; Crespo *et al.*, 2013; Mateus *et al.*, 2014; Nunes, 2014; Santos, 2014; Leal, 2015).

2.3.2 - Life Cycle

The life cycle of the family Ancylostomatidae is direct, unless there are paratenic hosts, in which case the cycle is indirect (Ballweber, 2001). Typical hookworm eggs (morula 2-8 cell) are passed in faeces, under optimal conditions, they embryonate (L1) within 24-48h and infective L3 develops in about a week (Prociv & Croese, 1996).

The most common route of transmission of *A. caninum* is percutaneous, where L3 penetrate the skin through hair follicles and are carried through the blood or lymphatic vessels to the heart and lungs. They change to L4 and penetrate alveoli, ascend to the trachea, are swallowed and reach the small intestine where they attach to the intestinal wall and mature to adults (Prociv & Croese, 1996; Ballweber, 2001). Some larvae become arrested in muscle and are reactivated by immunity decrease and can recolonize the intestine (Katagiri & Oliveira-Siqueira, 2007). Pregnancy is a cause of reactivation, leading to a migration of the larvae to the mammary gland. This transmammary infection is another important route of transmission, primary in puppies (Ballweber, 2001). A bitch previously infected and harbouring somatic larvae can infect her offspring for three consecutive pregnancies (Urquhart *et al.*, 1996). Other routes of transmission are ingestion of infective third-stage larvae from the environment or in paratenic hosts, and transplacental (Zajac & Conboy, 2012). In the case of *U. stenocephala*, the main transmission route is ingestion of L3 or paratenic hosts; percutaneous infection is uncommon and transmammary infection does not occur (Ballweber, 2001; Zajac & Conboy, 2012). The prepatent period varies between two to four weeks, depending on the route of transmission (Ballweber, 2001).

The severity of disease caused by these parasites depends on the route of infection, number and species of parasites (*U. stenocephala* is less pathogenic than *A. caninum* and clinical signs may not be present) and immune status of host (Ballweber, 2001; Katagiri & Oliveira-Siqueira, 2007). The disease is mostly common in dogs under one year old (Urquhart *et al.*, 1996). The most common problem, particularly in puppies, is anaemia, due to the hematophagous feedings of these parasites, which can lead to death (Zajac & Conboy, 2012). Diarrhoea is also present and can contain blood and mucous (Katagiri & Oliveira-Siqueira, 2007). Because of migrating larvae, dermatitis and pneumonia can also occur. Associated with *A. caninum*, clinical disease can be peracute (when puppies are infected by transmammary transmission), acute (when sudden exposure to overwhelming numbers of larvae occurs) and chronic (immunocompetent animals that are not exposed to overwhelming numbers of larvae) (Ballweber, 2001).

2.3.3 - Diagnosis

Ancylostomatidae infections are usually detected by faecal flotation, where strongyle-type eggs have an elliptical shape, smooth thin shell containing morula with 2-8 cells. *Ancylostoma* spp. and *Uncinaria* sp. eggs can be differentiated by size, being the latest, bigger (Zajac & Conboy, 2012). Table 5 shows the average egg size.

Table 5 – Egg size of *A. caninum* and *U. stenocephala* (adapted from Zajac & Conboy, 2012)

Specie	Length (µm)	Width (µm)
Ancylostoma caninum	52 – 79	28 – 58
Uncinaria stenocephala	71 - 92	35 - 58

2.3.4 - Treatment and Control

Both treatment and prophylaxis can be accomplished with regular anthelminthic drugs containing: pyrantel embonate, fenbendazole, febantel (Ballweber, 2001), mebendazol, nitroscanate, emodepside, moxidectin or milbemycin oxime 3 to 4 times a year. To prevent transmammary transmission, infected bitches can be treated with fenbendazole from the last third of pregnancy through the first stage of lactation (Alho *et al.*, 2010). Puppies should be treated at 2, 4, 6 and 8 weeks of age, to control transplacental and transmammary infections. Nursing bitches should be treated concurrently (Baños *et al.*, 1999). For encysted larvae, it is recommend emodepside and moxidectin, and to a lesser extent milbemycin oxime (Alho *et al.*, 2010). Treatment success should always be verified through regular faecal examinations (Bowman *et al.*, 2010).

Prevention of infection, both in humans and in companion animals, has in consideration regular deworming, not allowing pets to defecate in public places and picking-up the faeces of the dogs. Animal population should be controlled and the public should be educated about the disease (Ballweber, 2001).

2.3.5 - Zoonotic Risk

When the infective stage larvae penetrate the skin of humans, their migration causes progressive linear eruptions and pruritis, resulting in Cutaneous Larva Migrans (CLM) or creeping eruption (Robertson & Thompson, 2002). Although this disease can be caused by other Ancylostomatidae, it is most often associated with *A. braziliense* (Ballweber, 2001). Usually, the lesions are self-limiting but in heavily exposed humans, respiratory or intestinal symptoms have occurred (Prociv & Croese, 1996; Robertson & Thompson, 2002). People who walk around barefooted and have contact with soil or sand contaminated with infected faeces have a greater probability of getting the disease. Eosinophilic enteritis is associated with *A. caninum* and leads to abdominal pain, diarrhoea, abdominal distension, weight loss and rectal bleeding (Robertson & Thompson, 2002). Both conditions are more common in tropical climates (Robertson *et al.*, 2000; Robertson & Thompson, 2002). To reduce the chance of human infection, appropriate hygiene procedures such as washing hands, wearing enclosed footwear and not lying on areas where companion animals may have defecated should be taken (Robertson & Thompson, 2002).

2.4 - *Strongyloides stercoralis*

2.4.1 - Etiology

Strongyloides stercoralis belongs to the Phylum Nemathelminthes, Class Nematoda, Subclass Secernentea, Order Rhabditida, Family Strongyloididae (Ballweber, 2001). With a worldwide distribution (Ballweber, 2001), they can infect dogs, cats and humans (Urquhart *et al.*, 1996). This nematode is also known as “threadworm” and adults are very small in size, ranging from 0.7 to 2.2 mm (Foreyt, 2001).
In Portugal, the prevalence of *Strongyloides* ranges from 0.2% to 25.6% (faecal samples collected from public places and national park and kennels) (Gravata *et al.*, 2007; Silva, 2010; Santos, 2014).

2.4.2 - Life Cycle

The life cycle is direct, with a homogonic cycle (parasitic) or a heterogonic cycle (free-living). In the homogonic cycle, females lay eggs in the small intestine, L1 develop within and hatch, and are passed in the faeces. Infective L3, in the environment, penetrate the skin, are carried through the blood or lymphatic vessels to the lungs, migrate up the bronchi to the trachea, are swallowed and reach the small intestine where they attach to the intestinal wall and mature to adults. Another route of transmission is autoinfection, which occurs when L1, instead of passing in the faeces, stay in the intestine, develop to L3 and penetrate the mucosa and migrate as for percutaneous infection (Ballweber, 2001). In the heterogonic cycle, L3 initiate free living generations, when environmental conditions are satisfactory (Ballweber, 2001; Robertson & Thompson, 2002). The prepatent period is about two weeks (Bowman, Fogarty, & Barr, 2005).

Infection with this parasite is less common than with other helminths (Robertson & Thompson, 2002). Usually it is asymptomatic, however, dermatitis, catarrhal enteritis, severe diarrhoea, dehydration, and pneumonia may occur (Ballweber, 2001; Robertson & Thompson, 2002). In puppies and immunosuppressed dogs this infection could be fatal (Bowman *et al.*, 2005).

2.4.3 - Diagnosis

S. stercoralis infection may be diagnosed with faecal flotation (Ballweber, 2001), where oval eggs with thin shell, measuring 55 µm x 30 µm (Foreyt, 2001) and containing a L1, can be found. Baermann technique can be used to detect larvae (Ballweber, 2001). L1 measures from 280 µm to 310 µm in length (Kassai, 1999).

2.4.4 - Treatment and Control

Treatment with anthelmintics will not kill the migrating autoinfective L3 larvae in very young or immunosuppressed dogs, but remove adult *S. stercoralis* worms from the intestine (Overgaauw & van Knapen, 2000). It is described the administration of diethylcarbamazine, mebendazole, selamectine, fenbendazole, nitroscanate and a combination of febantel and pyrantel embonate (Alho *et al.*, 2010). Follow-up faecal examinations are advised as control of parasitological cure (Overgaauw & van Knapen, 2000).
Appropriate cleaning and elimination of faeces contributes to a better control (Baños *et al.*, 1999).

2.4.5 - Zoonotic Risk

S. stercoralis may contribute to CLM in humans (Ballweber, 2001). In the presence of a lower burden of worms, people may present mild intestinal signs (abdominal pain, diarrhoea, constipation). However, with a heavy burden, clinical signs include fever, liver tenderness, nausea, vomiting, weight loss and severe diarrhoea. In immunocompromised individuals, the infection can be fatal (Robertson & Thompson, 2002).

2.5 - *Trichuris vulpis*

2.5.1 - Etiology

Trichuris vulpis belongs to the Phylum Nemathelminthes, Class Nematoda, Subclass Adenophorea, Order Enoplida, Family Trichuridae. With a worldwide distribution, they can infect the caecum (and sometimes the colon) of canids (Ballweber, 2001). This nematode is also known as “whipworm” due to its thin two-thirds anterior ends (Figure 6a) (Elsheikha & Khan, 2011), and adults length ranges from 30 to 80 mm (Foreyt, 2001). Males have a single spicule in a sheath in the tail (Urquhart *et al.*, 1996).

In Portugal, the prevalence of *Trichuris vulpis* ranges from 1.1% to 49.5% (faecal samples collected from public places, national park, pets, farm and hunting dogs and kennels) (Crespo *et al.*, 2006; Maurício *et al.*, 2006; Gravata *et al.*, 2007; Silva, 2010; Ferreira *et al.*, 2011; Lebre, 2011; Frias, 2012; Crespo *et al.*, 2013; Mateus *et al.*, 2014; Neves *et al.*, 2014; Santos, 2014; Leal, 2015).

2.5.2 - Life Cycle

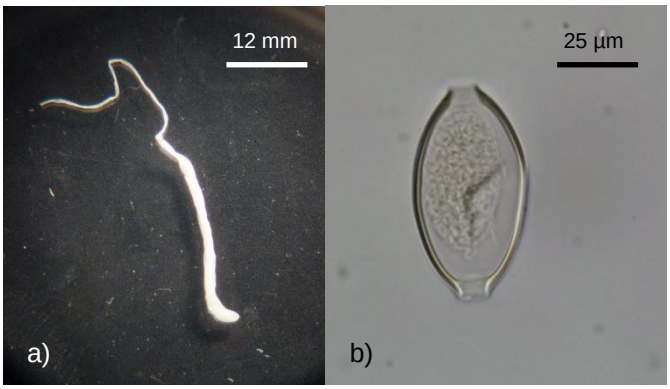
The life cycle is direct: eggs are passed in faeces and embryonate (L1) in about 3 weeks. The infection is acquired by ingestion of eggs containing infective L1. Larvae hatch in the small intestine, penetrate mucosal glands of caecum and colon and develop into immature adult stage. They return to lumen, embed their anterior end in the mucosa and mature (Ballweber, 2001). The eggs are very resistant to external environment conditions (Elsheikha & Khan, 2011). The prepatent period is eleven to twelve weeks (Ballweber, 2001).

Usually the infection is asymptomatic (Baños *et al.*, 1999), however, clinical signs include mucoid diarrhoea with blood, weight loss and, in heavy infections, enterocolitis may occur (Ballweber, 2001).

2.5.3 - Diagnosis

The laboratory diagnosis depends on detection by faecal flotation (Ballweber, 2001) of light brown lemon-shaped eggs, with transparent polar plugs (Figure 6b) (Bowman *et al.*, 2005; Elsheikha & Khan, 2011). *T. vulpis* eggs size is 70–80 µm × 30–40 µm (Ballweber, 2001).

Figure 6 – *Trichuris vulpis*: a) adult with a whip shape; b) egg (specimens courtesy of LPPD FVM-UL; original)



2.5.4 - Treatment and Control

For the treatment of trichuriosis, it is described the administration of fenbendazole, febantel (Ballweber, 2001), milbemycin oxime, oxibendazol and more recently emodepside and moxidectin. Because *Trichuris* only reach maturity in 3 months, it is necessary to repeat dosing, deworming animals monthly 3 days in a row (Alho *et al.*, 2010).
Appropriate hygiene procedures and appropriate cleaning and disinfection of the spaces contribute to a better control (Baños *et al.*, 1999).

2.5.5 - Zoonotic Risk

Despite of being rare, *T. vulpis* has been reported in humans (Ballweber, 2001), mainly in children and institutionalized patients. People can be asymptomatic or demonstrate diarrhoea and dysentery (Dunn, Columbus, Aldeen, Davis, & Carroll, 2002).

3 - Protozoa

Protozoans are unicellular organisms, with different sizes and shapes. Only a small number are parasitic (Ballweber, 2001), and a lower proportion is associated with disease (Bowman, 2014). For this study, flagellates (*Giardia*) and apicomplexan coccidia (*Cystoisospora*, *Cryptosporidium* and *Sarcocystis*) (ESCCAP, 2011) are described.
In 2004, *Giardia* and *Cryptosporidium* were included in the 'Neglected Diseases Initiative' of the World Health Organization (WHO) (Savioli, Smith, & Thompson, 2006).

3.1 - *Giardia* spp.

3.1.1 - Etiology

Giardia spp. belong to Flagellates in the protozoan group (Ballweber, 2001) and six species that infect a wide range of vertebrate hosts are recognized (Sprong, Cacciò, & Van Der Giessen, 2009). *Giardia duodenalis* (also known as *Giardia intestinalis* or *Giardia lamblia*) infects the small intestine of mammals (including pets) and is the only one to be found in humans (Ryan & Cacciò, 2013). It has a worldwide distribution (Ballweber, 2001) and it is considered a species complex (Tangtrongsup & Scorza, 2010), whose members, morphologically identical, can be molecularly classified into distinct assemblages (Ryan & Cacciò, 2013). *G. duodenalis* assemblages and sub-assemblages, proposed taxonomy and their host distribution are described in Table 6.

Table 6 - *Giardia duodenalis* assemblages and sub-assemblages, proposed taxonomy and their host distribution (adapted from Ryan & Cacciò, 2013)

Assemblages and Sub-assemblages	**Proposed taxonomy**	**Hosts**
Assemblage A	*Giardia duodenalis*	Humans and other primates, livestock, dogs, cats and some species of wild mammals
AI		Humans (also described in animals)
AII		Humans (also described in animals)
AIII		Animals
AIV		Animals
Assemblage B	*Giardia enterica*	Humans and other primates, dogs, cats and some species of wild mammals
BI		Animals
BII		Animals
BIII		Humans (also described in animals)
BIV		Humans (also described in animals)
Assemblage C	*Giardia canis*	Dogs and other canids
Assemblage D	*Giardia canis*	Dogs and other canids
Assemblage E	*Giardia bovis*	Hoofed livestock
Assemblage F	*Giardia cati*	Cats
Assemblage G	*Giardia simondi*	Rats
Assemblage H		Marine mammals

In dogs, around the world, the prevalence of *Giardia* sp. ranges from 1% to 39% (Anderson *et al.*, 2004), whereas in Europe, it varies from 0.3% to 36% (Bowman & Lucio-Forster, 2010). The prevalence rises up to 100% in breeding establishments and kennels (Anderson *et al.*, 2004). In Portugal, infection rates vary from 7.4% to 55.9% for *Giardia* sp. in dogs (samples collected from public places, pets and kennels) (Ferreira *et al.*, 2011; Lebre, 2011; Fernandes, 2012; Neves *et al.*, 2014; Nunes, 2014; Santos, 2014; Leal, 2015).

3.1.2 - Life Cycle

Giardia sp. has a direct life cycle. Cysts containing two trophozoites, in result of asexual reproduction, are passed in faeces (Ballweber, 2001). On ingestion, free trophozoites attach to the surface of enterocytes of the small intestine, although they can also remain free (Esch & Petersen, 2013), and multiply through binary fission (Ballweber, 2001). Subsequently, as each trophozoite transits toward the colon, it will form a cyst (Ballweber, Xiao, Bowman, Kahn, & Cama, 2010). However, trophozoites can also be found in faeces, particularly in

acute infections (Ballweber, 2001), having a very short survival time outside the host (Tangtrongsup & Scorza, 2010). Cysts are immediately infectious, are very resistant and can survive several months in the environment (Ryan & Cacciò, 2013) in wet and cold conditions, but are susceptible to desiccation (Tangtrongsup & Scorza, 2010) with a decrease in numbers during winter. The prepatent period can be from four to sixteen days and patency can persist for months (ESCCAP, 2011).

Ingestion of cysts leads to infection, either by faecal-oral route directly from infected individuals (Ryan & Cacciò, 2013) or through contaminated food, water, fomites or self-grooming (CAPC, 2013). A few cysts can initiate infection (ESCCAP, 2011). Outbreaks are most frequently waterborne caused by contamination of drinking water (Sprong *et al.*, 2009). Asymptomatic, as well as sick individuals, are sources of infection, and pregnant or lactating bitches can be sources for their offspring (Vega, 1999).

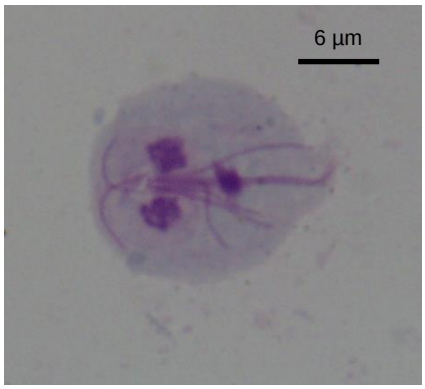
Although *Giardia* infection is common, most infected pets are asymptomatic (Tangtrongsup & Scorza, 2010). The attachment of the parasite to enterocytes causes villous atrophy and crypt hyperplasia, leading to a decrease in the absorptive surface area of the intestine (Ballweber, 2001) which, in turn, results in hypersecretion (Tangtrongsup & Scorza, 2010), maldigestion, malabsorption and diarrhoea (CAPC, 2013). Clinical signs include intermittent acute or chronic (ESCCAP, 2011), soft to watery diarrhoea with mucous on the surface, strong odour and steatorrhea, abdominal discomfort (Tangtrongsup & Scorza, 2010), anorexia, vomiting, weight loss and lethargy (ESCCAP, 2011).
Immunocompromised diseases or coexisting infections may potentiate the development of clinical signs (Tangtrongsup & Scorza, 2010). Severity of the disease is dependent on parasite factors (large number of ingested cysts and ingestion of cysts instead of trophozoites are more pathogenic) and host factors (Vega, 1999). Maturation of the immune system (Anderson *et al.*, 2004) and previous exposure (since this induces partial immunity) (ESCCAP, 2011) are consistent with the fact that puppies are more susceptible to acquire *Giardia* infections (Tangtrongsup & Scorza, 2010).
Asymptomatic carriers function as a reservoir and may spread the infections (Sprong *et al.*, 2009), because of that, giardiosis is usually associated with kennels (Robertson & Thompson, 2002).

3.1.3 - Diagnosis

The diagnosis of *Giardia* infection can be performed either by detection of trophozoites or cysts in faeces (Ballweber, 2001).

The trophozoite (Figure 7), the active and motile form (Tangtrongsup & Scorza, 2010), is bilaterally symmetrical with a pyriform body (Zajac & Conboy, 2012), measuring approximately 9-21 µm long by 5-12 µm wide (ESCCAP, 2011). There are two anterior nuclei, eight flagella, two dark-staining median bodies, and a ventral, adhesive disc (Zajac & Conboy, 2012), which facilitates attachment to the intestinal mucosa (Urquhart *et al.*, 1996).

Figure 7 – *Giardia* sp. trophozoite (specimen courtesy of LPPD FVM-UL; original)



Cysts are ellipsoidal to ovoid, non-motile, and contain two to four nuclei. They measure 8-15 µm by 7-10 µm and have a thick retractile wall (ESCCAP, 2011; CAPC, 2013).

Giardia cysts can be detected by simple or centrifugal faecal flotation using zinc sulphate (Ballweber, 2001), which is the laboratory method of choice for practitioners (Irwin, 2002). Saturated sugar solution can also be used but, it will collapse the cyst in a characteristic way. A drop of Lugol's iodine may be added to a better visualization of morphology (CAPC, 2013).

Direct saline smear is used for detection of motile trophozoites in diarrheic faeces (CAPC, 2013), preferably within 20 minutes of sample collection (Ballweber, 2001).

Several Enzyme-Linked Immunosorbent Assays (ELISA) test kits, which allow the detection of antigen in faeces, and direct immunofluorescent antibody (IFA) test kits (that require immunofluorescent microscope) are also available (Ballweber, 2001).

Giardia DNA in faeces can be isolated by Polymerase Chain Reaction (PCR) to assess the *G. duodenalis* assemblage, since it remains expensive for commercial diagnostic tests (Savioli *et al.*, 2006; Tangtrongsup & Scorza, 2010).

According to the CAPC recommendations, symptomatic dogs should be tested with a combination of centrifugal faecal flotation, direct smear and ELISA optimized for use in companion animals (CAPC, 2013). Since *Giardia* shedding is intermittent (giardiosis should not be excluded based on a single negative faecal exam) (Ballweber, 2001) and in order to improve detection, three faecal samples collected at 3 to 5 day period should be examined (ESCCAP, 2011).

3.1.4 - Treatment and Control

Dogs infected with giardiosis can be treated with metronidazole (ESCCAP, 2011) and fenbendazole (CAPC, 2013). Tinidazole is also effective (ESCCAP, 2011). A combination of febantel, pyrantel embonate and praziquantel, administered daily for 3 days, doses according to the formulation, can be used (CAPC, 2013), mainly when a concurrent infection with nematodes or cestodes is suspected (Tangtrongsup & Scorza, 2010). When clinical signs and cyst shedding persist, treatment with fenbendazole can be repeated (ESCCAP, 2011) and a combination with fenbendazole and metronidazole can be administered for 5 days (CAPC, 2013).

When there is a detection of *Giardia* cysts, treatment should be employed, however, it does not necessarily imply that this parasite is the cause of the diarrhoea in the pet (Irwin, 2002). When the appropriated treatment fails, reinfection of the dog from its environment, *Giardia* resistance, co-infections or another underlying disease, can be the cause of disease (Irwin, 2002; ESCCAP, 2011).

Treatment may not be required in asymptomatic dogs. If treatment is desired, the dog without clinical signs, as well as other co-habitant dogs may be treated with a single course of anti-giardial therapy (CAPC, 2013).

Vaccines against *Giardia* can reduce cyst shedding and prevent clinical signs (Tangtrongsup & Scorza, 2010), however, routine vaccination is not part of a treatment protocol (CAPC, 2013).

Dogs should be shampooing, concomitant with treatment, to remove adhering faeces and cysts and reduce re-infections (ESCCAP, 2011; CAPC, 2013). Daily and appropriately removal of faeces (CAPC, 2013), and suitable hygienic measures (including personal hygiene of the animal carers) avoid the spreading of cysts (ESCCAP, 2011). Cleaning followed by disinfection of kennels with quaternary ammonium compounds (cysts are susceptible) should be performed (Ballweber, 2001) and surfaces should be left to dry thoroughly after cleaning (CAPC, 2013). Correct water treatment (levels of chlorine in drinking water are inadequate to inactivate cysts) will prevent the transmission of this protozoa (Tangtrongsup & Scorza, 2010).

3.1.5 - Zoonotic Risk

The role of pets as a source of human giardiosis has not been conclusively demonstrated (Ballweber *et al.*, 2010). Assemblages A and B are suggested to have zoonotic potential (Sprong *et al.*, 2009). It is believed that person-to-person transmission is more important than zoonotic transmission, and that humans are the main reservoir of human infection

(Robertson & Thompson, 2002). However, immunocompromised people should limit their exposure to infected pets (CAPC, 2013) since dogs can carry strains of *Giardia* which are potentially infective to humans (Robertson & Thompson, 2002).

Human giardiosis can have a wide spectrum of clinical manifestations that range from asymptomatic to acute or chronic diarrhoea, dehydration, abdominal pain, nausea, vomiting and weight loss (Ryan & Cacciò, 2013). The severity of infection is determinated by host factors, such as immune status, nutritional status, and age (Ferreira *et al.*, 2013), being children and immunocompromised people the most susceptible (Sprong *et al.*, 2009; Bowman & Lucio-Forster, 2010). In developed countries, this disease is associated with outbreaks of diarrhoea in child-care centres (Irwin, 2002). Giardiosis is associated with travel to endemic areas, mainly developing countries (Macpherson, 2005; Bowman & Lucio-Forster, 2010), and especially with water-borne outbreaks (Bowman & Lucio-Forster, 2010). Sewage discharges, which contain environmental resistant cysts excreted by animals and humans, contaminate water sources (Savioli *et al.*, 2006). To prevent infection, proper sanitation of water sources, appropriate treatment of drinking water and personal hygiene (hand washing, proper disposal and handling of waste, not allowing children with diarrhoea to participate in recreational water activities) should be implemented (Esch & Petersen, 2013).

3.2 - *Cystoisospora* spp.

3.2.1 - Etiology

Cystoisospora spp. belong to Apicomplexans in the protozoan group (Ballweber, 2001) and are host-specific (ESCCAP, 2011). Species that have dogs as a definitive host are listed in Table 7. *C. ohioensis*, *C. burrowsi*, and *C. neorivolta* are often referred to as *C. ohioensis*-complex because they cannot be separated morphologically and because *C. ohioensis* was the first named (Lindsay, Dubey, & Blagburn, 1997; ESCCAP, 2011).

Table 7 - Some species within genus *Cystoisospora* that infect dogs

Species	**Oocyst Size (µm) (Dubey, Lindsay, & Lappin, 2009)**	**Localization (Bowman *et al.*, 2005)**
Cystoisospora canis	38 x 30	Distal third of small intestine
Cystoisospora ohioensis	24 x 20	Intestinal cells of the jejunum, and epithelial cells of small intestine
Cystoisospora burrowsi	20 x 17	Lamina propria of the posterior small intestine
Cystoisospora neorivolta	24 x 20	Lamina propria of the posterior small intestine

In Portugal, the prevalence of *Cystoisospora* spp. ranges from 0.2% to 13.5% (faecal samples collected from public places, national park, pets, and kennels) (Crespo *et al.*, 2006; Maurício *et al.*, 2006; Gravata *et al.*, 2007; Silva, 2010; Ferreira *et al.*, 2011; Lebre, 2011; Rosa *et al.*, 2011; Crespo *et al.*, 2013; Mateus *et al.*, 2014; Neves *et al.*, 2014).

3.2.2 - Life Cycle

The life cycle of *Cystoisospora* spp. is direct, unless there are paratenic host, in which case the cycle is indirect. Oocysts are passed in faeces and sporulate within two to four days, resulting in two sporocysts, each of which contains four sporozoites (Ballweber, 2001). On ingestion, oocysts excyst and free sporozoites invade the intestine. When a paratenic host ingest sporulated oocysts, sporozoites penetrate the intestinal wall and invade extraintestinal tissues where they encyst (Dubey *et al.*, 2009). By ingesting these paratenic hosts, the definitive host becomes infected (Ballweber, 2001; Dubey *et al.*, 2009). The prepatent period can be from six to ten days, when oocysts are ingested, or it can be shortened when paratenic hosts are ingested (ESCCAP, 2011).

Usually it is asymptomatic, however, diarrhoea, abdominal pain, dehydration, anorexia, and weight loss may occur (Ballweber, 2001). In severe cases the faeces can contain blood (ESCCAP, 2011). Very young puppies are more susceptable to this disease (Ballweber, 2001).

3.2.3 - Diagnosis

The laboratory diagnosis depends on detection by faecal flotation of unsporulated oocysts (Ballweber, 2001) in freshly excreted faeces (Dubey *et al.*, 2009). However, they sporulate partially by the time of faecal examination, and oocysts with two sporocysts and no sporozoite are found (Dubey *et al.*, 2009; ESCCAP, 2011). *C. canis* is the only species that can be identified by microscopical examination of oocysts due to its size and shape. Because the oocysts of the other three species of *Cystoisospora* may overlap in size, specific identification requires either molecular methods or histological examination of tissues (Baker, 2007; Dubey *et al.*, 2009).

3.2.4 - Treatment and Control

The use of sulphonamides (usually sulfadimethoxine), daily for 5-7 days, for the treatment of *Cystoisospora* spp., only controls diarrhoea and does not prevent oocyst shedding (Ballweber, 2001; ESCCAP, 2011). Toltrazuril and diclazuril, in a single application, are the

drugs used for reducing oocyst excretion. An association of toltrazuril and emodepside can be used if a co-infection of coccidian and roundworms is present. Treatment should include all litter mates and in-contact puppies of the infected one (ESCCAP, 2011).
Control of transmission of the parasite includes suitable hygienic measures (including personal hygiene of the animal carers) and daily removal of faeces (ESCCAP, 2011).

3.2.5 - Zoonotic Risk

Humans are not susceptable to cystoisosporosis (ESCCAP, 2011).

3.3 - *Cryptosporidium* spp.

3.3.1 - Etiology

Cryptosporidium spp. belong to Apicomplexans in the protozoan group and it has a worldwide distribution (Ballweber, 2001). Species that infect dogs includes *Cryptosporidium canis* and *C. parvum* (found mainly in calves but also in dogs, cats and humans) (ESCCAP, 2011).

Prevalence rates in dogs vary from 0% to 44.8% (Bowman & Lucio-Forster, 2010). In Portugal, the prevalence of *Cryptosporidium* spp. in dogs ranges from 3.1% to 17.6% (samples collected from a national park and kennels) (Silva, 2010; Lebre, 2011; Santos, 2014; Leal, 2015).

3.3.2 - Life Cycle

Cryptosporidium spp. has a direct life cycle (Ballweber, 2001). Sporulated oocysts containing four sporozoites are passed in faeces (Ballweber, 2001; Bowman, 2014). On ingestion, free sporozoites invade the epithelium of the small intestine (ESCCAP, 2011) and have an intracellular, extracytoplasmic location. After undergoing through schizogony and gametogony, oocysts are produced (Baker, 2007). Sporulation of oocysts occurs in the intestines and they are excreted with the faeces already in the infective form (ESCCAP, 2011). There are two different types of oocysts produced: those thin-walled that rupture internally and contribute to autoinfection (roughly 20%) and, those thick-walled that pass in the faeces (Ballweber, 2001; Scorza & Tangtrongsup, 2010). Excretion lasts from 25 to 80 days (ESCCAP, 2011) and oocysts are extremely resistant to environmental conditions (Ballweber, 2001). Routes of infection include coprophagia, grooming, ingestion of infected

preys and ingestion of contaminated food or water (Scorza & Tangtrongsup, 2010). The prepatent period can last from two to fourteen days for *C. canis* (ESCCAP, 2011).

Usually the infection is asymptomatic, however, watery diarrhoea, abdominal pain, vomiting and elevated body temperature may occur, mainly in puppies (ESCCAP, 2011). In immune-competent individuals, this disease is self-limiting and lasts from one to two weeks (Bowman & Lucio-Forster, 2010).
Clinical signs are more severe in immunocompromised individuals or with other underlying conditions (distemper or parvovirus; co-infection with *Giardia* spp., lymphoma, inflammatory bowel disease) (Irwin, 2002; Baker, 2007; Scorza & Tangtrongsup, 2010) in which the infection can become chronic and lead to malabsorption and death (Bowman & Lucio-Forster, 2010).

3.3.3 - Diagnosis

Cryptosporidium infections can be detected by simple or centrifugal faecal flotation using a concentrated sucrose solution (Ballweber, 2001). Here *Cryptosporidium* oocysts appear round-oval, colourless, measuring 5.0 µm x 4.5 µm for *C. parvum* and 5.0 µm x 4.7 µm for *C. canis* (ESCCAP, 2011).
Faecal smear and staining (Heine, safranin, acid-fast staining, such as Ziehl-Neelsen) (ESCCAP, 2011; Bowman, 2014) is the method of choice. Oocysts are easier to visualize and appear small, round and red or orange (ESCCAP, 2011).
Microscopy allows the detection of other concurrent parasitic infections (Bowman & Lucio-Forster, 2010) but it requires a trained technician for detection of infection (Irwin, 2002). Since shedding of oocyst occurs intermittently and dogs shed a relatively low number of oocysts, a single negative result may not be sufficient to eliminate this diagnosis (Scorza & Tangtrongsup, 2010).
There are also several direct IFA assays available for detection of *Cryptosporidium* oocysts that have better sensitivity and specificity, but these require a fluorescence microscope (Scorza & Tangtrongsup, 2010).
ELISAs for the detection of *C. parvum* antigens in humans are available and can be used, with the inconvenient of false negative results, due to other *Cryptosporidium* species, in small animals (Scorza & Tangtrongsup, 2010).
Molecular genetic techniques are the only that determined *Cryptosporidium* species since oocysts are morphologically and antigenically indistinguishable (Bowman & Lucio-Forster, 2010), however, PCR tests are not commercially available (ESCCAP, 2011).

3.3.4 - Treatment and Control

There are no drugs approved for the treatment of cryptosporidiosis in companion animals. Supportive treatment (fluids and antidiarrhoeals) is sufficient since the infection usually resolves spontaneously (ESCCAP, 2011). In dogs, chronic infections have been treated with paramomycin (Baker, 2007; Scorza & Tangtrongsup, 2010). The use of azithromycin and tylosin is also described. Nitazoxanide, a compound used for the treatment of giardiosis and cryptosporidiosis in humans, has also been administered to some small animals (Scorza & Tangtrongsup, 2010).

Control of transmission of the parasite includes strict hygienic measures (including personal hygiene of the animal carers) since *Cryptosporidium* oocysts are highly resistant (ESCCAP, 2011). Separation of dogs with diarrhoea from normal animals should be performed (Scorza & Tangtrongsup, 2010).

3.3.5 - Zoonotic Risk

Humans are infected mostly by *Cryptosporidium hominis* and *Cryptosporidium parvum* (about 90%), with the other *Cryptosporidium* species (including *C. canis*) having a higher prevalence in developing countries. Routes of human infection include: anthroponotic (human-to-human), zoonotic (animal-to-human), foodborne (ingestion of contaminated food) and waterborne (ingestion of contaminated water) (Xiao, 2010). Anthroponotic transmission is the most common route of *Cryptosporidium* infection in developing countries (Bowman & Lucio-Forster, 2010).

C. canis infections do not appear to be a significant source of zoonotic exposure, except in immunocompromised individuals (ESCCAP, 2011). In these people, in addition to ileum infections, also gastric, respiratory and conjunctival infections have been reported (Scorza & Tangtrongsup, 2010).

Potential sources of infections are: swimming pools, contaminated drinking water, travel to lower levels of hygiene places and contact with children. The prevention of the disease by immunocompromised individuals, in addition to the aforementioned preventive care, is made by avoiding direct contact with young animals and with pet faeces (Bowman & Lucio-Forster, 2010). Animal handlers should practice effective hygiene protocols, such as hand washing (ESCCAP, 2011).

3.4 - *Sarcocystis* spp.

3.4.1 - Etiology

Sarcocystis spp. belong to Apicomplexans in the protozoan group, has a worldwide distribution (Ballweber, 2001) and several species are found in the small intestine of dog as definitive host (Table 8) (ESCCAP, 2011).

Table 8 - Some species within *Sarcocystis* spp. that have dogs as definitive hosts (adapted from Ballweber, 2001)

Species	Intermediate Host	Definitive Host
Sarcocystis cruzi	Cattle	Canids
Sarcocystis fayeri	Horse	Dog
Sarcocystis bertrami	Horse	Dog
Sarcocystis tenella	Sheep	Canids
Sarcocystis arieticanis	Sheep	Dog
Sarcocystis gigantea	Sheep	Dog
Sarcocystis capracanis	Goat	Canids
Sarcocystis miescheriana	Pig	Canids

In Portugal, the prevalence of *Sarcocystis* spp. ranges from 0.5% to 2.6% (faecal samples collected from public places and national park) (Maurício *et al.*, 2006; Silva, 2010).

3.4.2 - Life Cycle

The life cycle of *Sarcocystis* spp. is indirect. Sporulated oocysts containing two sporocysts with four sporozoites or free sporocysts are passed in faeces (Ballweber, 2001). The intermediate host ingest these forms, from the pasture or contaminated water or fodder, and sporozoites develop extra-intestinally into tissue cysts (ESCCAP, 2011), which requires 1-2 months (Ballweber, 2001). The definitive host becomes infected when ingesting meat containing tissue cysts. Sexual development takes place in the intestinal epithelium and leads to formation of oocysts that sporulate while still in the intestinal tract. The prepatent period can be from 8 to 33 days in dogs (ESCCAP, 2011).

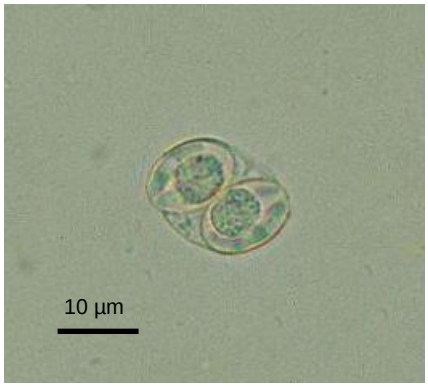
Sarcocystis spp. is generally non-pathogenic in the definitive host (ESCCAP, 2011). After reinfection, dogs develop some degree of species-specific immunity (ESCCAP, 2011). Disease is shown in the intermediate host, mostly in cattle (Ballweber, 2001; Zajac & Conboy, 2012), and clinical signs include fever, anaemia, anorexia, lymphadenopathy, emaciation, hair loss in the tail and abortion (Ballweber, 2001; Bowman, 2014).

3.4.3 - Diagnosis

Sarcocystis infections in the definitive host are usually detected by simple or centrifugal faecal flotation (Zajac & Conboy, 2012). Sporulated oocysts have a dumbbell shape, with thin oocyst wall and measure 15–20 µm × 12–16 µm in size (Figure 8) (Ballweber, 2001) but are rarely seen (Zajac & Conboy, 2012). The oocyst wall usually ruptures during intestinal passage so that the two sporocysts, each with four sporozoites, are released and are the forms usually found in the faeces (ESCCAP, 2011; Bowman, 2014). Sporocysts with smooth, thick, clear cyst wall measure 12–15 µm × 8–10 µm (Ballweber, 2001; Zajac & Conboy, 2012). Infectious forms from the different species are morphologically indistinguishable (ESCCAP, 2011).

In the intermediate hosts, the diagnosis is made based on skeletal or cardiac tissue cyst morphology in histologic section and on molecular methods (Ballweber, 2001; ESCCAP, 2011).

Figure 8 – *Sarcocystis* sp. sporulated oocyst (adapted from Zajac & Conboy, 2012)



3.4.4 - Treatment and Control

Generally domestic animals are not treated for this kind of parasitic infection (ESCCAP, 2011).

Control in intermediate host consists of preventing canine faecal contamination of animal feed and pastures. Control in definitive host consists of freezing (-20 °C for at least 4 days) or cooking meat to be fed to dogs (Ballweber, 2001; ESCCAP, 2011).

3.4.5 - Zoonotic Risk

Humans are not susceptable to *Sarcocystis* spp. involving dogs. Human sarcocystosis occurs when infected beef or pork are ingested (ESCCAP, 2011).

IV – Gastrointestinal parasite risk in dog parks in the Lisbon area

1 - Objectives

The goals of this study were:

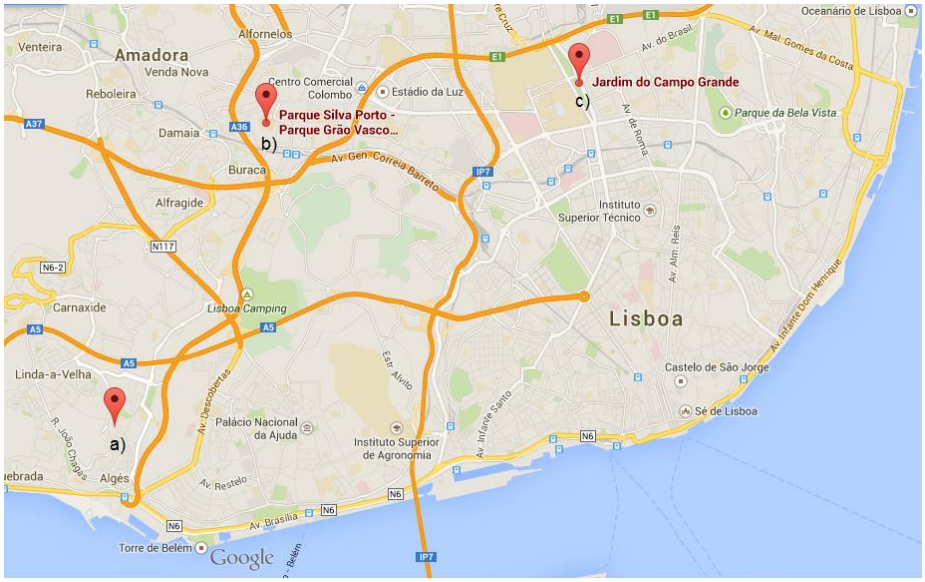
a) Assessment of parasitic population of urban dogs attending canine parks.
b) Association of soil parasite contamination of these same places, with data obtained from faecal samples, into a complete environmental contamination assessment.
c) Recognition of the behaviour and risk factors for owners and park-attending dogs, taking into account the identification and characterization of the pet, the walking to the dog-attending park, the veterinary care and the owner-pet relationship.
d) Correlation of data obtained regarding Animal Health and Public Health within the concept One Health, since the places studied are prone to transmission of zoonotic agents among animals and between animals and humans.

2 - Material and Methods

2.1 - Sampling Places

For this project, three dog-attending parks were chosen to be studied, one in Oeiras (Algés) and two in Lisbon (Benfica and Campo Grande) (Figure 9). Benfica and Campo Grande are the only canine parks present in Lisbon and Algés was chosen because of its proximity to this county. Each park is going to be described, subsequently, according to data from each civil parish.

Figure 9 – Map with the locations of the analysed dog parks: a) Algés; b) Benfica; c) Campo Grande (Google maps)



2.1.1 - Dog park of Algés

This dog park (Figure 10), opened June 18th 2012, is located in Algés, Oeiras, in a residential area, Quinta da Formiga, and has an area of 350 m^2.

Algés belongs to the civil parish named União das Freguesias de Algés, Linda-a-Velha e Cruz Quebrada-Dafundo, with 48 665 inhabitants and 1693 dogs licensed.

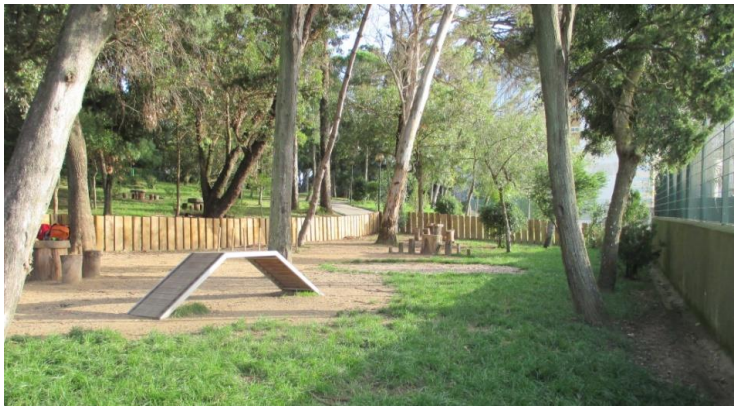
Figure 10 – Dog park of Algés (courtesy of Luís Carvalho)

2.1.2 - Dog park of Benfica

Silva Porto Park (also known as Mata de Benfica) is a public park, in Benfica, Lisbon, with 5 hectares where the dog park (Benficanino) is located within (Figure 11). This dog park, opened September 13th 2013, and has an area of 465 m^2.

Benfica is a civil parish located in Lisbon, with 36 821 inhabitants and there are about 400 dogs licensed.

Figure 11 – Dog park Benficanino (original)



2.1.3 - Dog park of Campo Grande

Campo Grande Park is a public park, in Campo Grande, Lisbon, with 11.1 hectares, which is divided in two zones, the north zone with 6 hectares and the south zone with 5 hectares. In the north zone it is located the dog park (Figure 12). It was opened November 2014 and has an area of 1120 m^2.

Campo Grande belongs to the civil parish of Alvalade, with 31 813 inhabitants and about 1700 dogs licensed.

Figure 12 – Dog park of Campo Grande (original)

Features of the three dog-attending parks studied are listed in Table 9.

Table 9 – Features of the three dog-attending parks

	Algés	**Benfica**	**Campo Grande**
Area	350 m^2	465 m^2	1120 m^2
Fence	106 cm high	100 cm high	122 cm high
Double-gated entry	Yes	Yes	Yes
Agility equipment	Little	Yes	Yes
Shade	No	Yes	Yes
Water source	No	Only for dogs	For both dogs and owners
Benches	Yes	Yes	Yes
Covered garbage cans	Yes	Yes	Yes
Waste bags	No	No	No
Rules and requirements	No	Yes	Yes

Although all three dog-attending parks are fenced and have a double gate entry, sometimes this was left open and stray dogs could enter the space. No control to prevent the presence of stray cats was available.

2.2 - Samples

The samples (both faecal and soil samples) were collected from October 6th to December 12th 2014. Faecal samples were collected once every 15 days and soil samples once a month. The weather conditions experienced up to 5 days before the sampling day are summarized in Table 10. The questionnaires were filled in the same period of time.

Table 10 – Weather conditions up to 5 days before the sampling day for the Lisbon area (data obtained from Weather Underground, 2015)

Sampling day	Samples collected	Average temperature	Average precipitation	Average humidity
1st (October 1st fortnight)	Faecal Soil	16-24 ºC	0.19 mm	57.9%
2nd (October 2nd fortnight)	Faecal	14-21 ºC	0.41 mm	67.3%
3rd (November 1st fortnight)	Faecal Soil	13-20 ºC	0.40 mm	64.0%
4th (November 2nd fortnight)	Faecal	10-17 ºC	0.35 mm	71.8%
5th (December 1st fortnight)	Faecal Soil	9-16 ºC	0.30 mm	71.7%
6th (December 2nd fortnight)	Faecal	8-14 ºC	0.28 mm	67.7%

2.2.1 - Faecal samples

In total, 369 faecal samples were collected from the three dog-attending parks. The distribution of the number of samples is shown in Table 11. Each visit, they were collected between 20 to 25 faecal samples distributed randomly throughout the parks.

Table 11 - Distribution of the number of faecal samples through the different dog-attending parks

Dog-attending park	Number of samples
Algés	125
Benfica	124
Campo Grande	120
Total	369

Fresh faecal samples found in the parks in the visited period were collected for this study. When, at the time of visit, faecal samples present in the fenced dog park were scarce (due to, for example, cleaning by municipal services), faecal samples present in the surroundings of the parks were collected instead. Each sample, large enough to perform the intended analyses, was collected in an individual plastic bag identified according to the place and date and numbered for later identification. Samples were chilled in cooler bag until arrival at the laboratory where they were stored in a refrigerator (at 4ºC) and examined within two weeks.

2.2.2 - Soil samples

In total, 18 soil samples were collected from the three dog-attending parks, 6 samples from each park.

Two samples were collected, each month, per park. One included, soil samples from three different places with grass (places where there are evidences that dogs use it frequently, for example, scratched soil, a pole nearly by, or a place where there is collection of faecal samples), in a total of approximately 250 grams, with a gardening spade, to a 0-5 cm depth (evidence of more helminthic egg occurrence). The other included, soil samples from two different places with gravel (places where there are evidences that dogs use it frequently), in a total of approximately 250 g, with a gardening spade, to a 0-5 cm depth. Every sample was collected in an individual plastic bag identified according to the place and date and numbered for later identification. Samples were chilled in cooler bag until arrival at the laboratory where they were stored in a refrigerator (at 4ºC) and examined within two weeks.

The places that were chosen for sampling were the same as the ones in the previous month.

2.2.3 - Questionnaires

2.2.3.1 - Survey Design

In order to achieve one of the goals proposed, a survey was carried out for dog owners walking their pet in one of the three dog-attending parks.

For the development of the questions, the survey design guidelines of Dohoo, Martin, & Stryhn (2003), and the works of Overgaauw *et al.* (2009), Matos (2013), and Smith, Semeniuk, Kutz, & Massolo (2014) were consulted.

One of the considerations for the design of the survey was that it did not require much time of the dog owners so the number of answered surveys would be the higher possible. The majority of the questions are closed. Open questions were left to answers where there could be a wide variety of possibilities (such as age, breed, when there was the possibility of other answers than the stated ones, other visited parks, or when specification of drug active

ingredients was requested). Breeds were denominated according to the *Fédération Cynologique Internationale*. The Portuguese language is used as spoken by the everyday people. Questions are direct and easy to understand. The questionnaire is to be exposed by the interviewer and completed in accordance with the responses of the owners.

2.2.3.2 - Survey Pre-testing

A panel composed by a veterinarian, two parasitologists, and an epidemiologist evaluated the survey to ensure that all important subjects were covered. After that, a pre-test was developed. For the pre-test, 10 questionnaires were performed to dog owners that attend canine parks. The purpose of this pre-test was to evaluate the perceptiveness of the questions, to train the interviewer regarding the language and its consistency, and the duration of the survey. Appropriated modifications were made.

2.2.3.3 - Survey Test and Validation

Surveys used in the pre-test were already approaching the final version of the survey, presented in Annex 3. Three people reviewed the final survey. One with veterinary education and two non-veterinarians. No changes were considered as necessary.
The questionnaire consisted of 31 questions arranged within five sections: identification and characterization of the pet, the walking to the dog-attending park, the veterinary care and the owner-pet relationship.

2.2.3.4 - Application

From October to December 2014, dog owners with their animals present at the three studied dog-attending parks were approached opportunistically and asked to participate in a questionnaire. A total of 102 surveys were conducted; an average of 34 per park. Each survey corresponds to a different animal. 93 different owners answered the questionnaires, since some of them had more than one animal present with them.
The interviews took place both on weekdays and weekends, throughout the day (mornings, afternoons and evenings), taking each survey less than 5 minutes to be answered.

2.3 - Laboratory techniques used in the analysis of samples

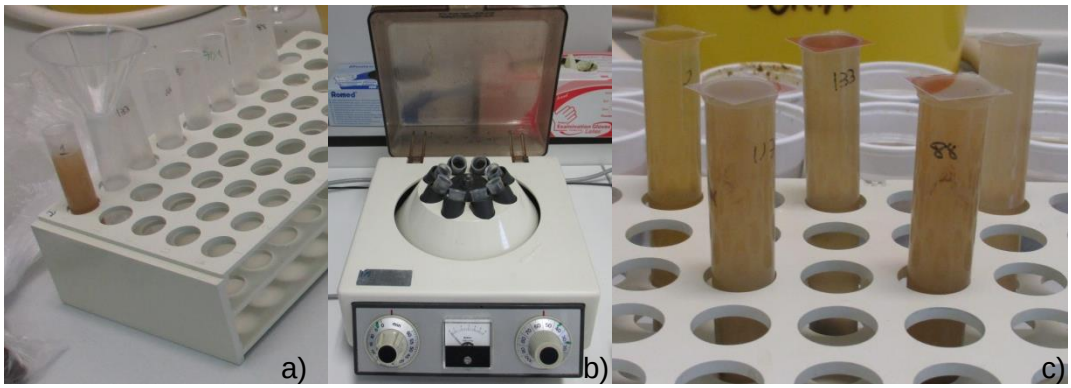
2.3.1 - Macroscopic examination

Each faecal sample was macroscopically observed for adult parasites or proglottids of tapeworms and qualitative analyses were performed. Consistency, colour and presence or absence of mucous were recorded. Consistency of the samples was characterized into one of four groups: shaped, semi soft, soft and liquid stools.
Also, part of each faecal sample was placed into 1.0 ml Eppendorf tubes to be frozen for future analysis.

2.3.2 - Centrifugal Sedimentation/Flotation (CSF) technique for faecal samples

A modified version of the technique used by Dryden, Payne, Ridley, & Smith, (2005) was used. For each faecal sample, 3-5 g of faeces were homogenised in approximately 55 ml of water with a stirring rod and then were filtrated, using a strainer and a funnel, into a centrifuge tube (filling up to approximately 0.5 cm from the top) (Figure 13a). The tubes were centrifuged for 3 minutes at 3000 rpm (Figure 13b) and the supernatant was discarded after. A third of the tube was filled with 25% sucrose solution and then vortexed, filling another third of the tube with more sucrose solution afterwards, and centrifuged again for 3 minutes at 3000 rpm. The tubes were placed in a tube rack and filled with sucrose solution until creating a convex meniscus. A cover slide was placed immediately on top of the tubes (Figure 13c), which was taken off after a minimum period of 25 minutes, and placed on a slide for observation under an optical microscope at 100x-400x magnification.

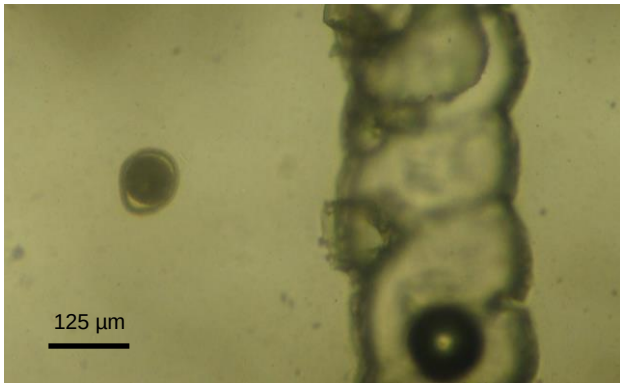
Figure 13 – CSF technique: a) filling of the centrifuge tube with the homogenised sample; b) centrifuge; c) cover slides on top of the tubes (original)



2.3.3 - McMaster counting technique

The McMaster counting technique, proposed by Thienpont, Rochette, & Vanparijs (1986), a quantitative analysis, was performed only on CSF-positive samples. Two grams of faeces were weighed, added to 28 ml of 25% sucrose solution and stirred. The faecal suspension was filtered through a strainer and both compartments of the McMaster counting chamber were filled. The counting chamber was allowed to stand for 5 minutes and then was examined under the compound microscope at 100x magnification, focusing on the grids (Figure 14). Every egg within the engraved area of both chambers was counted and multiplied by 50 for the total eggs per gram of faeces (epg).

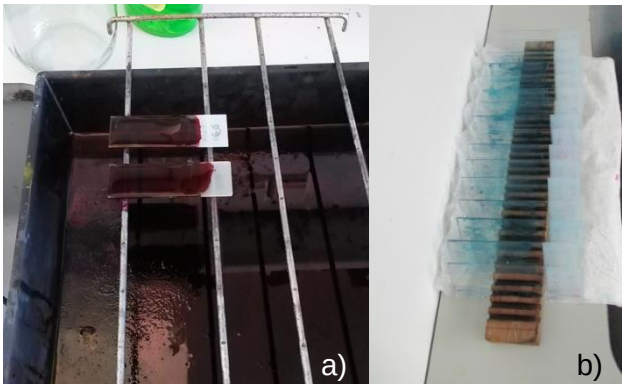
Figure 14 – *Toxocara* sp. egg in a McMaster chamber (original)



2.3.4 - Faecal smear

A faecal smear, stained by the modified Ziehl-Neelsen technique, a variation of that proposed by Casemore, Armstrong, & Sands (1985), was performed for each sample. A small amount of faeces was spread over a slide, using a stirring rod, to form a thin layer. After letting it dry, all smears were fixed with methanol for 1 minute. Thereafter, the slides were covered with fuchsine (Figure 15a) for 10 minutes and washed off with running water. They were subsequently washed with 1% hydrochloric alcohol to remove excess fuchsine, and washed again with running water. Subsequently, the slides were covered with 0.4% malachite green for 30 seconds and washed again with running water and, finally left to air drying (Figure 15b). Faecal smears were made within two weeks from sampling day, however, their observation was only made within two months. The smears were observed at 1000x magnification, using oil immersion, for the detection of *Giardia* sp. cysts and *Cryptosporidium* spp. oocysts. In each slide a minimum of 50 fields were observed.

Figure 15 – Faecal smears: a) covered with fuchsine; b) air drying (original)



2.3.5 - Sieving and Centrifugal Sedimentation/Flotation (CSF) technique for soil samples

All the soil samples were mixed thoroughly when collected and were analysed using the Sieving and Centrifugal Sedimentation/Flotation technique, a modified process from the one proposed by Santarém, Magoti, & Sichieri (2009), also used by Otero *et al.* (2014). From each soil sample, 100 g were weighted into a plastic bag, equal volume of Tween 20 at 5% was added and everything was mixed thoroughly for 10 minutes. The samples were allowed to stand overnight. One Cisa Cedacería Industrial™ and five RETSCH™ sieves (sizes 1.000 mm, 0.500 mm, 0.250 mm, 0.125 mm, 0.063 mm and 0.020 mm) (Figure 16a) were assembled in decreasing order. The first sieves served to retain the not required soil constituents, while the latter two had a pore diameter slightly less than the width of the searched nematode eggs. The samples were sieved with the help of running water. When the contents of the sieve above were washed well, the upper sieve was removed and the work continued on the next sieve. Everything was repeated for the next sieves until the last two (0.063 mm and 0.020 mm). The remaining soil in these sieves was put in a sedimentation glass and was filled completely with water (Figure 16b). A drop of detergent was put on the top of the sedimentation glass and stir gently, so the eggs that may be attached to the walls of the glass get free. After allowing it to stand for 12 hours, the supernatant was discarded. With a Pasteur pipette, the superficial sediment was collected until fill a quarter of a centrifuge tube, the rest of the tube was filled with water until a third of the top. The tubes were centrifuged for 3 minutes at 3000 rpm and the supernatant was discarded after. A third of the tube was filled with 25% sucrose solution and then vortexed. Another third of the tube was filled with more sucrose solution and centrifuged again for 3 minutes at 3000 rpm. The tubes were placed in a tube rack and filled with sucrose solution until creating a convex meniscus. A cover slide was placed immediately on top of the tubes, which was taken off after a minimum period of 25 minutes, and placed on a slide for observation under an optical microscope at 100x-400x magnification.

Figure 16 - Sieving and CSF technique: a) sieves used; b) sedimentation glasses with the sieved samples (original)

Several eggs were measured using optical microscope Olympus DP10, BX50F model, with a metric ocular.

2.4 - Data Analysis

Results were recorded in a Microsoft Excel 2010® spread sheet and statistically analysed using R program, version 3.1.3 (R Core Team, 2014), using the extension R Commander.
For the categorical variables, absolute and relative frequencies and confidence intervals of 95% were formulated using VassarStats (2015). Cohen's kappa coefficient was also calculated using the same website. Pearson's chi-squared test (χ^2) and Fisher's exact test (FET) were used to evaluate the association between categorical and binominal variables.
Shapiro-Wilk normality test was performed regarding all the quantitative variables. The number of epg suffered a logarithmic transformation for a parametric data analysis, namely using analysis of variance (ANOVA) to assess if significant differences were present. The 5% significance level was used.

3 - Results

3.1 - Faecal Samples

3.1.1 - Macroscopic examination

Macroscopic observation of faeces did not allow the identification of adult parasites or proglottids of tapeworms in any of the samples (n=369).
Detailed characterization of qualitative analyses for consistency, colour and mucous of samples from each park is presented in Table 24 (Annex 4).

3.1.2 - Parasitic population

Overall, 33.1% (122/369) of faecal samples had at least one parasitic element. Algés, Benfica and Campo Grande dog-attending parks showed positive results in 35.2% (44/125), 31.5% (39/124) and 32.5% (39/120), respectively.
No cestode egg was found in the samples. Among the nematodes, the family Ancylostomatidae is noteworthy (16.5%, 61/369). *Toxascaris leonina* had 1.1% (4/369) and *Toxocara* spp. only 0.5% (2/369). Regarding protozoa, *Cryptosporidium* spp. (11.9%, 44/369) and *Giardia* sp. (11.4%, 42/369) are the most prevalent. *Cystoisospora* spp. corresponds to 1.1% (4/369) and *Sarcocystis* spp. had the lowest prevalence with only one positive sample (0.3%).
The overall prevalence for protozoa was 23.6% (87/369) and for nematode was 16.8% (62/369).

In Table 12 are represented the absolute frequency and its prevalence of the distribution of each parasite specie/genus/family according to each dog-attending park and in total, with a confidence interval of 95% (CI 95%).

Concerning the comparison of the prevalence distribution of parasites by the three canine parks, it is worth mentioning *Toxascaris leonina*, which had significant difference (p<0.05).

Table 12 – Parasite absolute frequency and its prevalence per park - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=125)	Benfica (n=124)	Campo Grande (n=120)	Total (n=369)
Ancylostomatidae	18 14.4% (9.0-22.1%)	23 18.5% (12.4-26.7%)	20 16.7% (10.7-24.8%)	61 16.5% (13.0-20.8%)
Cryptosporidium spp.	15 12.0% (7.1-19.3%)	19 15.3% (9.7-23.2%)	10 8.3% (4.3-15.2%)	44 11.9% (8.9-15.8%)
Giardia sp.	20 16.0% (10.3-23.9%)	8 6.5% (3.0-12.7%)	14 11.7% (6.8-19.1%)	42 11.4% (8.4-15.2%)
Cystoisospora spp.	1 0.8% (0.0-5.0%)	2 1.6% (0.3-6.3%)	1 0.8% (0.0-5.2%)	4 1.1% (0.4-2.9%)
Toxascaris leonina	-	-	4 3.3% (1.1-8.8%)	4 1.1% (0.4-2.9%)
Toxocara spp.	1 0.8% (0.0-5.0%)	1 0.8% (0.0-5.1%)	-	2 0.5% (0.1-2.2%)
Sarcocystis spp.	1 0.8% (0.0-5.0%)	-	-	1 0.3% (0.0-1.7%)
Total of positive samples	44 35.2% (27.0-44.3%)	39 31.5% (23.6-40.5%)	39 32.5% (24.4-41.7%)	122 33.1% (28.3-38.2%)

Eggs and (oo)cysts found in this work, by the CSF and modified Ziehl-Neelsen stain techniques are showed from Figure 17 to Figure 22.

Figure 17 – Ancylostomatidae eggs: a) unembryonated; b) embryonated (originals)

In the samples that were Ancylostomatidae positive, unembryonated and/or embryonated, eggs were found. Some of these eggs fit in the size for *Uncinaria stenocephala* (e.g. 47.5 x 85 µm), others fit in the size for *Ancylostoma caninum* (e.g. 25 x 70 µm).

Figure 18 – *Cryptosporidium* spp. oocysts in faecal smear (original)

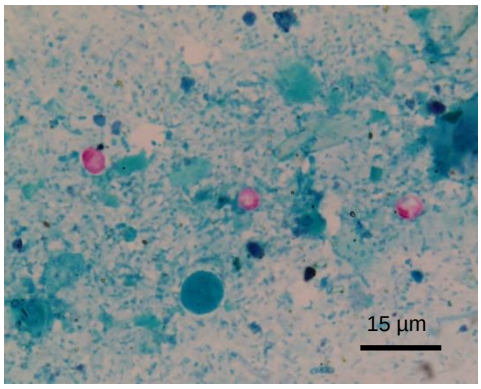


Figure 19 – *Giardia* sp. cysts in faecal smear (original)

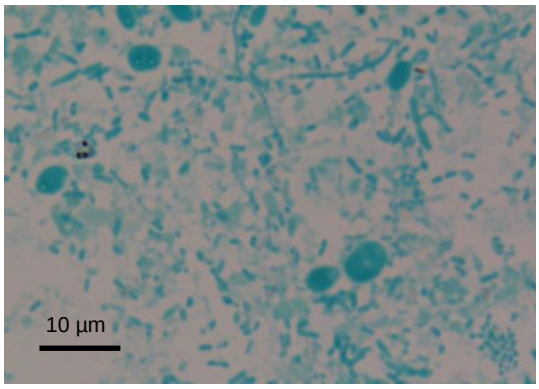
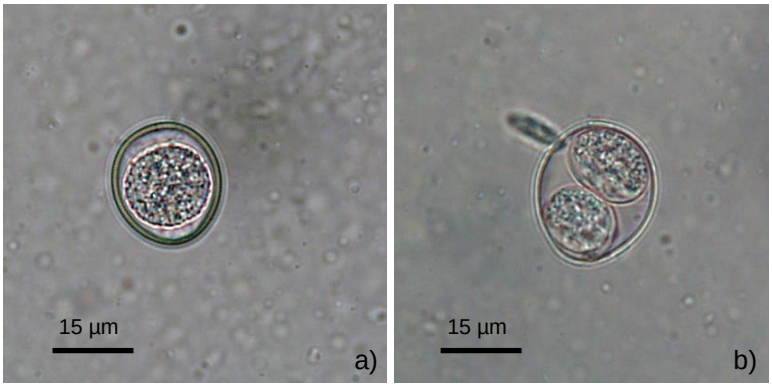


Figure 20 – *Cystoisospora* spp. oocysts: a) unsporulated; b) sporulated (originals)



Positive *Cystoisospora* spp. samples had unsporulated and/or sporulated oocysts. The sizes from all samples are consistent with species from the *C. ohioensis*-complex.

Figure 21 – Unembryonated *Toxascaris leonina* egg (original)

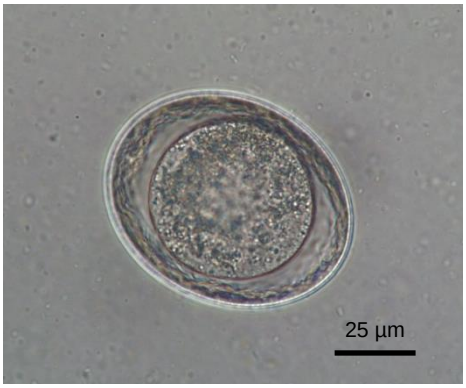
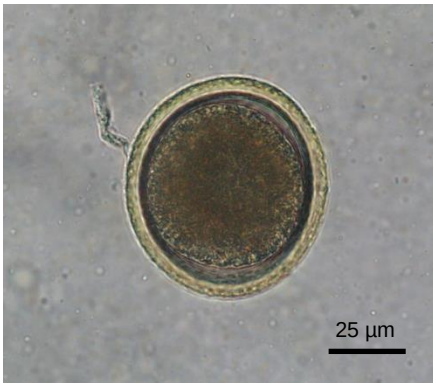


Figure 22 - Unembryonated *Toxocara* sp. egg (original)



All *Toxascaris leonina* eggs were unembryonated. Considering *Toxocara* spp., both samples contained unembryonated eggs that were smaller than the average size for *T. canis* eggs. Only sporocysts from *Sarcocystis* spp. were found.

3.1.3 - Parasitic associations

Using the two qualitative laboratory techniques, samples tested positive for more than one type of egg/(oo)cyst in 9.2% (34/369) (Table 13), mainly due to the association of Ancylostomatidae and *Cryptosporidium* spp. (6.2%, 23/369). *Toxascaris leonina* was always associated with Ancylostomatidae, one sample had only *Toxacara* spp. eggs, and *Cystoisospora* spp. was always solely present.

Table 13 – Number and its prevalence of different type of eggs/(oo)cysts per park - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=125)	Benfica (n=124)	Campo Grande (n=120)	Total (n=369)
One type of egg/(oo)cyst	34 27.2% (19.8-36.0%)	25 20.2% (13.7-28.5%)	29 24.2% (17.0-33.0%)	88 23.9% (19.7-28.6%)
More than one type of egg/(oo)cyst	10 8.0% (4.1-14.6%)	14 11.3% (6.5-18.5%)	10 8.3% (4.3-15.2%)	34 9.2% (6.6-12.8%)

3.1.4 - Quantitative estimate of epg

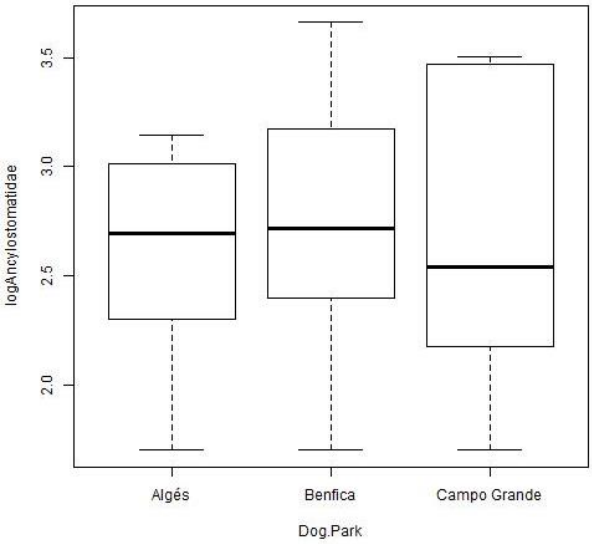
Using the McMaster counting technique, only Ancylostomatidae, *Toxocara* spp. and *Toxascaris leonina* eggs were found. From 61 samples with Ancylostomatidae eggs to CSF technique, only 36 had countable eggs. One *Toxocara* spp. positive and all four samples with *Toxascaris leonina* had countable eggs.

Table 14 displays the average intensities and amplitude, for each park, of Ancylostomatidae epg and Graphic 1 shows the bloxplot of the logarithmic transformation of the same family. No differences were significant when comparing the dog park with the Ancylostomatidae epg.

Table 14 – Average intensities with standard deviation and amplitude of Ancylostomatidae infection, per park and in sum of all parks, expressed in number of epg

	Average intensity (epg)	Amplitude (epg)
Algés (n=8)	625.0±509.2	50-1400
Benfica (n=18)	1113.9±1308.7	50-4600
Campo Grande (n=10)	1140.0±1383.4	50-3200
Total (n=36)	1012.5±1191.7	50-4600

Graphic 1 – Boxplot of the logarithmic transformation of Ancylostomatidae epg within the three dog-attending parks

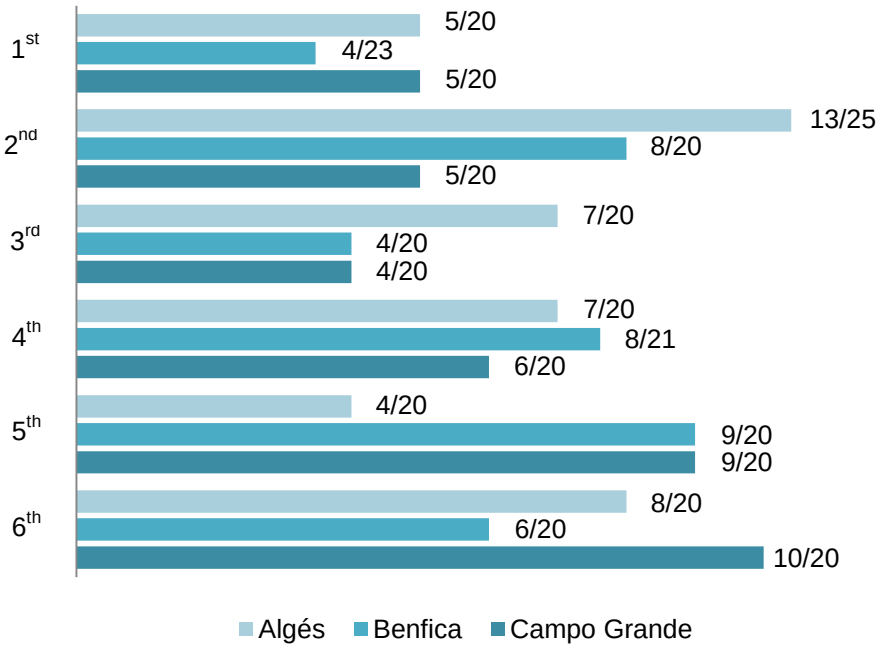


The only one sample, from Benfica dog park, that presented *Toxocara* spp. had 50 epg. The samples with *Toxascaris leonina* eggs, all from Campo Grande, had an average intensity of 187.5 ± 213.6 epg and amplitude ranging from 50 to 500 epg.
No other significant differences were found.

3.1.5 - Parasitic relationship with sampling day

The prevalence of positive samples, for each dog park, according to the sampling day is shown on Graphic 2.

Graphic 2 – Distribution of positive samples, per park, according to the sampling day



Sampling days: 1st (October 1st fortnight), 2nd (October 2nd fortnight), 3rd (November 1st fortnight), 4th (November 2nd fortnight), 5th (December 1st fortnight), 6th (December 2nd fortnight)

The distribution of each parasite's positivity according to the sampling day is displayed on Table 15.

Table 15 - Distribution of each parasite positivity according to the sampling day (CI95%)

	1st (n=63)	2nd (n=65)	3rd (n=60)	4th (n=61)	5th (n=60)	6th (n=60)
Ancylostomatidae	1 1.6% (0.1-9.7%)	8 12.3% (5.8-23.4%)	4 6.7% (2.2-17.0%)	18 29.5% (18.9-42.7%)	20 33.3% (22.0-46.8%)	10 16.7% (8.7-29.0%)
Cryptosporidium spp.	6 9.5% (3.9-20.2%)	8 12.3% (5.8-23.4%)	3 5.0% (1.3-14.8%)	15 24.6% (14.9-37.6%)	9 15.0% (7.5-27.1%)	3 5.0% (1.3-14.8%)
Giardia sp.	9 14.3% (7.1-25.9%)	12 18.5% (10.3-30.4%)	8 13.3% (6.3-25.1%)	1 1.6% (0.1-10.0%)	4 6.7% (2.2-17.0%)	8 13.3% (6.3-25.1%)
Cystoisospora spp.	-	1 1.5% (0.1-9.4%)	-	-	-	3 5.0% (1.3-14.8%)
Toxascaris leonina	-	-	-	-	-	4 6.7% (2.2-17.0%)
Toxocara spp.	-	-	-	1 1.6% (0.1-10.0%)	-	1 1.7% (0.1-10.1%)
Sarcocystis spp.	-	1 1.5% (0.1-9.4%)	-	-	-	-

Concerning the sampling day, significant differences are present regarding *Giardia* sp. (p<0.05), Ancylostomatidae (p<0.001), *Cryptosporidium* spp. (p<0.05), and *Toxascaris leonina* (p<0.01).

3.1.6 - Parasitic relationship with qualitative macroscopic examination of faecal samples

The prevalence of *Giardia* sp. in shaped stools was 7.7% (21/271), 27.5% (11/40) in semi soft stools, and 17.9% (10/56) in soft stools.

Regarding faecal colour, *Giardia* sp. was present in 2 of 3 samples of greenish brown, 14.3% (1/7) of coppery brown, 14.0% (8/57) of light brown, 9.1% (25/274) of brown and 21.4% (6/28) of dark brown stools.

There are significant differences when relating *Giardia* sp. with consistency (p<0.01), and colour (p<0.05) of the faecal sample.

3.2 - Soil Samples

Overall, 27.8% (5/18) of soil samples were contaminated with unembryonated and/or embryonated Ancylostomatidae eggs (Figure 23) (Table 16). One sample from Benfica e another from Campo Grande had also the presence of *Heterakis* spp. eggs (Figure 24) (11.1%).

Table 16 – Ancylostomatidae prevalence in soil samples per park - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=6)	Benfica (n=6)	Campo Grande (n=6)	Total (n=18)
Ancylostomatidae	2 33.3% (6.0-75.9%)	1 16.7% (0.9-63.5%)	2 33.3% (6.0-75.9%)	5 27.8% (10.7-53.6%)

Figure 23 – Embryonated Ancylostomatidae eggs found in soil samples (original)

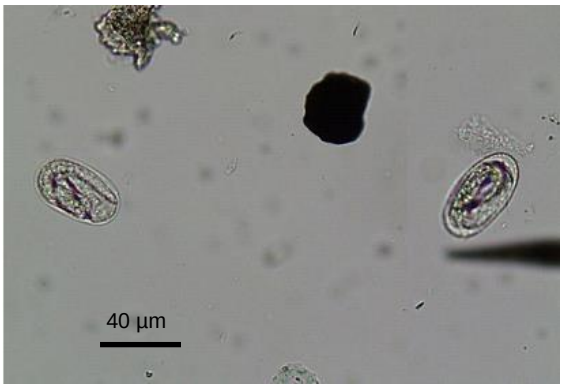
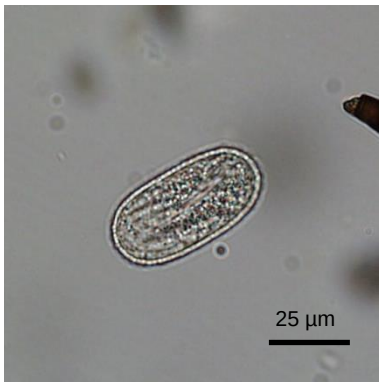


Figure 24 – *Heterakis* sp. egg found in soil samples (original)



The type of analysed sample (grass or gravel) has influence in their positivity (p<0.05), with grass samples having all the eggs found. The sampling day did not have significant differences regarding the presence of Ancylostomatidae eggs.

Ancylostomatidae eggs were found both on the 0.063 mm and 0.020 mm sieves. Three samples were only positive regarding the 0.020 mm sieve and the other two samples were positive in both sieves. Cohen's kappa coefficient (κ=0.49) was used to determinate the agreement within the sieves. Moderate agreement (according to Landies & Koch, 1977) or good agreement (according to Fleiss, 1981) is present. *Heterakis* spp. eggs were only found in the 0.020 mm sieve, due to their small size.

3.3 - Questionnaires

A total of 102 surveys were conducted; with 34 per park, each of which corresponding to a different animal.

3.3.1 - Identification of the pet

The distribution of animals according to the gender and the neutering state is showed in Table 17. The sample comprises similar proportion of neutering state in females but a higher percentage of non-castrated males, in relation to castrated ones.

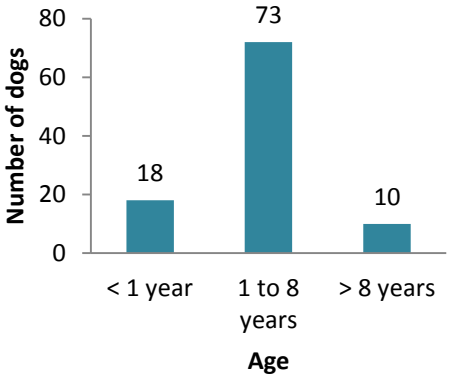
Table 17 - Distribution of dogs according to the gender and the neutering state (n=102) (CI95%)

	Castrated	Non-castrated	Total
Female	23 22.6% (15.1-32.1%)	21 20.6% (13.5-30.0%)	45* 44.1% (34.4-54.3%)
Male	7 6.9% (3.0-14.1%)	50 49.0% (39.1-59.1%)	57 55.9% (45.7-65.6%)
Total	30 29.4% (21.0-39.4%)	71 69.6% (59.6-78.1%)	

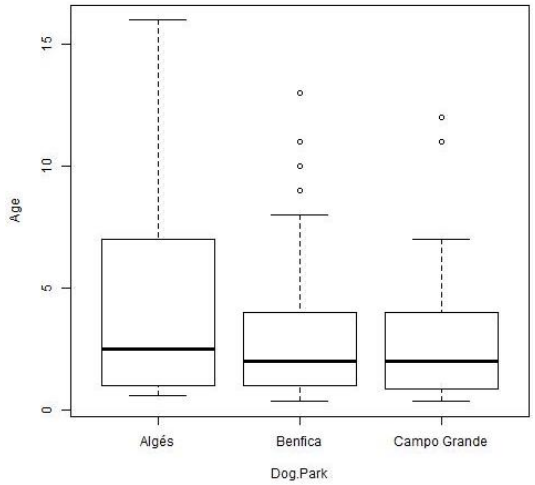
* one female had no data about neutering state

Dogs were arranged in three age categories (Graphic 3): young dogs aged less than one year; adult dogs aged between one and eight years; and old dogs aged more than eight years. The overall average age is 3.5 ± 3.5 years (from 4 months to 16 years). Graphic 4 shows the distribution of ages for each park.

Graphic 3 - Distribution of dogs by age group (n=101)



Graphic 4 - Distribution of ages for each park (n=101)



The distribution of animals according to the breed is showed in Table 18. The majority of the surveys were from undetermined breed dogs (47.1%, 48/102). Twenty-six different pure breeds, of which three Portuguese, were found, with the most prevalent being Beagle and Labrador Retriever (each with 9.8%, 10/102).

Table 18 - Distribution of dogs according to the breed (n=102)

Breed	Absolute frequency	Relative frequency	Breed	Absolute frequency	Relative frequency
Undetermined	48	47.1%	Castro Laboreiro Dog	1	1.0%
Beagle	10	9.8%	German Shepherd Dog	1	1.0%
Labrador Retriever	10	9.8%	German Spitz	1	1.0%
Dalmatian	3	2.9%	Golden Retriever	1	1.0%
Jack Russell Terrier	3	2.9%	Great Dane	1	1.0%
Rhodesian Ridgeback	3	2.9%	Greyhound	1	1.0%
Chow Chow	2	2.0%	Poodle	1	1.0%
Dachshund	2	2.0%	Pug	1	1.0%
English Cocker Spaniel	2	2.0%	Saint Miguel Cattle Dog	1	1.0%
French Bulldog	2	2.0%	Shih Tzu	1	1.0%
Portuguese Podengo	2	2.0%	West Highland White Terrier	1	1.0%
Border Collie	1	1.0%	White Swiss Shepherd Dog	1	1.0%
Boxer	1	1.0%	Yorkshire Terrier	1	1.0%

3.3.2 - Characterization of the pet

Regarding co-habitants, 59.8% (61/102) of the dogs did not live with other animal; 25.5% (26/102) lived with at least a dog (from 1 to 4 dogs); 15.7% (16/102) with at least a cat (from 1 to 4 cats) and 8.8% (9/102) with other kind of animal (birds, rabbit or guinea pig). Four animals lived with both dogs and cats; six pets lived with both dogs and birds; one animal lived only with a rabbit and another one with only a guinea pig.

Commercial food gathered 99.0% (101/102) of the nutrition and 20.6% (21/102) of people also feed their animals with cooked homemade food. 1.0% (1/102) was fed with raw food.

75.5% (77/102) of the pets spend most of the day indoors, 17.6% (18/102) outdoors, and 6.9% (7/102) both.

All dogs that have contact with animals outdoors (89.2%, 91/102), have contact with other dogs, 5.9% (6/102) also have contact with cats and 1.0% (1/102) have contact with cows.

Regarding daily walking, 64.7% (66/102) do it both on the streets and in parks, and 1.0% (1/102) also use an area of open ground. 17.6% (18/102) only walks on streets and 16.7 (17/102) only walks on parks.

3.3.3 - Characterization of walking to the dog-attending park

About the walks to the dog-attending park, 50.0% (51/102) of the dogs visit the park daily and 20.6% (21/102) did not attend the park on a regular basis (Table 19).

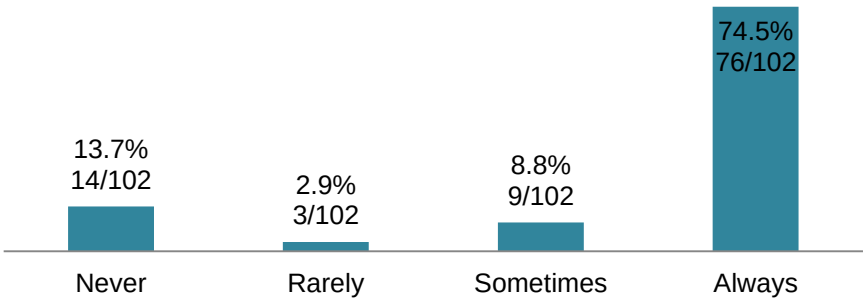
Table 19 - Distribution of dogs according to the frequency of walking to each park - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=34)	Benfica (n=34)	Campo Grande (n=34)	Total (n=102)
Rarely 0-3x/year	-	-	10 29.4% (15.7-47.7%)	10 9.8% (5.1-17.7%)
Occasionally <1x/month	-	1 2.9% (0.2-17.1%)	1 2.9% (0.2-17.1%)	2 2.0% (0.3-7.6%)
Infrequently 1-3 days/month	1 2.9% (0.2-17.1%)	3 8.8% (2.3-24.8%)	5 14.7% (5.6-31.8%)	9 8.8% (4.4-16.5%)
Regularly 1x/week	6 17.6% (7.4-35.2%)	3 8.8% (2.3-24.8%)	3 8.8% (2.3-24.8%)	12 11.8% (6.5-20.0%)
Often 2-6 days/week	3 8.8% (2.3-24.8%)	11 32.4% (18.0-50.6%)	4 11.8% (3.8-28.4%)	18 17.6% (11.1-26.7%)
Everyday 1x/day	24 70.6% (52.3-84.3%)	16 47.1% (30.2-64.6%)	11 32.4% (18.0-50.6%)	51 50.0% (40.0-60.0%)

In 60.8% (62/102) of the surveys, dogs attended that park both on weekdays and weekends; 22.6% (23/102) only on weekends and 16.7% (17/102) only on weekdays.

On-leash walking was present in 17.6% (18/102) of the inquired, off-leash in 57.8% (59/102) and both on and off-leash walking was performed on 24.5% (25/102) of dogs.
Dogs are allowed to always have off-leash time in 74.5% (76/102), and 11.8% (12/102) may have an off-leash period of time (Graphic 5).

Graphic 5 - Distribution of frequency of off-leash dog



94.1% (96/102) of the owners claimed faecal collection of their pets, however, 9.4% (9/96) of these admitted not to always do it.

Of the inquired people, 41.2% (42/102) visited other parks with their dog. Although in the questionnaires it was asked for other canine parks, owners have enumerated all public parks they attend with their dog. Table 20 shows the distribution of dogs according to other parks visited, by each park.

Table 20 - Distribution of dogs according to other parks visited, by each park studied - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=34)	Benfica (n=34)	Campo Grande (n=34)	Total (n=102)
Other parks visited	8 23.5% (9.3-37.8%)	18 52.9% (36.1-69.7%)	16 47.1% (30.3-63.9%)	42 41.2% (31.7-51.4%)

There was a significant difference (p<0.05) between the studied dog park and visits to other parks.

Other parks visited, common to the three dog-attending parks studied, are: Monsanto (with one answer in Algés, nine in Benfica and three in Campo Grande), Campo Grande (with one answer in Algés and two in Benfica), both in Lisbon, and Parque da Paz, in Almada (with one in Benfica and another in Campo Grande).

The dog park in Algés, had more regular attendants, with only four other parks being visited by them. Four questionnaires referred Miraflores, three Linda-a-Velha, both in Oeiras, one in Alcântara, Lisbon and another one in Loures.

Regarding Benfica's dog park, other parks visited include: Parque das Nações, Mercado de Benfica, both in Lisbon, and Venda Nova, in Amadora, each with two positive answers and Parque Eduardo VII, in Lisbon, and Brandoa, in Almada, with one questionnaire each.

People that attend the canine park in Campo Grande, frequent a bigger range of other parks. Three dogs attended Parque das Conchas in Lisbon. With two answers each: Avenida de Roma, Belém and Parque de Alvalade, all in Lisbon. Benfica, Campo Pequeno, Campolide, Estrela, Príncipe Real, Telheiras, Vale Grande, all in Lisbon; Caparica in Almada; Arco do Cego, in Odivelas; Amadora, Sintra, Cascais, and Barreiro, gathered one questionnaire each.

The studied park was visited more often than the other parks in 71.4% (30/42) of cases. Frequency of walking of those who visited other parks is described on Table 21.

Table 21 – Distribution of frequency of walking to other parks, by each park studied - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=8)	Benfica (n=18)	Campo Grande (n=16)	Total (n=42)
Rarely 0-3x/year	-	-	1 6.3% (0.3-32.3%)	1 2.4% (0.1-14.1%)
Occasionally <1x/month	3 37.5% (10.2-74.1%)	9 50.0% (26.8-73.2%)	1 6.3% (0.3-32.3%)	13 31.0% (18.1-47.2%)
Infrequently 1-3 days/month	3 37.5% (10.2-74.1%)	3 16.7% (4.4-42.3%)	4 25.0% (8.3-52.6%)	10 23.8% (12.6-39.8%)
Regularly 1x/week	2 25.0% (4.5-64.4%)	2 11.1% (2.0-36.1%)	2 12.5% (2.2-39.6%)	6 14.3% (6.0-29.2%)
Often 2-6 days/week	-	3 16.7% (4.4-42.3%)	4 25.0% (8.3-52.6%)	7 16.7% (7.5-32.0%)
Everyday 1x/day	-	1 5.6% (0.3-29.4%)	4 25.0% (8.3-52.6%)	5 11.9% (4.6-26.4%)

Of the 42 people that attend other parks with their dog, 64.3% (27/42) did it on weekends, 26.2% (11/42) both on weekdays as well as weekends, and 9.5% (4/42) only on weekdays.
On-leash walking was present in 21.4% (9/42) of the inquired, off-leash in 52.4% (22/42) and both on and off-leash walking was performed on 26.2% (11/42) of dogs.
Dogs are allowed to always have off-leash time in 71.4% (30/42), 9.5% (4/42) may be off-leash sometimes, and 19.1% (8/42) are never off-leash.

3.3.4 - Veterinary care

Regarding animal health care, 94.1% (95/101) of dogs were consulted by the veterinarian in the last 12 months. Concerning dog anthelmintic treatment, 89.9% (89/99) were dewormed in the previous six months, but only 27.7% (23/83) at least four times a year (Table 22). Owners that dewormed their dogs every month, have young dogs with less than one year old.

Table 22 – Distribution of frequency of internal deworming by the three dog parks - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=30)	Benfica (n=28)	Campo Grande (n=25)	Total (n=83)
1x/year	6 20.0% (8.4-39.1%)	3 10.7% (2.8-29.4%)	2 8.0% (1.4-27.5%)	11 13.3% (7.1-22.9%)
2x/year	15 50.0% (31.7-68.3%)	14 50.0% (31.1-68.9%)	5 20.0% (7.6-41.3%)	34 41.0% (30.5-52.3%)
3x/year	5 16.7% (6.3-35.5%)	3 10.7% (2.8-29.4%)	4 16.0% (5.3-36.9%)	12 14.5% (8.0-24.3%)
4x/year	3 10.0% (2.6-27.7%)	7 25.0% (11.4-45.2%)	6 24.0% (10.2-45.5%)	16 19.3% (11.8-29.7%)
6x/year	1 3.3% (0.2-19.1%)	-	-	1 1.2% (0.1-7.5%)
monthly	-	1 3.6% (0.2-20.2%)	5 20.0% (7.6-41.3%)	6 7.2% (3.0-15.7%)
other	-	-	3 12.0% (3.2-32.3%)	3 3.6% (0.9-10.9%)

Only 22 people referred which internal deworming product is used in their dogs. A combination of febantel, pyrantel embonate and praziquantel was the most used anthelmintic drug (Table 23). Young dogs were treated using febantel, pyrantel, praziquantel (5 and 6 months old); pyrantel (7 months old); febantel, pyrantel (8 months old) and emodepside, praziquantel (11 months old).

Table 23 - Distribution of deworming product used by the studied dogs from each park - Algés, Benfica and Campo Grande - and in total (CI95%)

	Algés (n=7)	Benfica (n=5)	Campo Grande (n=10)	Total (n=22)
Febantel, pyrantel, praziquantel	6 85.7% (42.0-99.3%)	4 80.0% (29.9-99.0%)	6 60.0% (27.4-86.3%)	16 72.7% (49.6-88.4%)
Febantel, pyrantel	-	1 20.0% (1.1-70.1%)	-	1 4.6% (0.2-24.9%)
Fenbendazole, praziquantel	-	-	1 10.0% (0.5-45.9%)	1 4.6% (0.2-24.9%)
Emodepside, praziquantel	1 14.3% (0.8-58.0%)	-	-	1 4.6% (0.2-24.9%)
Pyrantel	-	-	1 10.0% (0.5-45.9%)	1 4.6% (0.2-24.9%)
Milbemycin oxime, praziquantel	-	-	1 10.0% (0.5-45.9%)	1 4.6% (0.2-24.9%)
Selamectin	-	-	1 10.0% (0.5-45.9%)	1 4.6% (0.2-24.9%)

3.3.5 - Characterization of the owner-pet relationship

In 75.5% (77/102) of the households the dog was allowed to lick the owners' faces, 82.4% (84/102) to visit the owners' bedroom and 43.1% (44/102) to sleep with the owners in bed.

4 - Discussion

This is the first known study about parasitic agents and characterization of dog population from dog-attending parks in Lisbon, and also in Portugal. These places are mostly frequented by pet dogs, although it is possible for stray dogs to visit them punctually. Thus, these results concern animals that have close contact with people and can transmit zoonotic agents. Several sampling days for each park were chosen to have a better representation of the parasitic population, although, there is still a possibility of sampling the same animal more than once.

4.1 - Faecal Samples

4.1.1 - Parasitic population

The parasites found in most samples were the nematodes of the family Ancylostomatidae, in 61 of 369 samples (16.5%), followed by protozoans *Cryptosporidium* spp., in 44 of 369 samples (11.9%) and *Giardia* sp., in 42 of 369 samples (11.4%). With lower prevalence, *Toxascaris leonina* and *Cystoisospora* spp., each found in 4 of 369 samples (1.1%), *Toxocara* spp., in 2 of 369 samples (0.5%) and *Sarcocystis* spp., in only one sample (0.3%). Of all samples collected, 247 did not allow the detection of any parasite by any of the used methods (66.9%).
Interestingly, one of the rules set out in the canine park of Benfica is that infected dogs are not allowed to enter the park; however, 31.5% (39/124) of faecal samples had at least one parasitic agent.

False negatives results should always be considered, especially when using less sensitive techniques. This is especially the case for *Taenia* eggs (Robertson *et al.*, 2000). Although centrifugation (which was used in this work) has a better chance of recovering parasitic eggs (Dryden *et al.*, 2005), a sedimentation technique could also increase the sensitivity in parasite detection (mainly in tapeworms) (Robertson *et al.*, 2000).
Adding to the presence of false negatives, several parasites such as *Giardia* have an intermittent shedding which leads to an underestimate prevalence of infection (Robertson *et al.*, 2000).
On the other hand, coprophagic behaviour, shown by some dogs, can lead to an overestimation of the prevalence of patent infections. False positive results can be due to the passage of some parasite eggs through the gastrointestinal tract without being digested. Prevalence estimates can be affected if dogs consume faeces from other dogs or even other species (e.g. cats), when they contain parasites that are morphologically difficult to distinguish from eggs of dog parasites (Nijsse *et al.*, 2014).

In this study, protozoans had a prevalence of 23.6% (87/369) whereas nematodes were of 16.8% (62/369). Prevalence of intestinal helminths is declining due to owners' awareness and adoption of strategic treatments with anthelmintics. For this same reason, since enteric protozoa are unaffected by the routine anthelmintics, and because more sensitive diagnostic techniques are being used, protozoa prevalence is arising (Robertson *et al.*, 2000).

In this study, no cestodes were found in any of the samples.
Parasites from Taeniidae family have an indirect life cycle and need an intermediate host (lagomorphs and cattle for *Taenia* spp. and ruminants and rodents for *Echinococcus* spp.), which are more common in rural areas. Due to the existence of sheepdogs and predatory habits more prevalent in these areas (Acedo, Quílez, & del Cacho, 1999), it was not expected to be found in these samples from urban dogs. *Dipylidium caninum*'s life cycle is related to the presence of fleas and lice, which should be more controlled in dogs that inhabit people's homes.
Other studies in Portugal show a relatively low prevalence of these parasites. Regarding Taeniidae and *Dipylidium caninum* eggs, in Peniche it was found a prevalence of 0.2% (1/648) in urban areas and 2.0% (1/50) in rural areas (Crespo *et al.*, 2006), in Santarém 0.3% (1/384) for both (Crespo *et al.*, 2013), and in Setúbal 0.2% (1/648) and 0.6% (4/648) (Gravata *et al.*, 2007), respectively, all from faecal samples collected from public spaces. All of them were carried out through several seasons of the year, considering therefore much diversity of climate conditions that can comprise the ideal conditions of the development of parasites. In kennels, a study in Lisbon (Lebre, 2011) showed a prevalence of 0.6% (1/179), using zinc sulphate, for Taeniidae and another one from Vila Franca de Xira (Santos, 2014) with the higher prevalence recorded, 10.0% (8/80). Furthermore, in Lisbon, 1.1% (2/179) of samples corresponded to *D. caninum* (Lebre, 2011).

Toxocara spp. had a prevalence of 0.5% (2/369) in this study. This result is lower than those found in other works: 1.1% (7/648) in Setúbal (Gravata *et al.*, 2007), 2.9% (19/648) in urban areas and 4.0% (2/50) in rural areas of Peniche (Crespo *et al.*, 2006), 3.1% (12/384) of ascarids in Santarém (Crespo *et al.*, 2013), and 39.5% (17/43) in Óbidos (Rosa *et al.*, 2011). In kennels, *Toxocara* spp. had a prevalence of 1.6% (2/124) in Lisbon, by zinc sulphate flotation technique (Lebre, 2011), and 15.0% (12/80) in Vila Franca de Xira (Santos, 2014). In a study from Oporto, using zinc sulphate, Neves *et al.* (2014) found 5.1% (9/175) positive asymptomatic animals and 7.8% (15/193) dogs with gastrointestinal clinical signs, that were presented at the veterinary hospital.

Young animals are the most prone to *Toxocara* spp. infections (Robertson *et al.*, 2000), and generally these animals are not taken to public places, nor to spaces of canine socialization, because they do not have completed the full vaccination plan and they are still not immunized. For this reason, it is predictable that this parasite is not the most common found in faecal samples from dog-attending parks.

However, positive samples of *Toxocara* spp. in dog-attending parks, even with low intensity of epg (in this case, the only one that could be counted had 50 epg) are of major concern for public health, due to the human syndrome of Visceral Larva Migrans.

Otero *et al.* (2014) studied the presence of parasitic forms in soil and faecal samples from public parks and playgrounds. Soil samples were positive in 63.2% (12/19) of parks and faecal samples in 15.8% (3/19) of those. The dog-attending park in Algés was also included with no positive sample (soil or faecal). In the playground of Mata de Benfica, 9 in 10 (90%) soil samples were positive and in Campo Grande Park 7 in 9 (78%) soil samples and 3 in 8 (38%) faecal samples were positive for *Toxocara* spp. eggs.
Regarding Algés, the higher percentage of positive faecal samples (1/125) in this study can be explained by the total number of samples examined (125 compared to only 10 faecal samples). The same can be applied to Benfica, although the same area was not studied (in the present study the dog-attending park and in the other, the playground). Campo Grande Park (where the dog-attending park is within) was found to have *Toxocara* spp. positive faecal samples, and therefore, the dog-attending park would also be expected to have. One explanation for this absence can be due to the fact that, stray dogs (whose deworming is not implemented) always have access to the entire park, whereas they only have access to the dog-attending park when the door is left open.

The role of the red fox and the Iberian wolf in spreading this parasite, can not be underestimated. Two studies in Portugal (Guerra *et al.*, 2012; Silva, 2010) have shown the presence of *Toxocara* spp. in these wild carnivores. With the ongoing growing of red fox populations and their invasion of urban areas (Deplazes *et al.*, 2011), their role in the spread of this and other zoonotic parasites at the urban areas, should be studied.

Concerning *Toxascaris leonina*, the prevalence was 1.1% (4/369). This result complies with other works from Portugal: 0.6% (4/648) in samples from Setúbal public spaces (Gravata *et al.*, 2007), using zinc sulphate flotation technique, 1.6% (2/124) in a kennel from Lisbon, (Lebre, 2011) and 0.5% (1/193) in dogs with gastrointestinal clinical signs from Oporto (Neves *et al.*, 2014) were found.

All positive samples were from Campo Grande and from the same sampling day, which can lead to wonder if all samples are not from the same individual. Although faecal contamination intensity ranged from 50 to 500 epg, the zoonotic risk of this parasite is of less importance than other helminths.

Parasites from Ancylostomatidae family are the most prevalent nematodes in Portugal. 16.5% (61/369) of them were found in this study, which is higher than several national researches: in Óbidos they were present in 2.3% (1/43) (Rosa *et al.*, 2011), 6.0% (39/648) of faecal samples collected from urban public spaces in Peniche (Crespo *et al.*, 2006), 6.0% (39/648) in Setúbal (Gravata *et al.*, 2007), 8.9% (34/384) in public spaces from Santarém (Crespo *et al.*, 2013) and 9.7% (12/124) in a kennel from Lisbon, with the use of zinc sulphate as a flotation fluid (Lebre, 2011). On the other hand, higher prevalences were observed in kennels from Vila Franca de Xira (31.3%, 25/80) (Santos, 2014), rural areas, farm and hunting dogs from Ponte de Lima (44.6%, 264/592) (Mateus *et al.*, 2014) and Peneda Gerês National Park (53.8%, 21/39) (Silva, 2010).

The members of Ancylostomatidae family are zoonotic agents responsible for CLM, with *Ancylostoma caninum* being more pathogenic than *Uncinaria stenocephala* (Bowman, 2014). Despite human infections being more common in tropical climates, in developing countries where people walk around barefooted, its presence in dog-attending parks poses a risk factor for the population, as it can infect humans by simple direct cutaneous contact.

A. caninum is more common in central and southern Europe and *U. stenocephala* in colder climates (ESCCAP, 2010), but both have been found in Portugal.
Despite different egg measures present in this study, no assumption regarding species can be made solely by egg size, being necessary genetic studies for a solid conclusion.
In this study, more positive samples were collected on 4th and 5th sampling days (18/61 and 20/60, respectively). This can be explained by the low moderate temperatures (more suitable for *U. stenocephala*, though) and high humidity, optimal conditions for these parasites (Robertson & Thompson, 2002; CAPC, 2013).

Countable eggs were found in samples from all studied dog-attending parks. Algés had the lowest average intensity of epg and in spite of Benfica being the park with the faecal samples with the highest epg, Campo Grande had a bigger average parasite burden.

Strongyloides stercoralis was not found in this work. Few national studies have reported its prevalence: 0.2% (1/648) in samples from Setúbal public spaces (Gravata *et al.*, 2007), 7.5% (6/80) in kennels from Vila Franca de Xira (Santos, 2014) and in Peneda Gerês National Park, 25.6% (10/39) of dogs were infected (Silva, 2010).

Regarding *Trichuris vulpis*, which is a parasite that is frequently found in Portugal, prevalence ranges from 1.1% (2/175) in healthy dogs from Oporto (Neves *et al.*, 2014) and 18.0% (9/50) in faecal samples collected from rural areas in Peniche (Crespo *et al.*, 2006). However, in Ponte de Lima, *T. vulpis* was positive in 49.5% (50/101) samples from hunting dogs (Mateus *et al.*, 2014). Heavy infections tends to be geographically localized or in kennels (ESCCAP, 2010) and this can explain the lack of positive results in the present study. This parasite does not have the zoonotic importance attributed to other parasitic agents.

Giardia sp. was found in 11.4% (42/369) of all faecal samples, using faecal smears stained by the modified Ziehl-Neelsen technique.
Using the same technique for faecal smears, prevalence of 8.5% (10/118) and 32.5% (26/80) were found in samples collected from Beja public parks (Nunes, 2014) and kennels from Vila Franca de Xira (Santos, 2014), respectively.
In Oporto, *Giardia* sp. was found in 7.4% (13/175) of healthy dogs and 15.5% (30/193) dogs with gastrointestinal signs (Neves *et al.*, 2014) and in 1.3% (1/77) and 61.2% (30/49) in household and kennels dogs from Évora, respectively (Ferreira *et al.*, 2011), using zinc sulphate as a flotation fluid.
Using direct IFA, 19.8% (25/126) of dogs in kennels from Bragança (Leal, 2015) and 55.9% (19/34) from Lisbon (Lebre, 2011) were found positive. In kennels in Viseu, three different techniques were implemented to determine prevalence of this parasite: 17.6% (9/51) with faecal smear, 19.6% (10/51) with zinc sulphate as a flotation fluid, and 21.6% (11/51) with Speed® *Giardia* (Fernandes, 2012).
In parks attending dogs from Calgary, Canada, using direct IFA, *Giardia* sp. was present in 24.7% of 251 samples (Smith *et al.*, 2014). A study from Colorado, United States of America, demonstrated a prevalence of *Giardia* sp. of 7.6% in a group of 66 dogs that attended canine parks, compared to 0% in the group of 63 dogs that did not frequent those same parks, using direct IFA (Wang, Ruch-Gallie, Scorza, Lin, & Lappin, 2012).

Ziehl-Neelsen technique in faecal smears is not much referenced for detection of *Giardia* sp. but it was chosen in this study because of its common use for *Cryptosporidium* spp. diagnosis. This method of detection is easy and well suited to general practice, but it has a low sensitivity, even when performed by trained technicians (Irwin, 2002). Zinc sulphate flotation is the method of choice for practitioners, because of higher sensitivity; however, it also requires an experienced technician. Optic microscopy also allows identification of other parasites that may be present. Direct IFA is considered to be the reference standard for detection of this organism in faeces (Tangtrongsup & Scorza, 2010).

Intermittent shedding of *Giardia* sp. occurs and false negative results can appear (Irwin, 2002). For diagnosis purpose, three faecal samples from the same dog, collected at 3 to 5 day period should be examined (ESCCAP, 2011). As this study was conducted with random samples of unknown dogs, it is possible that there may be some false negative results, not only because of the sensitivity of the technique used, but also due to the possibility of infected animals being checked in a period of cyst excretion absence.

Results for *Giardia* sp., in this study are in accordance with some national researches that also used Ziehl-Neelsen technique in faecal smears (Fernandes, 2012; Nunes, 2014). Higher prevalence was found in studies using a more sensitive method of detection, or in kennels, places more associated to giardiosis due to a large concentration of animals and environmental contamination.

Multiple dogs in a household increase the chance of infection with *Giardia* sp. Due to the fact that enteric protozoan are not affected by most common deworming, may contribute to high prevalence of *Giardia* sp., which colonise the intestine left by helminths (Robertson *et al.*, 2000).

Infection in dogs is more common in winter (Ballweber *et al.*, 2010), with most survival time occurring around 4ºC (Olson, O'Handley, Ralston, McAllister, & Thompson, 2004), and disease is more typically associated to water-borne outbreaks (Bowman & Lucio-Forster, 2010). In this study, more positive samples were collected on 2nd sampling day (12/65), due to, perhaps, heavy rain spreading contaminated water, as the same happens with *Cryptosporidium* spp.

Despite the fact that obvious clinical disease (e.g. presence of diarrhoea) is unusual in infection with *Giardia* sp. (Irwin, 2002), in the present study, 21.4% (21/98) of faecal samples that had lack of consistency were positive to this protozoa, against 7.7% (21/271) of positive shaped samples, which was also observed in the study by Neves *et al.* (2014), 7.4% in apparently healthy animals and 15.5% in dogs with gastrointestinal signs. Nunes (2014) showed a prevalence of *Giardia* sp. of 14.3% (1/7) in soft faecal samples and of 25.0% (1/4) in diarrhoeal stools. It was also found, in this study, that 14.0% (8/57) of positive samples had a lighter colour, which can suggest steatorrhea, a sign that can also be present in giardiosis (Tangtrongsup & Scorza, 2010). No other specific faecal colour is described for *Giardia* sp. infections, which can be seen by the wide range of colours presented by *Giardia* sp. samples, in this study.

Giardia duodenalis is an intestinal protozoa common of humans and dogs. Despite dogs have their group of organisms within the *Giardia* specie (assemblages C and D), while humans have their own anthroponic group (assemblages A and B), the first ones have also been identified to be infected, in a lesser extent, by assemblages A and B (Ryan & Cacciò, 2013). Zoonotic potential of *Giardia* sp. is still under debate (Sprong *et al.*, 2009), due to genetic differences in assemblages A and B subtypes (Ryan & Cacciò, 2013).
Two transmission cycles occur in domestic urban environments: transmission of dog-specific genotypes among large density of dogs living together and infections with potentially zoonotic assemblages in household dogs. However, some studies prove the otherwise (Ryan & Cacciò, 2013).
The risk of human infection through companion animals is low, comparing with other sources of contamination, except in severe immunosuppression (Bowman & Lucio-Forster, 2010).

In a study of preschool children in Lisbon by Ferreira *et al.* (2013), 2.5% of 316 children were presented in the faeces with cysts of *Giardia* sp. belonging to assemblages A and B, most likely with anthroponic transmission. However transmission from pets can not be excluded.
Ferreira *et al.* (2011) found, in dogs from Évora, 96.4% (27/28) of *Giardia* positive samples belonging to dog assemblages, C and D, only one sample with zoonotic potential, belonging to assemblage B. In another study from Portugal, 73.3% (22/30) of canine faecal samples studied were isolated with assemblage A (Eduardo, 2008).

In the present study, it would be interesting to perform identification by molecular biology of assemblages present in positive samples of *Giardia* sp., regarding public health, in particular for those who frequent dog-attending parks.

Treatment should be considered in asymptomatic dogs, not only because of the potential zoonotic risk of this parasite, but also due to the cyst excretion by these animals and environmental contamination (Bowman & Lucio-Forster, 2010). *Giardia* sp. cysts are immediately infective when passed in faeces (Ryan & Cacciò, 2013).

Cystoisospora spp. oocysts were found in four samples (1.1%). Because this parasite is more frequent in young animals, a low prevalence was expected in this study. In Peniche, 0.2% (1/648) of samples from urban public spaces were positive (Crespo *et al.*, 2006) and 13.5% (26/193) of dogs with gastrointestinal clinical signs had *Cystoisospora* spp. in Oporto (Neves *et al.*, 2014). Intermediate prevalence was recorded in Setúbal (2.2%, 14/648) (Gravata *et al.*, 2007), in Óbidos (2.3%, 1/43) (Rosa *et al.*, 2011) and in a kennel in Lisbon, using zinc sulphate as the flotation fluid (12.1%, 15/124) (Lebre, 2011).

Cryptosporidium spp. was found in 11.9% (44/369) of all faecal samples, using faecal smears stained by the modified Ziehl-Neelsen technique.
In kennels from Vila Franca de Xira, 11.3% (9/80) samples were positive for *Cryptosporidium* spp. using faecal smears stained by the same technique (Santos, 2014). The conjoined use of modified Ziehl-Neelsen stain faecal smears and direct IFA allowed detection in 13.5%, from 39 samples from Peneda Gerês National Park (Silva, 2010). In kennels from Bragança, prevalence of 3.1% (3/97) was found (Leal, 2015), and 17.6% (6/34) from Lisbon, (Lebre, 2011), using direct IFA. In parks attending dogs from Calgary, Canada, *Cryptosporidium* spp. was present in 14.7% of 251 samples (Smith *et al.*, 2014). In the Colorado study, *Cryptosporidium* spp. was present in 4.8% of 66 park-attending dogs. No oocyst was observed in the non park-attending dogs (Wang *et al.*, 2012).

Cryptosporidium spp. oocysts detection by traditional coprological methods (in this case stained faecal smear) is difficult and sometimes they are identified only by the most experienced technicians (Irwin, 2002). Rapid antigen tests provide an higher sensitivity and specificity (Scorza & Tangtrongsup, 2010). This means that the prevalence in these dog-attending parks may be devalued.
As cited above, gastrointestinal protozoan are not affected by most common deworming (and this parasite is even less sensitive to them), which may contribute to its high values.

Infective thick wall oocysts, which are expelled in the faeces of infected animals, present a high environmental resistance that perpetuates dissemination and transmission of the disease (Scorza & Tangtrongsup, 2010).
Cryptosporidium spp., such as *Giardia* sp., is frequently associated to water-borne outbreaks. A studied from Lisbon, studied both untreated and treated water samples for the detection of *Cryptosporidium* spp. and *Giardia* sp. using IFA. *Cryptosporidium* spp. oocysts were found in 53.6% (37/69) and 41.5% (44/106) of untreated and treated water samples, respectively. *Giardia* sp. cysts were detected in 58.0% (40/69) and 25.6% (27/106), of untreated and treated water analysed, respectively (Lobo, Xiao, Antunes, & Matos, 2009).

In this study, more positive samples were collected on 4th sampling day (15/61). This can be explained by the average temperatures (most survival time is around 15 ºC) and infections tend to be most common in the rainy season since rainfall presumably results in greater spread of contaminated surface water (Dillingham, Lima, & Guerrant, 2002; Alum, Absar, Asaad, Rubino, & Ijaz, 2014).

Infection by *Cryptosporidium* spp. in dogs is mainly associated with *C. canis* whereas in humans is *C. hominis*. Dogs are also quite refractory to infection with *C. parvum*. The risk of human infection by this protozoa from dogs, is considered to be limited in immunocompetent individuals, with most infections being acquired through anthroponotic transmission (Bowman & Lucio-Forster, 2010). Although zoonotic potential appears to be minimal, it has not yet been conclusively refused by the scientific community. Genotypic PCR isolation could be performed in order to access to *Cryptosporidium* species that can be found in the studied dog-attending parks.

The decision to implement a treatment protocol in asymptomatic dogs, when the diagnosis of cryptosporidiosis is made, is an issue, since these animals are shedding oocysts and contaminating the environment (Bowman & Lucio-Forster, 2010).

Park attending dogs are more likely to be infected with the protozoa, *Giardia* sp. or *Cryptosporidium* spp. (Smith *et al.*, 2014; Wang *et al.*, 2012).

Only two works in Portugal studied *Sarcocystis* spp.; one from Azambuja, with 0.5%, with 2 positive samples in a total of 432 (Maurício *et al.*, 2006) and another one from Peneda Gerês National Park, with 2.6% (1/39) (Silva, 2010). In the present study, sporocysts of *Sarcocystis* spp. were found in only one sample (0.3%). This result can be explained by the indirect life cycle of this parasite and the necessity of having an intermediate host.

In the present study, it was found that samples with intestinal parasites, many (68.9%, 84/122) had shaped faeces. This may be due to the presence of chronic and sub-clinical infections or infections with low worm burden. Still, the clinical signs differ depending on the parasitic agent, and may or may not include diarrhoea, and the detection of any parasite does not necessarily mean that it is the cause of the diarrhoea.

Anthelmintic deworming was expected to be effective, in this population. Pet dogs should have a regular preventive treatment, which is sorely needed for reducing transmission of these parasites, as well as, the environmental contamination. However, the high prevalence of parasites, mainly helminths, which are covered by regular deworming products, shows a lack of prophylactic care by owners. This situation has been recently reported by Matos (2013).

4.1.2 - Parasitic association

From the dog-attending parks studied, 9.2% (34/369) of all samples were associations with more than one type of egg/(oo)cyst. From these, only two samples were detected with three different parasites, being the rest infected with only two. Infections with more than one type of egg/(oo)cyst is referred instead of mix infections, due to the possibility of coprophagia. This behaviour can lead to false positive results by detection of eggs that are only passing through the gastrointestinal tract.

In kennels from Vila Franca de Xira, parasitic association occurred in 12.5% (10/80) (Santos, 2014) and from Lisbon in 5.6% (7/124) (Lebre, 2011). In Oporto, 1.7% (3/175) in healthy dogs and 6.2% (12/193) in dogs with clinical gastrointestinal sigs had more than one type of egg/(oo)cyst (Neves *et al.*, 2014). In Évora, 6.5% (5/77) of household dogs and 16.3% (8/49) of kennel dogs presented multiple parasitic associations (Ferreira *et al.*, 2011).

Ancylostomatidae and *Cryptosporidium* spp. were the parasitic association most often found, 6.2% (23/369) from all faecal samples analysed. This may be due to respective rates of individual infection, since these parasites had higher prevalence. Three samples had associations with only protozoa (*Cryptosporidium* + *Giardia* and *Sarcocystis* + *Giardia*) and four were presented with only nematodes (Ancylostomatidae + *Toxascaris leonina*).

4.2 - Soil Samples

Heterakis spp. eggs found were not considered relevant since this parasite infects birds and easily contaminates this kind of space and sample.
Concerning soil samples, only Ancylostomatidae eggs were found. They may not survive several weeks in the environment (Prociv & Croese, 1996), which means that the environmental contamination comes from relatively recent infected dog faeces.

Toxocara spp. eggs have high resistance to environmental conditions and remain infective for years (Overgaauw & van Knapen, 2013). Despite negative soil samples, it does not mean *Toxocara* spp. eggs are not present in the environment. Positive faecal samples can lead to an environmental contamination of the soil and be washed away by rainwater, if owners do not pick up their dog faeces (which was observed during the sampling days of this work). The study by Otero *et al.* (2014) has analysed the same public parks, where the dog-attending park is located, and proved that there is soil contamination of those places.

There was a significant difference between the type of soil sample. Gravel samples did not have any of eggs found. One explanation for this is the large size of the grains of gravel. Since these were quite big, they did not retain any of the eggs and they could be deposited deeper. Hereupon, only grass samples had parasitic eggs, namely Ancylostomatidae.

Regarding the agreement within the sieves, moderate agreement, according to Landies & Koch, 1977, or good agreement, according to Fleiss, 1981 (Houe, Ersbøll, & Toft, 2004), is present. This means that both sieves retrieved eggs in positive samples, and showed no eggs in negative results. Because of theirs elliptical shape, and even though their length allowed them to be recovered from the 0.063 mm sieve, their width could lead to their recovery only in the 0.020 mm sieve.

Soil contamination with parasitic agents is a major concern for animal and public health. This represents a significant source of infection for humans and, in particular children. They are the most affected because of the practice of pica and geophagia (Robertson & Thompson, 2002), and if they are brought to contaminated dog-attending parks, greater exposure occurs and the risk of infection increases.

4.3 - Questionnaires

4.3.1 - Identification of the pet

The high percentage of non-castrated male demonstrates the mentality that is still observed in Portugal, mainly supported by men, for not castrating male pets. Both Benfica and Campo Grande canine parks prohibit the attendance of females in estrus.
Minimal age observed was 4 months, which is in accordance with the complete vaccination plan. The rules of Benfica dog-attending park discourage the presence of animals younger than 6 months, however two surveyed dogs from this park, had 4 and 5 months of age.

4.3.2 - Characterization of the pet

In this study, 25.5% (26/102) of the animals lived with at least a dog. Matos (2013) observed, in a survey made to people in a veterinary hospital, that owners took care in applying equal deworming to all the animals when their dog co-habited with other dogs.
In the present study, one person (1.0%) made a point of stating that her dog was fed with raw food, however, data about frequency of deworming was not available. The consumption of raw food is a risk factor for acquiring parasitic infections, either by ingestion of infected intermediate or paratenic hosts.

From all dogs, 25 spend most of the day outdoors or both indoors and outdoors. Outdoors, dogs have access to more potential sources of infection, such as, contaminated soil and water.
Contact with other dogs outdoors (89.2%, 91/102) and daily walking on streets and/or parks lead to the possibility of acquiring parasite infection due to the contact with other animals that may not be correctly dewormed and/or with environmental contamination.

4.3.3 - Characterization of walking to the dog-attending park

In the study made by Smith *et al.* (2014), infection with at least one enteric parasite was positively associated with frequency of park use, off-leash activity and visits of more than one park. Furthermore, *Giardia* sp. intensity was also positively associated to the same factors. In the present study, 50.0% (51/102) of the studied dogs visit the park daily, 82.4% (84/102) of them have off leash activity and 41.2% (42/102) frequented other parks. This demonstrates that dogs that attend the three dog parks studied here, have behaviour risk factors to parasitic infection.

Dog park in Algés, had more faithful attendants, with only 8 people referring going to other parks. It may be due to the fact that this park is in a residential area and people who attend it do so for a matter of convenience.
Owners that go with their dog to Benfica canine park, are the ones who attend other parks more often (18/42), however, Campo Grande dogs', visit a wider variety of other parks. Both parks are in the centre of Lisbon, which allows a frequency by people from all over the city.

Despite 94.1% (96/102) of the owners claimed faecal collection of their pets, it was common to see 10-20 faecal samples on the environment of every dog space in every site and sampling day. In the study of Matos (2013) 63.3% (119/188) of the owners, and of Overgaauw *et al.* (2009) about 61% from 152 dog owners, affirmed to collect their dog faeces. Comparing the value obtained with the cited studies, it may be overestimated due to the negative prejudice associated with the knowledge that that person does not pick up their dog's faeces.
Both Benfica and Campo Grande canine parks demand, as a rule for the proper attendance of those parks, the collection of canine excrement. This is essential to reduce and manage risk of infections. Algés park lacks any kind of information on this sense.

4.3.4 - Veterinary care

Regarding animal health care, 94.1% (95/101) of dogs were consulted by the veterinarian in the last 12 months. Smith *et al.* (2014) showed that *Cryptosporidium* spp. infections intensity was associated with visiting the veterinarian in the previous 12 months, in park-attending dogs, suggesting that heavily infected dogs may have been symptomatic.

Concerning dog anthelmintic treatment, 89.9% (89/99) of dogs were dewormed in the previous six months. In studies from veterinary hospitals, Matos (2013) and Nabais (2008) presented 90.9% (221/243) (dogs with 1 year or older) and 96.5% (194/201) of dogs internally dewormed, respectively, which is in accordance with our findings.
Regarding the frequency of internal deworming, only 27.7% (23/83) of dogs were dewormed in the recommended periodicity, at least four times a year (ESCCAP, 2010). This result is in agreement with 25.7% (47/183) found by Matos (2013). Dogs dewormed every six months are the most common, not only in this study (41.0%, 34/83), but also in other studies cited above, 30.6%, 56/183 in Matos (2013) and 29.9%, 60/201 in Nabais (2008).

The combination of febantel, pyrantel embonate and praziquantel, was the most used anthelmintic drug (72.7%, 16/22), as the same was observed by Matos (2013) and Nabais (2008). This association of drugs has spectrum of action, according to the manufacturer, against nematodes (ascarids, Ancylostomatidae, *Trichuris vulpis*), cestodes (*Echinococcus granulosus*, *Dipylidium caninum*, *Taenia* spp., *Multiceps multiceps* and *Mesocestoides* spp.), and *Giardia* spp.. This drug is the most complete, since it has additional action against protozoa (*Giardia*), notably due to febantel, which after liver metabolization will transform to fenbendazole, the active ingredient (Anderson *et al*., 2004).
Products that were referred during the questionnaire, with narrow spectrum of action include febantel and pyrantel embonate (1/22), only against ascarids, Ancylostomatidae and *Trichuris vulpis*; and pyrantel embonate (1/22), against the same nematodes as the previous except *Trichuris vulpis*. These products were used in dogs with 8 and 7 months of age, respectively. At this age, more complete products can be used to protect against a broader range of parasites. Selamectin was also an used product (1/22), with a spectrum of action only against *Toxocara canis*.

In places where different parasitic agents have been found, some animals reveal a lack of protection against some of them. Proper anthelminthic products should be used in the recommended periodicity, so that environmental contamination and, mainly, dog and possibly human infections can be reduced.

4.3.5 - Characterization of the owner-pet relationship

In the present study, 75.5% (77/102) of the households the dog was allowed to lick the owners' faces, 82.4% (84/102) to visit the owners' bedroom and 43.1% (44/102) to sleep with the owners in bed. Higher close physical contact with pet dogs in this population contrasts with a study by Overgaauw *et al.* (2009), in the Netherlands, where 50% out of 212 pets were allowed by the owner to lick their faces, 60% visited the bedroom and 18% (from 152) of dogs were allowed to sleep with the owner in bed.

The majority of the owners have close physical contact with their dogs, increasing the transmission risk of zoonotic agents. Therefore, an additional effort of information must be done by veterinarians, towards a safer relationship between pet animals and humans.

V - Conclusion

Finalizing this work, objectives were achieved. Approximately one third of dog faecal samples were infected with parasitic agents, with Ancylostomatidae (16.5%), *Cryptosporidium* spp. (11.9%) and *Giardia* sp. (11.4%) being the most prevalent. Two different nematodes with proven zoonotic effect (family Ancylostomatidae and *Toxocara* spp.), and two protozoa, that are possibly zoonotic (*Cryptosporidium* spp. and *Giardia* sp.), were observed. Furthermore, soil contamination with the zoonotic agents belonging to Ancylostomatidae family, was present in all of those parks. The findings represent a menace to other dogs that attend those parks, but also, to humans.

Some parasites, in particular, protozoa, represent an increased risk, essentially in immunocompromised individuals. Since the genotype of *Giardia* cysts found was not possible to be assessed, the risk of zoonotic transmission of these samples can not be measured. Although, they are always a source of infection to those dogs that visit these places.

Having in mind that the majority of those that enter these parks are pet dogs, the prevalence of parasites is higher than the expected, demonstrating that responsible pet ownership is not being fulfilled.

Surveys suggest (and are reinforced by the results from analysed faecal and soil samples) that few dogs are dewormed with the recommended schedule, despite the frequent contact with other dogs and environment in urban dog-attending parks. Protozoa treatment is a serious concern, since regular antiparasitic drugs do not have action over them.

Some behaviour risk factors to parasitic infection are present in this dog population, mainly frequency of park use, off-leash activity and visits of more than one park. The most concerning factor is the observation of several faecal samples on the environment of every dog space, even though 94.1% of the owners claimed collection of their pets faeces. This preventive measure should be performed by every attendant, since it is an extremely important (and easy) way to reduce environmental contamination by parasitic agents. The majority of the owners have close physical contact with their dogs, increasing the transmission risk of zoonotic agents.

However, we should notice too, the lack of waste bags (in the three dog parks) and of rules of use (in Algés park), both situations needing a better evaluation by municipal authorities towards a better and civilized use of these public places.

All these findings highlight the need to raise public awareness about potential risks and preventive procedures and increase the promotion of faecal removal control measures and effective deworming practices, towards a better status of Animal and Public Health under the scope of “One World, One Health”.

The outcomes of this study provide information for further epidemiological investigations and disease control interventions to increase awareness of dog owners, public park managers, veterinarians, medical doctors and municipal authorities.

VI - Further Studies

Future studies, regarding dog-attending parks are needed, not only in Lisbon but in other cities from Portugal and also in the world. Preferably using the same analytical methods that make the results comparable.

Individual dogs should be studied (faecal samples accompanied by the questionnaire). This would allow demonstrating which are the risk factors for that particular population on the dog-attending parks.

More sensitive and specific coprological techniques should be applied and genotyping of *Giardia* cyst would give more information about the real zoonotic risk from these dogs. It would also be interesting to assess parasitic population and genetic characterization of those agents found in the owners of the dogs.

The time of sampling should also be extended to a minimum of one year, to evaluate peaks of parasite activity during all seasons.

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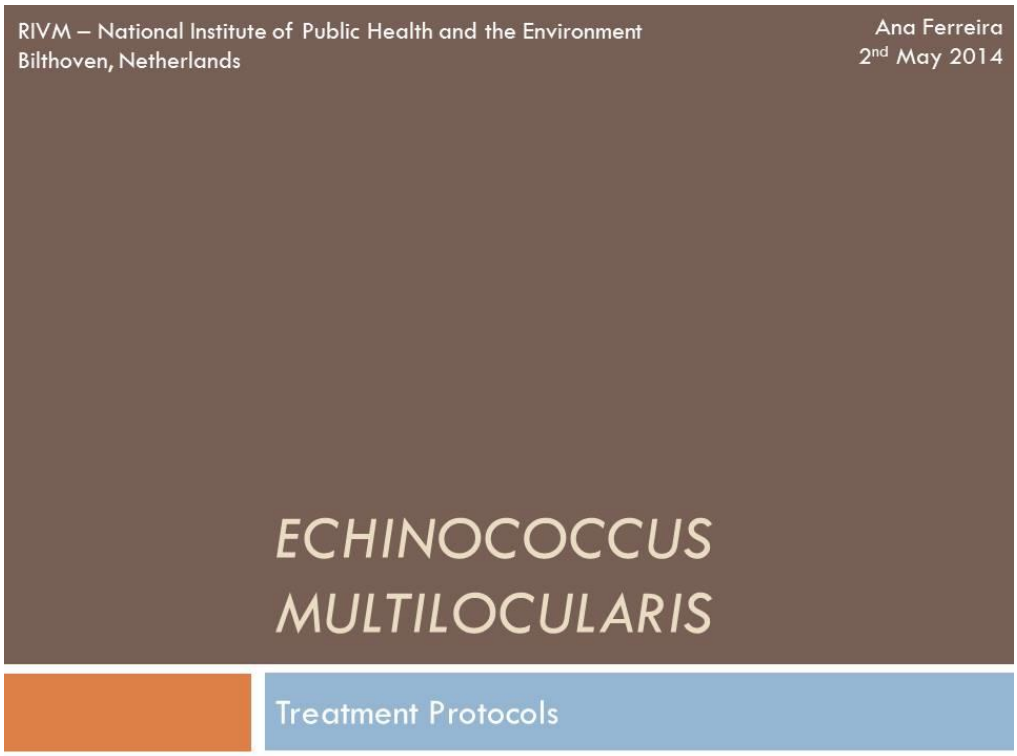
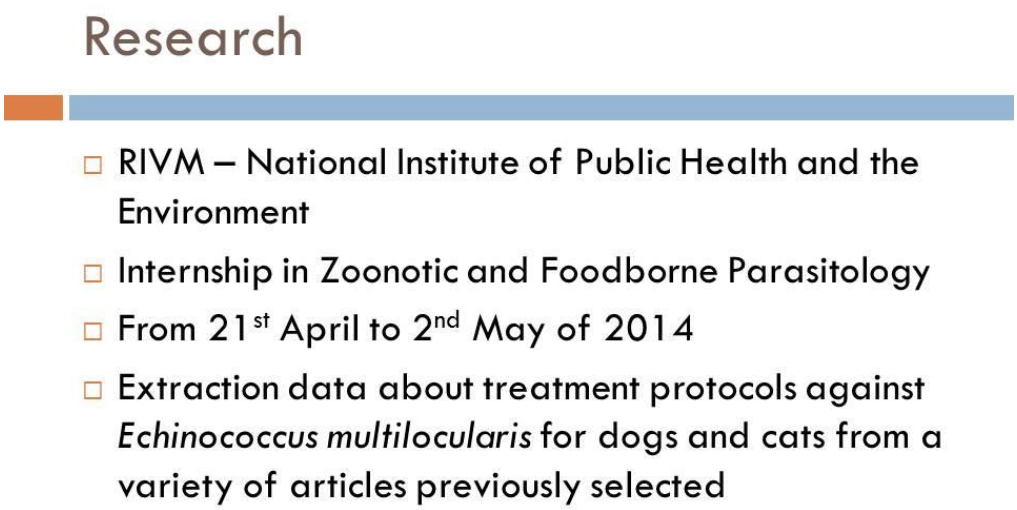
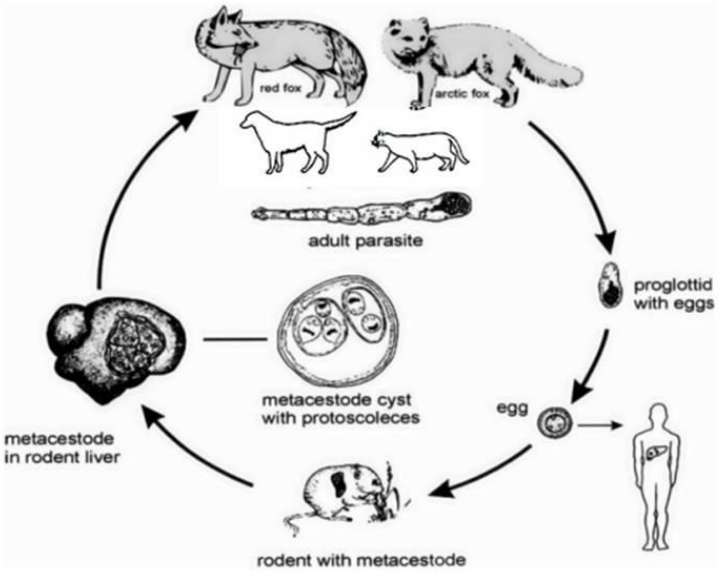
VIII - Annexes

Annex 1 – Presentation about treatment protocols against *Echinococcus multilocularis* for dogs and cats



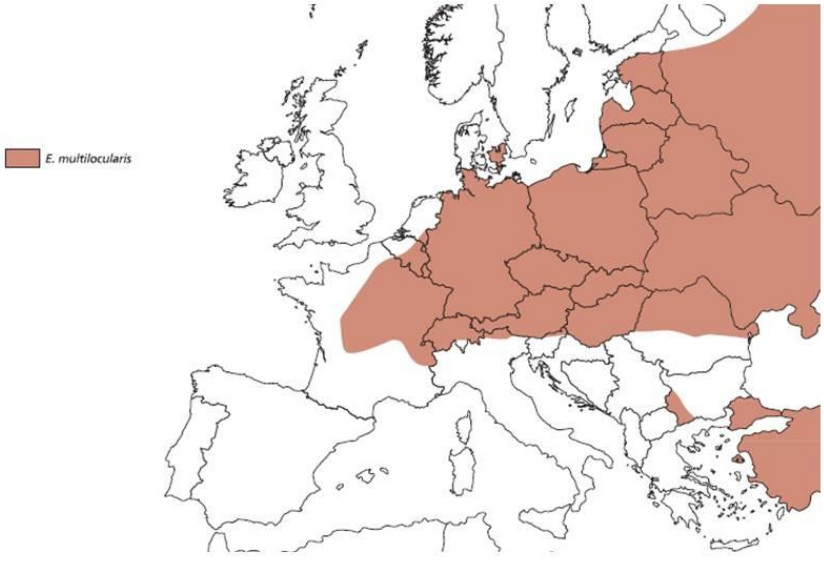


Life Cycle



http://openi.nlm.nih.gov/detailedresult.php?img=2889826_pntd.0000722.g001&req=4

Distribution of EM in the fox in Europe



ESCCAP Guideline – Worm Control in Dogs and Cats

Dogs - Effectiveness of Available E.M. Deworming Drugs

Autor	Target	Drug	Dose (mg/kg body weight)	Day of Intervention	Sample Time	Sample Size	Efficacy
Sakamoto 1977	Against **immature**	Praziquantel – Droncit - gelatine capsule, oral	0,1 mg/kg 0,25 mg/kg 0,5 mg/kg 1 mg/kg 2,5 mg/kg 5 mg/kg 10 mg/kg Once	25 dpi	27 dpi	5/40	0,1 mg/kg → 18,55% 0,25 mg/kg → 23,11% 0,5 mg/kg → 95,20% 1 mg/kg → 99,92% 2,5 mg/kg → 99,98% 5 mg/kg → 99,99% 10 mg/kg → 100%
Thomas 1977	Against **mature** and **immature**	Praziquantel – SC - Against mature – oral - Against mature – oral - Against immature	SC - Against mature 0,5 mg/kg 1 mg/kg Oral - Against mature 1 mg/kg Oral - Against immature 1 mg/kg 5 mg/kg Once				SC - Against mature 0,5 mg/kg → 88% 1 mg/kg → 100% Oral - Against mature 1 mg/kg → 100% Oral - Against immature 1 mg/kg → 95% 5 mg/kg → 100%
Andersen 1978	Against **immature**	Praziquantel – Droncit - injectable IM	5 mg/kg Once	21 dpi	28 dpi	9/18	100%

Dogs - Effectiveness of Available E.M. Deworming Drugs

Autor	Target	Drug	Dose (mg/kg body weight)	Day of Intervention	Sample Time	Sample Size	Efficacy
Andersen 1985	Against **immature**	Praziquantel and febantel - paste, oral	1mg/kg praziquantel + 10 mg/kg febantel 3 times	21, 22, 23 dpi	28 dpi	6/12	100%
Rommel 2001	Against **mature** and **immature**	Praziquantel – Droncit - gelatine capsules and tablets, oral	Capsules - Against immature 1 mg/kg 2,5 mg/kg Capsules - Against mature 2,5 mg/kg 5 mg/kg Tablets - Against immature 5 mg/kg 10 mg/kg Once			4-6/45	Capsules - Against immature 1 mg/kg → 82% 2,5 mg/kg → 97% Capsules - Against mature 2,5 mg/kg → 96% 5 mg/kg → 100% Tablets - Against immature 5 mg/kg → 100% 10 mg/kg → 100%
Eckert 2001		Epsiprantel – Cestex - oral	5,2-5,8 (5,4) mg/ kg 4,9 - 5,3 (5,1) mg/ kg (Dose recommended 5,5mg) Once	20 dpi	24 dpi	4/16	99,6% 99,9%
Schroeder 2009	Against **mature** and **immature**	Emodepside and praziquantel – Profender - tablets, oral	1 mg/kg emodepside + 5 mg/kg praziquantel Once	11 dpi or 21 dpi	25/26 dpi	8/24	11 dpi: 100% 21 dpi: 100%

Dogs – Treatment Protocols

Drug	Via	Dose (mg/kg body weight)	
		Immature	Mature
Praziquantel	oral	5 mg/kg	1-5 mg/kg
	SC		1 mg/kg
	IM	5 mg/kg (5,7 mg/kg)	
Epsiprantel	oral	5,5 mg/kg	
Praziquantel + febantel	oral	1mg/kg praziquantel + 10 mg/kg febantel	
Praziquantel + emodepside	oral	5 mg/kg praziquantel + 1 mg/kg emodepside	

Cats – Effectiveness of Available E.M. Deworming Drugs

Autor	Target	Drug	Dose (mg/kg body weight)	Day of Intervention	Sample Time	Sample Size	Efficacy
Andersen 1978	Against **immature**	Praziquantel - Droncit - injectable IM	5 mg/kg praziquantel Once	21 dpi	28 dpi	11/22	100%
Jenkins 2000	Against **mature** and **immature**	Praziquantel - Droncit - spot on	8mg/kg spot on 4% w/v once spread out over the skin (fur was parted) over about one square cm	10 dpi or 21 dpi	23 dpi	10/30	100%
Eckert 2001		Epsiprantel - Cestex - oral	2,7-2,8 (2,7) mg/kg 5,5 mg/kg (Dose recommended 2,75mg/kg) Once	20 dpi	26 dpi	5/15	100%
Jenkins 2003	Against **immature**	Praziquantel and milbemycin oxime - Milbemax - tablets, oral	5 mg/kg praziquantel + 2 mg/kg milbemycin oxime Once	16 dpi	21 dpi	10/20	100%
Charles 2005		Emodepside and praziquantel - Profender - topical solution	emodepside (2,14 % w/v) and praziquantel (5,58% w/v), once spread out over the skin of the neck (fur was parted)	21 dpi	23 dpi	9-11/60	98,5– 100% 39600 → 98,5% 10000 → 100% 20000 → 100%

Cats – Treatment Protocols

Drug	Via	Dose (mg/kg body weight)	
		Immature	Mature
Praziquantel	IM	5 mg/kg (5,7 mg/kg)	
	topic	8mg/kg spot on 4% w/v	
Epsiprantel	oral	2,75 mg/kg	
Praziquantel + milbemycin oxime	oral	5 mg/kg praziquantel + 2 mg/kg milbemycin oxime	
Praziquantel + emodepside	topic	5,58% w/v praziquantel + 2,14 % w/v emodepside	

Recommended Treatments

- Infection with *Echinococcus*
  - Animals should be treated on two consecutive days
  - Dogs should be shampooed to remove any parasite eggs adhering to the coat
- Prevention from endemic to non-endemic areas
  - Animals should be treated on two consecutive days
- Endemic areas (central and eastern Europe, northern and central Eurasia, Japan, North America):
  - Dogs should be treated once every 4 weeks

Notes

- Older studies → oral dose 1 mg/kg

  Recent studies → oral dose 5 mg/kg
  - different formulas?
  - resistance?

- IM dose 5 mg/kg in the study → 5,7 mg/kg is recommended

Annex 2 – Submitted and accepted abstracts for poster presentation

1. - 25th International Conference of the World Association for the Advancement of Veterinary Parasitology; Liverpool, United Kingdom; August 2015

Canine faecal contamination and parasitic risk assessment in Lisbon dog parks

Ferreira, A.[1]; Alho, A.M.[1], Otero, D. [1], Gomes, L.[1], Nijsse, R. [2], Overgaauw, P.[3], Madeira de Carvalho, L.M.[1]

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Dog parks fulfil the desire of dog owners to spend quality time with their dogs. These parks pose an exposure risk for zoonotic agents among dogs, humans and wildlife. Regarding the concept of One Health, it is important to characterize and assess the parasitic population in these places. However, in Lisbon, few studies investigating gastrointestinal infections in urban dogs have been performed and none in park-attending dogs.

In total, 369 fresh faecal samples were collected from three dog parks: 125 from Algés, 124 from Benfica and 120 from Campo Grande, from October to December 2014, every 15 days. Samples were analysed at the Laboratory of Parasitic Diseases (FMV-ULisboa) by Centrifugal Sedimentation/Flotation, McMaster (in the positives) and Modified Ziehl-Neelsen stained faecal smear techniques.

The overall prevalence for positive samples was 33% (122/369). Ancylostomatidae represent 17% (62/369), *Cryptosporidium* spp. 12% (44/369), *Giardia* spp. 11% (42/369), *Toxascaris leonina* and *Cystoisospora* spp. 1% (4/369) each, *Toxocara* spp. 0.5% (2/369) and *Sarcocystis* spp. 0.3% (1/369). Samples tested positive for more than one type of egg in 9.8% (36/369) mainly due to Ancylostomatidae and *Cryptosporidium* spp. Protozoa and nematode prevalence was, respectively, 24% (87/369) and 17% (62/369).

Approximately one third of these dog faecal samples were infected with parasitic agents, some of zoonotic concern, representing a menace to other animals and humans. These findings highlight the need to raise public awareness and increase the promotion of faecal removal control measures and effective deworming practices, towards a better status on Animal and Public Health.

2. - II Congreso Ibero-Americano de Epidemiología y Salud Pública; Santiago de Compostela, Spain; September, 2015

Owner behaviour and parasite risk factors in park-attending dogs: a public health concern?

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Objectives: Public parks and especially the new trend of public facilities for dogs, the so called dog parks or dog areas, represent a common destination for dogs and owners. However, they may pose a risk for the transmission of parasitic zoonotic agents among animals and humans, such as roundworm infections. Information regarding pet-owner relationship, dog-walking habits, husbandry practices and history of deworming care are currently unavailable. For that reason, a survey was performed to assess and characterize the behaviour and risk factors for owners and park-attending dogs, based on an owner inquiry.

Material and methods: From October to December 2014, a total of 102 enquiries were conducted among dog owners with 102 animals, visiting one of the two dog parks in Lisbon and one in Oeiras, respectively the parishes of Benfica and Campo Grande and Algés.

Results: Regarding animal health care, 93% of dogs were consulted by the veterinarian in the last 12 months. Concerning dog anthelmintic treatment, 87% were dewormed in the previous 6 months, but only 23% at least four times a year. Febantel, pyrantel embonate and praziquantel were the most used anthelmintic drugs. Additionally, 66% of the dogs visit the park daily, 12% once a week and 75% were always allowed to be off-leash. Despite 94% of the owners claimed faecal collection of their pets, it was common to see 10-20 faecal samples on the environment of every dog space. Regarding the pet-owner relationship, 75% of the dogs were allowed to lick their owners' faces, 82% to be in their bedroom and 43% to sleep in their bed. Also, 25% of the dogs share the house with at least one dog, 16% with at least one cat and 1% were fed with raw meat.

Discussion/conclusion: The findings suggest that few dogs are dewormed with the recommended schedule (minimum three-monthly), despite the frequent contact with soil and with other dogs in urban parks. The majority of the owners have close physical contact with their dogs, increasing the transmission risk of zoonotic agents. Although a high percentage of owners mention faeces collection, faecal droppings were common in the studied areas. Owner's education about potential risks and preventive practices is therefore required and should be promoted to assure responsible pet ownership under the scope of "One World, One Health". The outcomes of this study provide information for further epidemiological investigations and disease control interventions to increase awareness of dog owners, public park managers, veterinarians, medical doctors and municipal authorities.

Annex 3 – Questionnaire on Owner-Pet Relationship

Number: **Questionnaire on Owner-Pet Relationship** Date:

Canine Park: _____ **Access for stray animals:** Yes ☐ No ☐

IDENTIFICATION OF THE PET

♀☐ ♂☐ **Age**:_____ **Breed**:_____ **Castrated:** Yes ☐ No ☐

CHARACTERIZATION OF THE PET

- **House conditions**

Lives with other animals:

No ☐ Yes ☐

Dogs ☐ Number___

Cats ☐ Number___

Others: _____ Number___

- **Food conditions**

Nutrition:

Commercial food ☐

Cooked homemade food ☐

Raw homemade food ☐

Ingests what it picks on the street ☐

- **Day-to-day**

Spends most of the day:

Indoors ☐ Outdoors ☐

Contacts with animals outdoors: Yes ☐ No ☐

Which animals: Dogs ☐ Cats ☐ Others: _____

Walking (daily):

Walks on the street ☐

Parks/Green spaces ☐

Land/Area of open ground ☐

Does not walk – Backyard ☐

CHARACTERIZATION OF WALKING TO THE DOG-ATTENDING PARKS

- **Walking to the park**

How often do you come to this park:

Rarely 0-3x/year ☐

Occasionally <1x/month ☐

Infrequently 1-3 days/month ☐

Regularly 1x/week ☐

Often 2-6 days/week ☐

Everyday 1x/day ☐

When do you come to this park:

Weekdays ☐

Weekends ☐

Both ☐

Type of walking:

On-leash ☐

Off-leash ☐

Both ☐

Frequency of off-leash dog in this park:

Never ☐

Rarely ☐

Sometimes ☐

Always ☐

Picking-up the faeces of the dog: Yes ☐ No ☐ **Always:** Yes ☐ No ☐

- **Walking to other parks**

Do you visit any other dog-attending parks: Yes ☐ No ☐

Which parks: _____ **Location:** _____

Which one of the additional parks do you visit most often: _____

How often do you come to this park:

Rarely 0-3x/year ☐

Occasionally <1x/month ☐

Infrequently 1-3 days/month ☐

Regularly 1x/week ☐

Often 2-6 days/week ☐

Everyday 1x/day ☐

When do you come to this park:

Weekdays ☐

Weekends ☐

Both ☐

Type of walking:

On-leash ☐

Off-leash ☐

Both ☐

Frequency of off-leash dog in this park:

Never ☐

Rarely ☐

Sometimes ☐

Always ☐

VETERINARY CARE

Visit to the veterinarian within the last 12 months: Yes ☐ No ☐

Internal Deworming Frequency:

1x/year ☐ 2x/ year ☐ 3x/ year ☐ 4x/ year ☐ 5x/ year ☐ 6x/ year ☐ monthly ☐ other ☐

Internal Deworming in the last 6 months: Yes ☐ No ☐

Active principle /commercial brand: _____

CHARACTERIZATION OF THE OWNER-PET RELATIONSHIP

Is the pet allowed to:

Lick the owners' face? Yes ☐ No ☐

Visit the owners' bedroom? Yes ☐ No ☐

Sleep with the owners in bed? Yes ☐ No ☐

Annex 4 – Qualitative analysis for consistency, colour and mucous of faecal samples

Table 24 – Distribution of qualitative analysis for consistency, colour and mucous of faecal samples from each park - Algés, Benfica and Campo Grande - and in total (CI95%)

		Algés (n=125)	Benfica (n=124)	Campo Grande (n=120)	Total (n=369)
Consistency	Shaped	86 68.8% (59.8-76.6%)	108 87.1% (79.6-2.2%)	77 64.2% (54.9-72.6%)	271 73.4% (68.6-77.8%)
	Semi soft	19 15.2% (9.6-23.0%)	9 7.3% (3.6-13.7%)	12 10.0% (5.5-17.2%)	40 10.8% (8.0-14.6%)
	Soft	19 15.2% (9.6-23.0%)	7 5.7% (2.5-11.7%)	30 25.0% (17.8-33.9%)	56 15.2% (11.8-19.4%)
	Liquid	1 0.8% (0.0-5.0%)	-	1 0.8% (0.0-5.2%)	2 0.5% (0.1-2.2%)
Colour	Greenish brown	1 0.8% (0.0-5.0%)	1 0.8% (0.0-5.1%)	1 0.8% (0.0-5.2%)	3 0.8% (0.2-2.6%)
	Coppery brown	6 4.8% (2.0-10.6%)	-	1 0.8% (0.0-5.2%)	7 1.9% (0.8-4.1%)
	Light brown	22 17.6% (11.6-25.7%)	18 14.5% (9.1-22.2%)	17 14.2% (8.7-22.0%)	57 15.5% (12.0-19.6%)
	Brown	81 64.8% (55.7-73.0%)	102 82.3% (74.2-88.3%)	91 75.8% (67.0-83.0%)	274 74.3% (69.4-78.6%)
	Dark brown	15 12.0% (7.1-19.3%)	3 2.4% (0.6-7.4%)	10 8.3% (4.3-15.2%)	28 7.6% (5.2-10.9%)
Mucous	Present	4 3.2% (1.0-8.5%)	5 4.0% (1.5-9.6%)	16 13.3% (8.1-21.0%)	25 6.8% (4.5-10.0%)
	Absent	121 96.8% (91.5-99.0%)	119 96.0% (90.4-98.5%)	104 86.7% (79.0-92.0%)	344 93.2% (90.0-95.5%)