UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

THE FIRST EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF CARDIOPULMONARY AND GASTROINTESTINAL PARASITES IN CATS AND DOGS FROM THE ALGARVE REGION OF PORTUGAL USING THE FLOTAC TECHNIQUE

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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RESUMO

PRIMEIRO RASTREIO DE PARASITAS CARDIOPULMONARES E GASTROINTESTINAIS EM CÂES E GATOS DA REGIÂO DO ALGARVE UTILIZANDO A TÉCNICA DE FLOTAC

Apesar de nas últimas décadas terem surgido vários trabalhos de rastreio parasitológico em Portugal, atualmente continuam a existir poucos dados sobre a incidência de parasitas gastrointestinais e cardiopulmonares de carnívoros domésticos com relevância clinica no Algarve. Por esta razão foi realizado um estudo entre Fevereiro e Abril de 2016 utilizando a técnica do FLOTAC. No total foram colhidas 142 amostras de canis e gatis da região correspondendo a 66 cães e 76 gatos todos com > 6 meses de idade.

Foram detetadas formas de eliminação fecal de seis grupos de endoparasitas, quatro dos quais com potencial zoonótico. A prevalência global no estudo foi de 31%, com 21.2% das amostras de canídeo positivas e 39.5% de felídeo positivos para pelo menos um parasita. Infeções mistas com dois parasitas foram observadas em 2.6% das amostras de cão e 9.2% das de gato. *Toxocara* sp. foi o parasita mais frequente em cães evidenciando-se em 13.6% (OPG: 61.3 ± 74.65) das amostras e em 31.6% (OPG 523.92 ± 688.75) das de gato. Nos cães também foram detetadas amostras positivas para Ancylostomatidae (3%), Taeniidae (6%) e *Trichuris* spp. (2%) e nos gatos Ancylostomatidae (5.3%), *Aelurostrongylus abstrusus* (4%), *Cystoisospora* spp. (6.6%) e Taeniidae (1.3%).

A presença de parasitas com potencial zoonótico na população estudada bem como e elevada prevalência de *Toxocara* spp. é preocupante e alerta para a necessidade de desparasitação regular e medidas de controlo higio-sanitário adequadas, quer em animais com proprietário, quer em animais de abrigo.

Palavras chave: cão, gato, parasitas gastrointestinais, parasitas cardiopulmonares, zoonoses, FLOTAC.

ABSTRACT

THE FIRST EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF CARDIOPULMONARY AND GASTROINTESTINAL PARASITES IN CATS AND DOGS FROM THE ALGARVE REGION OF PORTUGAL USING THE FLOTAC TECHNIQUE

Although over the past decades various parasitologal surveys have been conducted in Portugal, at present scant information is available on the prevalence of clinically-relevant gastrointestinal and cardiopulmonary parasites in the Algarve. This study performed between February and April 2016 using the FLOTAC technique was undertaken to address this. Faecal samples were collected from 66 dogs and 76 cats > 6 months old from shelters in the region.

Faecal forms of six different groups of endoparasites were detected, four of which with zoonotic potential. The overall prevalence in both species was 31%, with 21.2% of the dog samples positive and 39.5% of the cat samples positive for at least one parasite. Concurrent infections with two parasites were detected in 2.6% of the dog samples and 9.2% of the cats. *Toxocara* spp. was the most common parasite found in 13.6% (EPG: 61.3 ± 74.65) of the dogs samples and 31.6% (EPG 523.92 ± 688.75) of the cats. Other parasites found in dogs were Ancylostomatidae (3%), Taeniidae (6%) and *Trichuris* spp. (2%) and in cats Ancylostomatidae (5.3%), *Aelurostrongylus abstrusus* (4%), *Cystoisospora* spp. (6.6%) and Taeniidae (1.3%) were found.

The presence of zoonotic parasites in the population studied as well as the elevated prevalence of *Toxocara* spp. overall are concerning and highlight the need for regular prophylaxis, hygiene and sanitary control measures in pets as well as shelter animals.

Key words: dog, cat, gastrointestinal parasites, cardiopulmonary parasites, zoonosis, FLOTAC.

INTRODUCTORY NOTE

The present document is the result of the work developed during the author's traineeship period as part of the requirements for the attribution of the Master's degree in Veterinary Medicine. The results of the research project were presented in the form of a paper and poster communication at the 7th International EFOMV congress in Lisbon, Portugal held on 26th and 27th of November, 2016). (Appendix IV)

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LIST OF SYMBOLS

% – Percentage

& – And

µm – Micrometer

= – equals

< – less than

/ – division

+ – sum of or addition

± – more or less than

x – multiplication

® – registered trademark

LIST OF ABBREVIATIONS

A. caninum – Ancylostoma caninum

A. brazilienze – Ancylostoma braziliense

A. ceylanicum – Ancylostoma ceylanicum

A. vasorum – Angiostrongylus vasorum

BID – bis in die (twice per day)

C. felis – Cystoisospora felis

C. rivolta – Cystoisospora rivolta

CLM – cutaneous larva migrans

CNS – central nervous system

E. granulosus – Echinococcus granulosus

E. multilocularis – Echinococcus multilocularis

EPG – eggs per gram

ESCCAP – European Scientific Counsel Companion Animal Parasites

FLOTAC – Flotation and centrifugation

FMV-UL – University of Lisbon Faculty of Veterinary Medicine

FS – Flotation solution

GI – Gastrointestinal

kg – kilogram

L1 – First-stage larva

L2 – Second-stage larva

L3 – Third-stage larva

L4 – Fourth-stage larva

L5 – Fifth-stage larva

LRTI - Lower respiratory tract infections

mg – milligram

NLM – neural larva migrans

OLM – ocular larva migrans

OPG – Oocysts per gram

PCR – Polymerase chain reaction

PI – post infection

SCT – sedimentation and counting technique

s.g. – Specific gravity

SID – semel in die (once per day)

sp. – specie

spp. – species

TID – ter in die (three times per day)

T. canis – *Toxocara canis*

T. cati – *Toxocara cati*

T. vulpis – *Trichuris vulpis*

VLM – visceral larva migrans

VRSACM – Vila Real de Santo António & Castro Marim

CHAPTER I - INTRODUCTION

Companion animals are responsible for transmitting some of the most significant zoonotic parasitic diseases affecting man (Baneth et al., 2015). Knowledge relating to the epidemiology, modes of transmission, life cycles, pathogenicity, prevention and treatment of these agents is therefore of great importance. Indeed healthy animals not only contribute to physical well-being and mental health, but they are also in part responsible for healthy people (Paul, King, & Carlin, 2010).

In most cases appropriate prophylaxis and good hygiene practices are sufficient to reduce propagation and eliminate infections. However, many factors can influence these including a lack of knowledge on the subject or financial constraints. Indeed a recent study in Portugal found that although the majority of pet owners surveyed do give antiparasitic drugs to their pets, they do so at irregular intervals rendering them ineffective (M. Matos, Alho, Owen, Nunes, & Madeira de Carvalho, 2015).

At present, little information is available on the prevalence and types of cardiopulmonary and gastrointestinal parasites affecting cats and dogs in the Algarve, the most Southern region of Continental Portugal. The epidemiological study described herein, was undertaken to address this and is the first time the FLOTAC technique, a new multivalent technique for qualitative and quantitative copromicroscopic analysis technique developed by Cringoli, Rinaldi, Maurelli, & Utzinger (2010), was used in the region.

It is hoped that the information gathered may ultimately contribute to a better understanding of the health status of the animals in the region and serve as a guide for local authorities, veterinarians and shelters to implement more strategic and targeted prophylactic treatments in the future.

The present dissertation includes an account of the clinical activities undertaken in the first part of the training period, followed by a literature review and discussion of the research project.

CHAPTER II - TRAINING PERIOD ACTIVITIES

2.1 Hospital Veterinário de Loulé

The first period supervised by Dr. Dário Santinha took place between the 14th of April and the 28th of August 2015 at the Loulé Veterinary Hospital in the Algarve, Portugal (around 720 hours). The Hospital, founded in 2012, is a full-service modern facility centrally located in the Algarve with a 24 hour emergency service providing routine medical and surgical care for dogs, cats and other companion animals. Great emphasis is directed towards the prevention of disease and this is promoted by encouraging owners to regularly deworm and vaccinate their pets, as well as informing them of the importance of a good diet and exercise.

The facility has a fully equipped laboratory, with modern diagnostic equipment including chemistry and haematology analysers, where routine clinical biochemistry, haemograms and blood smears can take place in house. Diagnostic imaging is catered for with digital x-ray equipment, a modern ultrasound machine with Doppler capabilities for colour flow visualization, as well as endoscopy equipment enabling state of the art internal investigation.

The hospital has comfortable separate dog, cat and isolation ward rooms, as well as an intensive care unit, where constant observation is possible and all needs catered for including the provision of oxygen to high dependency respiratory patients and monitoring using intensive care equipment. Surgery is performed in a modern operating theatre with state of the art anaesthetic monitoring equipment and the highest hygiene and sterilisation standards are adhered to for all procedures.

During the internship period the author had the opportunity to attend the consultations that took place and assist by: helping to restrain animals for examination or treatment, preparing and administering medication, gathering patient history, carrying out supervised physical examinations and position patients for some diagnostic imaging procedures.

The author also assisted in the care of the hospitalized patients by helping to monitor critically ill animals, as well as preparing and administering medication. Other duties included cleaning beds, preparing food, feeding, changing water and taking the patients that were able for walks. There was also the opportunity to help care for some

critically ill patients in the isolation ward which required special attention and care so as not to spread the contagious diseases to the remaining patients.

The number of surgeries performed was large and the author assisted in setting up the surgical suite as well as preparing the patients for surgery which included preparing pre-surgical medications. A variety of procedures were observed from routine spaying and neutering to more complex orthopaedic procedures as well as emergency surgeries including gastropexy for patients with gastric torsion. During less critical procedures the author was tasked with monitoring anaesthesia and post recovery.

Figure 1 The Loulé Veterinary Hospital

Figure 2 “Nina”, the Hospital's first client

2.2 Laboratory for Parasitic diseases FMV-ULisboa

The research component, supervised by Prof. Doctor Luis Madeira de Carvalho, took place between February and September 2015 and involved the collection of faecal samples from kennels and catteries in the Algarve followed by processing and analysis which took place at the University of Lisbon, Faculty of Veterinary Medicine’s laboratory for Parasitic diseases (FMV-UL). Work undertaken during the second period formed the basis for the research component in this document.

CHAPTER III - REVIEW OF LITERATURE

3.1 Nematodes

Nematodes, otherwise known as roundworms, are a large phylum with over 25,000 species described thus far (Zhang, 2013). These worm-like organisms are diverse and parasite a variety of animals and plants and can be found in a wide range of different environments. Morphologically they are bilaterally symmetrical and have tubular digestive systems with openings on both extremities. Sexual dimorphism occurs with males being smaller than females (Ballweber, 2001)

Most parasitic roundworms do not need an intermediate host for the development of their free-living stages and can therefore infect their final hosts directly. Pregnant females within the host produce thousands of eggs daily which are then excreted in the host faeces into the environment. If conditions are favourable the egg will hatch into a L1 larvae, these then feed on bacteria or other microorganisms. They then develop and molt twice transforming from L1 into L2 and then L3 larvae which for most species is the infective stage. Once ingested by the host the larvae migrate to their preferred site within the host, usually an organ, where they develop into adults and begin reproducing.

Gastrointestinal (GI) infections caused by nematodes are a major threat to human health and currently affect over half of the world's population, causing hundreds of thousands of deaths annually as well as great morbidity (Stepek, Buttle, Duce, & Behnke, 2006).

3.2 Ancylostomatidae

Family Ancylostomatidae has 7 genera including *Uncinaria* sp. and *Ancylostoma* sp. This family belongs to Phylum Nematoda, Class Secernentea, Order Strongylida, Superfamily Ancylostomatoidea and generally designated hookworms (Taylor, Coop, & Coop, 2016).

Adult hookworms are small intestine parasites that can cause varying degrees of blood loss. These small nematodes have large mouthparts at an angle to the rest of the body, hence the name (ESCCAP, 2010a) *Ancylostoma* spp. species of veterinary importance in Europe (Table 1) include *A. caninum* (dogs and foxes) which exists worldwide, *A.*

tubaeforme (cats) worldwide, *A. braziliense* (dogs and cats) that can be found in tropical and subtropical environments, *A*. *ceylanicum* (dogs and cats) can be found in many parts of Asia and *Uncinaria stenocephala* which affects dogs, foxes and rarely cats and usually exists in cooler northern temperate regions, including Northern America and Europe (Ballweber, 2001; Zajac & Conboy, 2012).

Table 1 Characteristics of the major hookworms affecting cats and dogs in Europe. Adapted from ESCCAP (2010)

Worm species	Pre-patent period	Patent period	Infective stages and route of infection	Distribution in Europe	Final hosts
Ancylostoma caninum	2-3 weeks	Can be prolonged depending on immune status (7 months to 2 years)	Ingestion of L3 from environment, larvae in bitches' milk or paratenic hosts. Percutaneous infection of larvae	Mainly southern Europe, sporadic in northern Europe	Dogs and foxes
Ancylostoma tubaeforme	2-3 weeks	Can be prolonged depending on immune status	Primary ingestion of larvae from soil. Some percutaneous infection	Continental Europe	Cats
Uncinaria stenocephala	3-4 weeks	Can be prolonged depending on immune status	L3 orally from environment	Predominantly central and northern Europe	Dogs, foxes and cats

3.2.1 Life cycle

As with most parasitic nematodes *A. caninum* life cycle is direct. Once the eggs have been excreted into the environment they develop and may hatch into filariform larvae and become infective within 5 to 8 days. Environmental conditions favourable to larval development include warmth (23ºC to 30ºC), humidity, shade and poorly-drained soil (Ballweber, 2001; Bowman, 2014). The host becomes infected either by ingesting the L3 larvae or by percutaneous infection where the larvae penetrate the skin. Once in the host the larvae migrate to the lungs via the bloodstream. Within the trachea and bronchi they then moult into L4 and are then swallowed, allowing them to enter the small intestine where a final moult occurs.

If infective larvae have been ingested, they either burrow into the oral mucosa, and then migrate to the lungs as mentioned previously, or they may progress directly to the intestine where the adult worms burrow their buccal capsules into the intestinal mucosa to obtain nutrients: mainly blood in the case of *Ancylostoma* spp*.* and plasma for *U. stenocephala.* This burrowing action ultimately damages the intestinal surface. In either case the pre-patent period is between 14 to 21 days (ESCCAP, 2010a). Dogs

infected with *Ancylostoma* sp. can excrete millions of eggs each day over a period of several weeks.

Although transplacental transmission does not occur, infected bitches can infect their offspring for up to three weeks. This is because *Ancylostoma* spp. larvae can become arrested at the L3 stage and remain so until the bitch is pregnant. In some susceptible females L3 larvae that have migrated to the lungs may become dormant (Taylor et al., 2016). Transmammary transmission only occurs in dogs infected with A. *caninum* (Bowman, Montgomery, Zajac, Eberhard, & Kazacos, 2010).

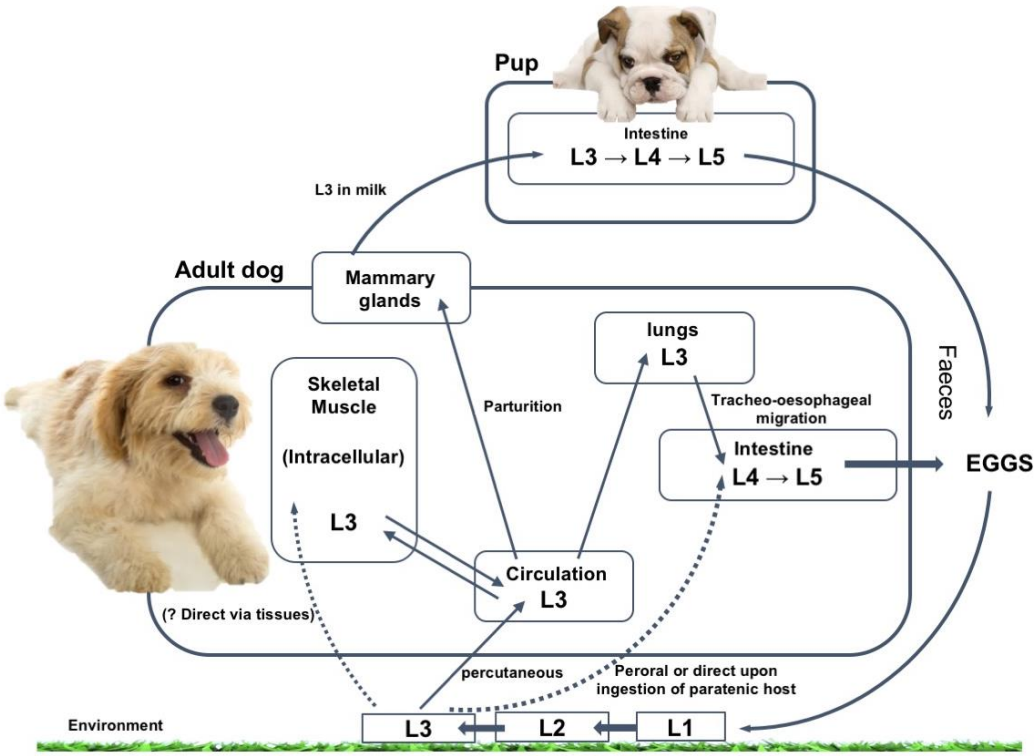


Figure 3 *Ancylostoma* spp. life cycle (original illustration adapted from Prociv & Croese (1996)).

3.2.2 Pathogenesis and clinical signs

Disease by *A. caninum* may take one of four forms (Ballweber, 2001; Taylor et al., 2016)

1. **Peracute**. This form occurs in very young pups due to the ingestion of infective larvae in the dam's milk. As few as 50 to 100 adult *A. caninum* may be fatal. The pups are born healthy but their condition deteriorates rapidly. Pups are particularly vulnerable due to their low iron reserves (Taylor et al., 2016).
2. **Acute hookworm disease** - the sudden exposure of older pups or adults to large numbers of infective larvae can cause severe anaemia and occasionally

respiratory embarrassment (Taylor et al., 2016) . The burden may be so great that clinical signs may precede the presence of eggs in the faeces by about 4 days (Bowman, 2014).

3. **Chronic (compensated)** - usually occurs in immunocompetent animals that have not been exposed to overwhelming numbers of larvae - clinical signs are not usually apparent. Hookworm eggs are present in the faeces and there are significant reductions in the erythrocyte count, haemoglobin or packed cell volume.
4. **Secondary (decompensated) hookworm disease** - occurs in older animals and may be secondary to other problems. Severe anaemia usually occurs; the animal may be malnourished or even emaciated.

Infected hosts may experience exhibit respiratory signs either due to lung damage caused by larvae or the anoxic effects secondary to the anaemia caused by blood loss due to the parasites. *Ancylostoma caninum*, *A. tubaeform* and *A. ceylanicum* are more pathogenic than other hookworms like *U. stenocephala* and *A. brasiliense* as they cause more blood loss and consequent anaemia (Bowman et al., 2010). If the infection is chronic the host may be emaciated and weak, there may also be a loss of appetite and occasionally pica. Cutaneous lesions, lameness and respiratory embarrassment have also been described (Taylor et al., 2016). Immunity although not complete, does develop following exposure (ESCCAP, 2010a).

3.2.3 Morphology and identification

The eggs are oval shaped and have characteristic thin shells that can be easily seen on flotation. *Ancylostoma* spp. eggs range from 52–79 × 28–58 µm whereas *Uncinaria* spp. have dimensions between 71–92 × 35–58 µm. (Zajac & Conboy, 2012). Ancylostomatidae eggs are however indistinguishable morphologically making epidemiological studies complicated (Bowman et al., 2010).

In dogs adult female worms can grow to 14-20mm while males can range from 11-13mm (Prociv & Croese, 1996). Buccal cavities also differ between species, whereas *Ancylostoma* spp. have sharp teeth, *Uninaria* spp. have cutting plates (Bowman, 2014). Depending on the species of *Ancylostoma* spp. the ventral margin of the stoma may have either one (*Ancylostoma brasiliense*), two (*Ancylostoma duodenale*) or three

pairs of sharp teeth (*A. caninum* and *A. tubaeforme*), (Bowman, 2014). The three teeth enable the differentiation between these and otherwise similar human parasites (Prociv & Croese, 1996).

3.2.4 Diagnosis

When diagnosing hookworm infections ante-mortem, clinical signs and patient history should be considered and upon suspicion, a haematological and faecal examination should be performed. Hookworm eggs can be detected morphologically using faecal flotation techniques (ESCCAP, 2010a). Depending on the technique used, a quantitative assessment of the burden may also be obtained. ESCCAP (2010) guidelines currently recommend the collection of between 3 and 6 g of fresh or fixed faeces for accurate diagnosis.

According to Zajac & Conboy (2012), “In the case of peracute hookworm disease, eggs will not be found on faecal flotation because the profound anaemia occurs before adults begin laying eggs”. Taylor et al. (2016), also noted that “suckled pups may show severe clinical signs before eggs are detected in the faeces.” Although high egg counts may help confirm the health problem is due to hookworm related disease, the presence of small numbers of hookworm eggs in faeces does not necessarily confirm that the underlying problem is hookworm disease (Taylor et al., 2016).

On post-mortem examination Ballweber (2001, p. 142) describes how adult worms are found in the small intestine, especially the jejunum and may be red and up to 2.8 cm in length with the anterior end bent dorsally resulting in a “hook”. The number of teeth in the buccal cavity enables differentiation.

3.2.5 Treatment and control

In acute infection or the chronic (compensated) form, the response to simple anthelmintic therapy is very effective and no further therapy beyond an adequate diet is necessary (Bowman, 2014). Anthelmintics such as mebendazole, fenbendazole or nitroscanate are effective at killing adult and developing intestinal stages. Pyrantel is also effective (Taylor et al., 2016), but may be becoming less so. High-level pyrantel resistance was found in some Brisbane isolates of *A. caninum* (Kopp, Kotze, McCarthy, & Coleman, 2007). Although macrocyclic lactones are effective, in severe cases anthelmintics alone may not be sufficient and supportive therapy may also be

necessary. In these cases a protein rich diet and parental iron are advised (Taylor et al., 2016) to encourage haematopoiesis and recovery vitamin B12 may also be given. Arrested fourth-stage larvae may be resistant to anthelmintic treatment and therefore anthelmintics must be given again once these larvae mature (Taylor et al., 2016).

To reduce the risk or transmammary infection bitches should be treated with an anthelmintic that is effective against somatic larvae (Taylor et al., 2016). To achieve this, from day 40 of gestation infected bitches can be treated fenbendazole 50mg SID through to day 14 of lactation. Alternatively, 4-9 days before giving birth ivermectin 0.5mg/kg can be given a second treatment should then be followed 10 days later (Ballweber, 2001). To reduce the risk of infection a drug appropriate for use in nursing pups should be given at least twice, once at the 1-2 week mark and then again two weeks later. This also helps to control ascarid infections (Taylor et al., 2016).

As with most parasitic agents, prophylaxis is the best form of action and may take the form of regular anthelmintic therapy which should take place at least quarterly for both weaned pups and adults (ESCCAP, 2010a). Good hygiene practices should also be encouraged (Taylor et al., 2016). In situations where young pups are present, bedding should be changed daily, flooring should be cleaned at least twice a week and kept as dry as possible (Ballweber, 2001). Following the removal of faeces with a shovel, the floor should be hosed with water and then a 1% solution of sodium hypochlorite should be sprayed on the paved surfaces (Taylor et al., 2016). To facilitate the cleaning process kennel floors should be crevice free, a tarmac or concrete flooring is recommended (Stepek et al., 2006; Bowman et al., 2010). In cases where and outbreak may have occurred earth runs can be treated with sodium borate as it is lethal to hookworm larvae (Taylor et al., 2016). The success of any regular hookworm treatment should be confirmed through regular faecal examinations. (Bowman et al., 2010)

3.2.6 Zoonotic risc

Hookworms species believed to be human specific include *Ancylostoma duodenale* and *Necator americanus*. However canine and feline hookworms, including *A. braziliense*, *A. caninum, A. ceylanicum* (Stepek et al., 2006) and *Uncinaria stenocephala* can cause zoonotic disease most notably cutaneous larva migrans or CLM (Stepek et al., 2006; Bowman et al., 2010). This occurs when nematode larvae,

penetrate the human's skin and begin migrating under it. Although these parasites perform complete migrations in their natural hosts, migrate to the lungs from where they are then swallowed and then enter the small intestine where they begin egg production. In paratenic hosts like humans their development generally stops at the lungs.

According to Bauerfeind, Graevenitz, Kimmig, Schiefer, & Schwarz, (2015 p. 431) In the classic form caused by *A. braziliense* "The burrows appear on the skin surface as elevated alterations, up to 2 mm wide, with surrounding erythema, oedema, and crusts". This may cause itching and some pain, secondary infections may develop due to the scratching. Symptoms may disappear between 2 to 8 weeks later or persist for up to 2 years if untreated (Bauerfeind et al., 2015).

In the case of *A. caninum* follicular alterations are more pronounced and pustules may also develop, this species also tends to spread in the organism due to the way it invades human blood vessels. In these cases Eosinophilic infiltrations as well as pneumonia have been described. (Bauerfeind et al., 2015). In Asia, Inpankaew et al., (2014) reported how *A. ceylanicum,* a hookworm of cats and dogs in Asia, is becoming the "second most common hookworm infecting humans".

As the main form of infection is via the skin, it is strongly recommended that humans wear appropriate footwear when walking in potentially infected areas. Effective treatments include Albendazole 400mg BID orally for 3 to 7 days or Ivermectin 200 micrograms/kg/ SID orally for two days (Bauerfeind et al., 2015).

3.3 *Toxocara* spp.

Toxocara spp. are important gastrointestinal helminths affecting domestic animals, some of which are also zoonotic. These roundworms or ascarids, with worldwide distribution, inhabit the small intestines of dogs, cats and other mammalian hosts and belong to Phylum Nematoda, Class Secernentea, Order Ascaridida, Superfamily Ascaridoidea, Family Ascarididae (Taylor et al., 2016). Common species found in Europe (Table 2) include *Toxocara canis* (Werner, 1782) which affects dogs and foxes, *Toxocara cati* (Schrank, 1788) that mainly infects cats and *Toxascaris leonina* that also affects dogs and foxes, as well as cats.

Table 2 Characteristics of roundworms or ascarids found in Europe. Adapted from ESCCAP (2010) with information from (Bowman, 2014)

Worm species	Pre-patent period	Patent period	Infective stages and route of infection	Distribution in Europe	Final hosts
Toxocara canis	Variable, typically 21 days after infection; 27-35 days after lactogenic infection; 32-39 days after ingestion of eggs	4-6 months except where immunity intervenes, for example in pups	Ingestion of embryonated eggs from soil or on fur, larvae in milk or paratenic hosts. *In utero* from dam	Worldwide	Dogs and foxes
Toxocara cati	Variable, usually around 6 weeks after ingestion of eggs.	4 – 6 months	Ingestion of embryonated eggs from soil or paratenic hosts. Transmammary transmission if cat acutely infected at end of pregnancy.	Worldwide	Cats and other wild felids
Toxascaris leonina	About 8 weeks	4 - 6 months	Ingestion of embryonated eggs from soil or larvae from paratenic hosts	Worldwide	Dogs, cats and foxes

3.3.1 Life cycle

The life cycles of both *T. canis* and *T. cati* are similar. In this review details of *T. canis* will be described with additional remarks on the differences to *T. cati*. There are currently four known pathways for *Toxocara* spp. infection in a suitable host: ingestion of embryonated eggs from the environment, ingestion of infected paratenic hosts, transplacental transmission and transmammary transmission.

The cycle begins when adults in the hosts' small intestine shed single-celled eggs which are then excreted into the environment during defecation. The eggs then undergo two moulting steps where structural modifications take place, developing into L3 which is the infective stage. According to Schnieder, Laabs, & Welz (2011) optimal conditions are temperatures between 25 and 30ºC and a relative humidity of 85-95%. If conditions or soil type are not optimal the development can take between 3 to 6

weeks or several months. The embryonated eggs may survive for more than a year in the environment if conditions are optimal (Overgaauw & Knapen, 2008).

Once ingested the eggs hatch and the larvae pierce the hosts gut wall within 2-4 hours, they then use the hosts blood vessels and undergo somatic migration throughout the body, remaining as somatic larvae in the tissues. This form of the disease is known as visceral larva migrans or VLM and according to Overgaauw & Knapen (2008) occurs in most adult dogs and cats with some degree of acquired immunity.

Parasite migration appears to depend on the hosts age as well as the infective load (Schnieder et al., 2011) In younger animals less than 3 months old, tracheal migration is most common. Once the L3 larvae have reached the lungs they then penetrate the alveoli, pass though the bronchioles and migrate up the trachea where they are then swallowed and mature in the intestinal tract as L4 stage larvae. It takes between 7-9 days following infection for larvae to be detected in the trachea and oesophagus (Schnieder et al., 2011). The larvae then mature in the small intestine into L5 adults which can take between 7 to 15 days post infection – PI. Significantly, Lee et al., (2010) describe how a low infective dose of 100 embryonated eggs consistently induces infection in adult dogs and how the risk of re-infection is the same for naïve dogs as it is for those infected transplacentally as puppies.

Although patent *Toxocara* infections are much more prevalent in young dogs and cats when compared to adults (Overgaauw & Knapen, 2008), dogs and cats of all ages can become infected with their specific *Toxocara* species through the ingestion of infective eggs or paratenic hosts (Lee et al., 2010). In older puppies and adults once the larvae become trapped in the lung capillaries they undergo somatic migration and re-enter the bloodstream. Somatic larvae then accumulate in tissues where they become arrested for long periods of time. As reviewed by Overgaauw & Knapen (2008) *T. canis* larvae are more commonly found in the central nervous system, whereas *T. cati* is more common in the muscles.

Transplacental transmission is the most common source of *T. canis* infection in dogs, but does not happen in kittens (Overgaauw & Knapen, 2008; Schnieder et al., 2011). It occurs when encysted larvae are reactivated in the final stages of pregnancy thereby enabling the transplacental and transmammary route to puppies. This reactivation is believed to be due to alterations in hormone levels in the final third of the gestation

period or the intrinsic immunosuppression that occurs in the periparturient period. Transplacental migrations cause infection of the foetus' liver and following birth tracheal migrations occur with the puppies shedding eggs within as little as two weeks (Overgaauw & Knapen, 2008; Schnieder et al., 2011)

Transmammary transmission occurs in both dogs and cats (Schnieder et al., 2011; Bowman, 2014). After activation, somatic *Toxocara* spp. larvae migrate to the milk ducts and are transmitted via the colostrum and the milk. Once ingested the larvae develop, but do not perform tracheal migrations. This route of infection is less significant than the transplacental mode in puppies, but is the primary mode of infection for kittens where faecal egg excretion can be observed 7 weeks following lactogenic transmission (Schnieder et al., 2011).

The ability of some larval nematodes to remain viable in paratenic hosts, namely prey species, enables the parasites continuing survival and distribution. This mode of transmission is effective as the parasite can develop somatically in a variety of vertebrates including rabbits and rodents. Once an infected paratenic host is ingested the larvae develop directly in their intestine. The fact that cats are more avid hunters than dogs may explain why higher rates of infection are found (Overgaauw & Knapen, 2008).

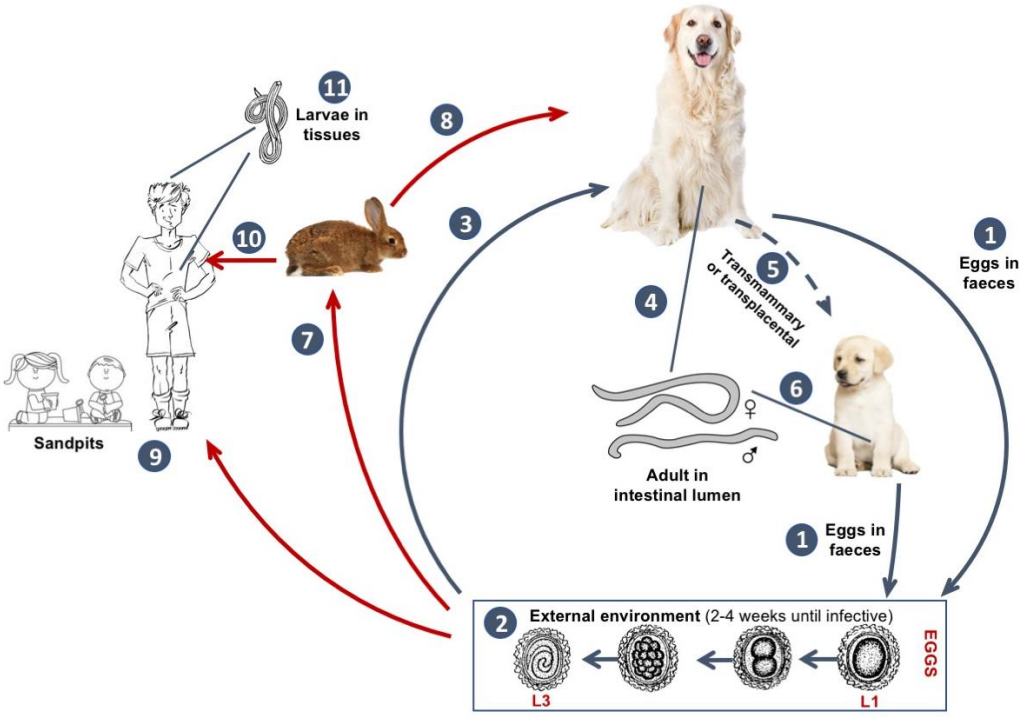


Figure 4 *Toxocara canis* life cycle (original illustration adapted from https://www.cdc.gov/parasites/toxocariasis/biology.html accessed on 10/05/17.)

(1) Definitive host sheds unembryonated eggs into the environment. (2) over a 2-4 week period the eggs develop into L3 and become infective. (3) Infected eggs ingested by dogs, hatch and penetrate the gut wall. In younger dogs migrations to the lungs, bronchial tree and oesophagus occur. (4) Adult worms mature into L5 and begin laying eggs in the small intestine. (5) During late pregnancy, encysted stages become reactivated and enable the transplacental and transmammary route to puppies. (7) After ingestion by paratenic hosts larvae hatch, penetrate the gut wall and migrate to various tissues where they encyst. (8) Dog ingests paratenic host and larvae develop into egg-laying adults in the small intestine. (9) Humans playing in infected sandpits act as accidental hosts through either the ingestion of infected soil or (10) infected paratenic hosts. Once ingested the eggs hatch and larvae penetrate the gut wall and circulate through the body to various tissues including: liver, lungs, brain, muscle, heart and eyes (11). (original figure) adapted from Toxocariasis life cycle: CDC - US. Department of health and human services - https://www.cdc.gov/parasites/toxocariasis/biology.html accessed on 10/05/17.

3.3.2 Pathogenesis and clinical signs

In the case of mild to moderate infections, during the pulmonary larval migration phase, there is little apparent damage to the tissues and external clinical signs may be absent (Taylor et al., 2016). The presence of adults in the intestine may cause mechanical damage to the small intestine and give the animal a bloated or pot-belly appearance, which may also cause stunted growth as well as occasional vomiting and diarrhoea (Taylor et al., 2016). In heavy infections affecting pups, the larval migrations can cause pulmonary damage and pulmonary oedema which may cause coughing, frothy nasal discharge and increased respiratory rate. (Taylor et al., 2016). The pups and kittens

may also exhibit mild mucoid enteritis, vomiting, diarrhoea and general unthriftiness (Ballweber, 2001). In the gut the parasites may cause mucoid enteritis which in rare cases can cause a partial or complete occlusion. *T. canis* is most lethal during the pulmonary phase and pups heavily infected transplacentally or lactogenically may die within 2-3 weeks of birth (Ballweber, 2001). Although nervous convulsions have been attributed to toxocarosis, it is not confirmed whether the parasite is responsible.

3.3.3 Morphology

Fresh *Toxocara* eggs are subspherical with a thick shelled wall, with a markedly pitted surface containing a round, single celled embryo (Uga et al., 2000). Although it is difficult to differentiate the eggs from both species, *T. canis* eggs are normally more subspherical that *T. cati* which tend to be more elliptical (Zajac & Conboy, 2012). Morphometric analysis of 442 eggs identified as either *T. canis* or *T. cati* by Fahrion, Schnyder, Wichert, & Deplazes (2011) revealed that *T. canis* eggs had dimensions of 62,3 x 72,7µm (mean values) while *T. cati* were slightly larger at 74,8 x 86,0µm (mean values). Differentiating both species by microscopy is difficult as described by Uga et al. (2000) who found that although both species have “markedly pitted surfaces” and that the “pitting in *T. canis* is more coarse than *T. cati”*, the differences were not absolute. These authors also found that egg measurement wasn’t sufficient to differentiate species as “approximately 90% of eggs measured were of similar size”.

In cats, it may also be necessary to differentiate between *T. cati* and *Toxascaris leonina*. Eggs of the latter often are slightly larger and range from 75-85 x 60-75µm, but similarly to *Toxocara* eggs they also contain a single celled embryo and a thick shelled wall, however unlike *T. cati* the embryo is light coloured. Whereas *Toxocara* eggs have rough outer shell walls, *Toxascaris* eggs have smoother outer shells.

Figure 5 Dividing *Toxocara* spp. egg, found in cat faeces (original). Scale bar 50µm.

Adult *T. canis* worms, most commonly seen in puppies, are cream coloured and can range from 10 to 15cm in length (Bowman, 2014). They have a glandular oesophageal bulb, cervical alae and their mouth openings are surrounded by three prominent lips. In fresh worms reproductive organs can appear white when viewed through the cuticle.

3.3.4 Diagnosis

Centrifugal or simple flotation techniques are indicated to detect eggs, in cases of severe infection a simple faecal smear with a drop of water enables the eggs to be seen. For accurate diagnosis 3 – 5g of faeces are required (ESCCAP, 2010a). The eggs observed are subspherical with a roughly pitted yellow-brown outer shell containing a large dark single cell. However, *T. canis* and *T. cati* eggs are not easily distinguished using these methods, suggesting that molecular techniques such as PCR may be more effective in determining the exact species infecting the host. Significantly, although it is assumed that eggs obtained comply with the species from which they are obtained, a study conducted by Fahrion et al., (2011) using PCR demonstrated that out of 35 dogs tested, 11 (31.5%) were in fact infected with *T. cati.* Notably, out of the 36 cats tested none were found to be infected with *T. canis.* It is also important to note that some dogs exhibit coprophagia making it difficult, at the time of diagnosis, to determine whether the animal has a patent infection or is simply passing ingested eggs back into the environment (Fahrion et al., 2011).

During the pulmonary phase, where the infection is heavy and larvae are migrating, a tentative diagnosis is possible simply based on the pneumonic signs which may appear in a litter within two weeks of birth (Bowman, 2014). According to Bowman (2014) on post-mortem puppies may appear pot-bellied, cachectic and poorly grown. Large numbers of white worms may be found in the small intestine are sometimes the stomach. The worms range from 3-10cm in length for *T. cati* and 10-18cm for *T. canis.* (Ballweber, 2001). The lungs of puppies with migrating *T. canis* larvae may also have focal haemorrhages.

3.3.5 Treatment and control

Due to the occurrence of transplacental transmission in pups, these should be treated with appropriate anthelmintics starting at 2 weeks of age, followed by 15 day intervals until two weeks after weaning, followed by monthly treatments for six months. Nursing females should be treated alongside their offspring's first treatment as they may have patent infections (ESCCAP, 2010a).

Monthly treatment is currently recommended by ESCCAP (2010) in high risk situations like where a family may have a pet and small children that share a common area such as a garden. This is because the pre-patent period, from the time of ingestion of larvae, either by paratenic hosts or infective eggs from the environment, is just over 4 weeks and therefore this regimen may minimise the risk. As the parasites are prolific egg layers and the eggs can survive for long periods of time in the environment regular anthelmintic therapy is required in particular if regular diagnostic testing, monthly to three monthly, is not performed (ESCCAP, 2010a). While anthelmintic treatment may be successful at removing adult worms it is not fully effective with juvenile or larval stages (Ballweber, 2001). Also, according to ESCCAP (2010) in the case of patent infections quarterly treatment may not be effective and in this case a monthly regimen may be proposed as it takes into account the parasite's biology. In all other cases, depending on the risk of multiple worm infections, a broad or narrow spectrum of activity anthelmintic should be given at least 4 times per year.

Effective anthelminthic drugs used to treatment *Toxocara* infections in dogs and cats include Macrocyclic lactones, Benzimidazoles and Pyrantel (ESCCAP, 2017).

3.3.6 Zoonotic risk

Both *T. canis* and *T. cati* can cause significant disease in people. (Lee et al., 2010). Humans may become infected (paratenic hosts) after ingesting embryonated eggs. This can occur for example in children who ingest contaminated objects or soil in areas such as gardens or sandpits. Insects can also act as transport hosts by spreading eggs from faeces they have fed on, to surfaces and foods. As with other mammals, humans may also be infected by ingesting other accidental hosts such as rabbits and chickens, which harbour L3 larval stages in their muscles and organs. The infective process is similar to domestic animals, whereby, upon ingestion the encysted larvae become reactivated and may migrate to human organs including the liver, lungs and eyes,

where tissue necrosis, chronic liver damage, oedema, haemorrhage and eosinophilia may occur. (Stepek et al., 2006)

Although humans infected with larval *Toxocara spp.* may be asymptomatic, a variety of symptoms may occur including VLM, OLM and neural larva migrans (NLM) (Bowman et al., 2010; Lee et al., 2010). Cases of NLM may be fatal or cause permanent neurological disease to intermediate hosts like man. To avoid infection, it is strictly recommended that high risk foods such as livers and other organs be thoroughly cooked. Regular deworming practices, strict handwashing and discouraging children from putting objects in their mouths are effective ways to reduce transmission.

3.4 *Trichuris* spp.

Trichuris spp. are intestinal parasites with importance in both veterinary and human medicine. They belong to Phylum Nematoda, Class Adenophorea, Order Enoplida, Superfamily Trichuroidea, Family Trichuridae (Taylor et al., 2016) and have a worldwide distribution. Commonly known as whipworms due to their characteristic whip shaped body the genus known as *Trichuris* includes a variety of nematodes affecting a variety of hosts from livestock to humans. For the sake of this review emphasis will be given to *Trichuris vulpis*, due to its distribution in central and southern Europe.

Table 3 *Trichuris vulpis* characteristics. Adapted from ESCCAP (2010)

	Pre-patent period	Patent period	Route of infection	Distribution in Europe	Final hosts
Trichuris vulpis	8 weeks	Up to 18 months	Ingestion of embryonated eggs	Everywhere but mainly in central a southern Europe	Dogs

3.4.1 Life cycle

After mating, adult females embedded in the mucosa of the caecum and colon lay non-infective single celled eggs which are then passed into the environment via faeces. Depending on moisture and temperature, the eggs then take up to 8 weeks to develop into their infective embryonated stage (Traversa, 2011). They then remain in the environment and don't hatch until ingested by a suitable host (Zajac & Conboy, 2012; Bowman, 2014).

After ingestion the egg plugs are lysed releasing the larvae which then penetrate the intestinal glands, during this process which takes up to two weeks the larvae moult and then colonise the large intestine becoming adults (Traversa, 2011). No extra intestinal migrations takes place (Bowman, 2014) and transplacental or transmammary transmission does not occur with this species (Traversa, 2011). The prepatent period takes around 8 weeks (ESCCAP, 2010a).

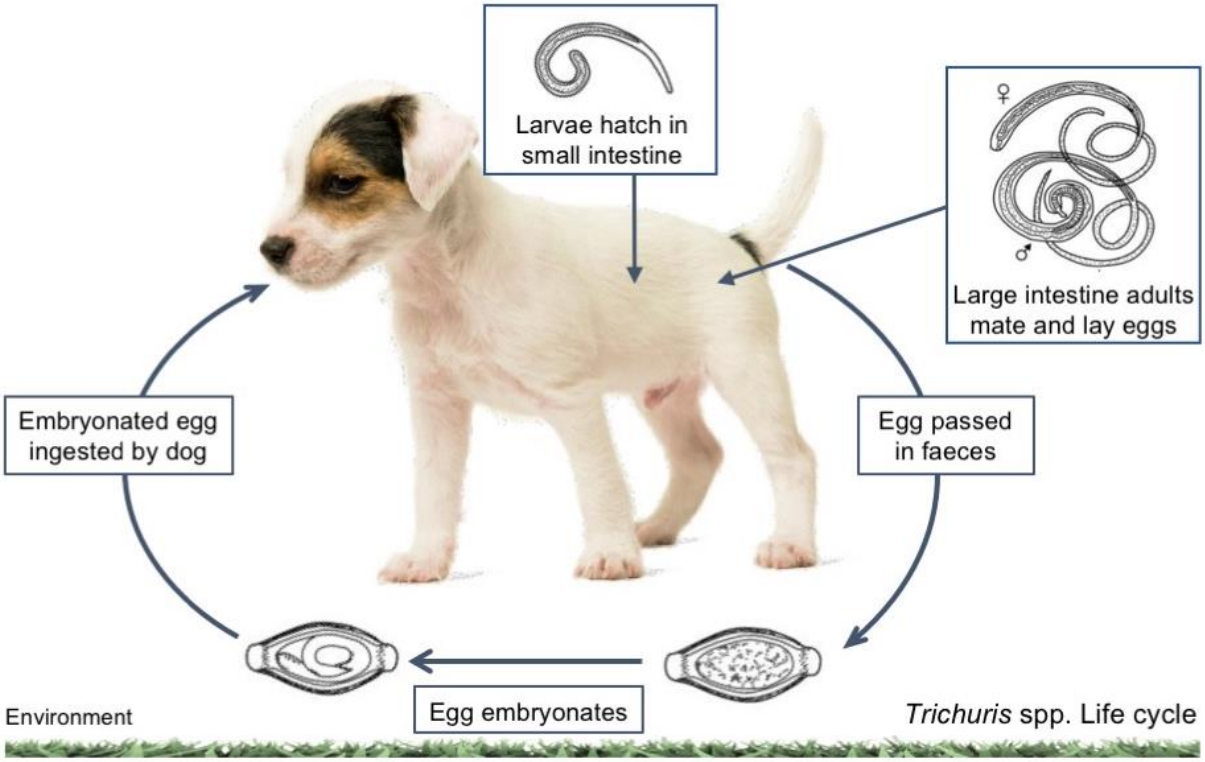


Figure 6 *Trichuris spp*. life cycle (original illustration adapted from ESCCAP, (2017)).

3.4.2 Pathogenesis and clinical signs

Most canine infections are unremarkable but heavy infections can cause diarrhoeal faeces, often containing mucus or blood, alternating with periods where normal stools are normal (Bowman, 2014). When less than 200 worms are present they can be found mainly in the cecum, as numbers increase they may also be found in the colon (Bowman, 2014).

As well as bloody diarrhoea, in heavy infections the dog may exhibit weight loss and unthriftiness (Zajac & Conboy, 2012). *Trichuris* spp. infections are rare in cats and believed to be non-pathogenic (Ballweber, 2001)

3.4.3 Morphology

Freshly passed *T. vulpis* eggs are brown, lemon-shaped with characteristic plugs which are symmetrical and have rings, the shell is thick and the wall is smooth (Di Cesare, Castagna, Meloni, Otranto, & Traversa, 2012). They measure 72–90 × 32–40µm (Márquez-Navarro et al., 2012; Zajac & Conboy, 2012) and can be differentiated from human *Trichuris trichiura* ova as these are typically much smaller measuring 50 to 56 x by 21 to 26 µm, (Dunn, Columbus, Aldeen, Davis, & Carroll, 2002).

Adult worms are whip-shaped and range from 4.5 – 7.5cm in length. The anterior end which embeds into the large intestine is thin and hairline while the posterior tail, which lies freely in the lumen, is thick and broad occupying about ¼ the length of the body (Traversa, 2011; Bowman, 2014).

3.4.4 Diagnosis

Due to the density of the eggs (s.g. = 1.15) detection by centrifugal flotation of faeces, with appropriate flotation solution, is more effective than simple flotation techniques. Current ESCCAP (2010) guidelines suggest collecting between 3 – 5g of fresh or fixed faeces for egg detection.

3.4.5 Treatment and control

As the eggs can survive and remain viable in cold and warm conditions especially wet and shady areas they can persist for long periods of time, thereby becoming a constant source reinfection for dogs living in these contaminated environments (Traversa, 2011). To reduce this risk animals should be treated regularly with effective anthelmintics and removed from the contaminated area until effective decontamination has taken place (ESCCAP, 2010a).

According to Bowman (2014) it is generally believed that parasitic larvae are more resistant than adults to anthelmintics, as *T. vulpis* larvae take up to 8 weeks to develop, a follow-up dose of anthelmintic should be given three times, at monthly internals after the initial treatment, in order to rid the host of the worms that survived the first treatment.

Due to the limited efficacy of current treatments to treat the human form of the disease caused by *Trichuris trichiura,* research has been made to find alternatives. A recent study by Partridge et al., (2017) discovered that a new class of compounds, known as dihydrobenzoxazepinones, not previously used as anthelmintics was effective and selective against *T. muris* and also interrupted the *Trichuris* life cycle by acting on the eggs. Due to the low toxicity against mouse cells, the authors suggest that these compounds may indeed be a novel pharmaceutical treatment against *Trichuris* in humans and domestic animals.

3.4.6 Zoonotic risk

Human infections by *T. vulpis* are rare (Márquez-Navarro et al., 2012; Zajac & Conboy, 2012). However cases have been described in man, these include that of a 49 year old patient with chronic diarrhoea and 5 dogs (Dunn et al., 2002) and a 9-year old Mexican child with symptoms of epistaxis lasting for over two years, with symptoms of rhinitis and a diagnosis of rhinitis (Márquez-Navarro et al., 2012). In the latter case although the child's pet's faeces were negative, out of 292 dogs living in the vicinity that were studied, 3,5% were found to be infected with *T. vulpis* suggesting that the patient may have been infected by dogs living close to her home. Indeed Traversa (2011) states that "its significance for human medicine is its controversial zoonotic ability".

Unlike *Ancylostoma* spp. where infections may occur due to skin penetration, as with *Ascaris, Trichuris* eggs must be me ingested in order to infect humans. Significantly although *T. vulpis* and the human form *T. trichiura* are morphologically similar, the former is nearly twice as large (Dunn et al., 2002). To control human infections apart from anthelmintic therapy it is essential that the canine sources also be treated in order to avoid reinfection (Dunn et al., 2002).

3.5 *Aelurostrongylus abstrusus*

Aelurostrongylus abstrusus otherwise known as feline lungworm, is a nematode belonging to Plylum Nematoda, Class Secernentea, Order Strongylida, Superfamily Metastrongyloidea (Taylor et al., 2016) that infects the terminal respiratory bronchioles and alveolar ducts of domestic cats worldwide (Traversa, Cesare & Conboy, 2010; Di Cesare et al., 2015). Often underdiagnosed the parasite can cause a variety of symptoms and may be fatal in cases of heavy infection (Zajac & Conboy, 2012). Recent studies suggest an increasing geographical distribution of the parasite. (Di Cesare et al., 2015) possibly due to epidemiological and biological factors relating to the gastropod mollusc intermediate hosts and altered development rate due to climate changes (Traversa et al., 2010; Di Cesare et al., 2015).

Table 4 Characteristics of *A. abstrusus* - ESCCAP (2010)

Worm species	Pre-patent period	Patent period	Infective stages and route of infection	Distribution in Europe	Final hosts
Aelurostrongylus abstrusus	7 - 9 weeks	Several years	Ingestion of infected intermediate host	Everywhere in Europe	Cats

3.5.1 Life cycle

The *Aelurostrongylus abstrusus* life cycle requires two hosts with cats as final hosts and gastropods as intermediate hosts (Figure 7). The first stage occurs in the environment and involves a variety of terrestrial molluscs such as slugs or snail species, while the second stage (an optional one) involves a variety of paratenic hosts including rodents, amphibians, birds and reptiles (Taylor et al., 2016). Infection begins when a cat either ingests an infected intermediate mollusc or an infected paratenic host such as a rodent or a bird.

On digestion, the infective L3 larvae are released and the larvae migrate to lungs via the lymphatic system or bloodstream. Once in the lungs, they moult a final time and then the adults remain in nodules in the terminal bronchioles, alveolar ducts and pulmonary alveoli (Traversa et al., 2010). After mating the eggs mature and the L1 larvae pass up the bronchial escalator and are either coughed out into the environment or swallowed and released via the faeces (Taylor et al., 2016). The L1 larvae then remain in the environment until they penetrate an intermediate mollusc host, such as a snail or slug, where they develop into infective L3 larvae. They then remain within this host until it is either ingested by a paratenic host or by a cat. The host becomes

infected either by ingesting the paratenic host, the most frequent form of infection, or by ingesting the intermediate host (Taylor et al., 2016). Alternatively L3 from infected molluscs may be released within the snails mucus into the environment where they can potentially contaminate a cat's food or infect other gastropods thereby increasing the number of infected intermediate hosts available to infect other definitive or paratenic hosts (Colella et al., 2015). This alternative method of transmission is known as *intermediasis.*

The pre-patent period can vary between 7 and 8 weeks (ESCCAP, 2010a). Although larvae may not be present in the faeces patency can last for several years (ESCCAP, 2010a; Taylor et al., 2016).

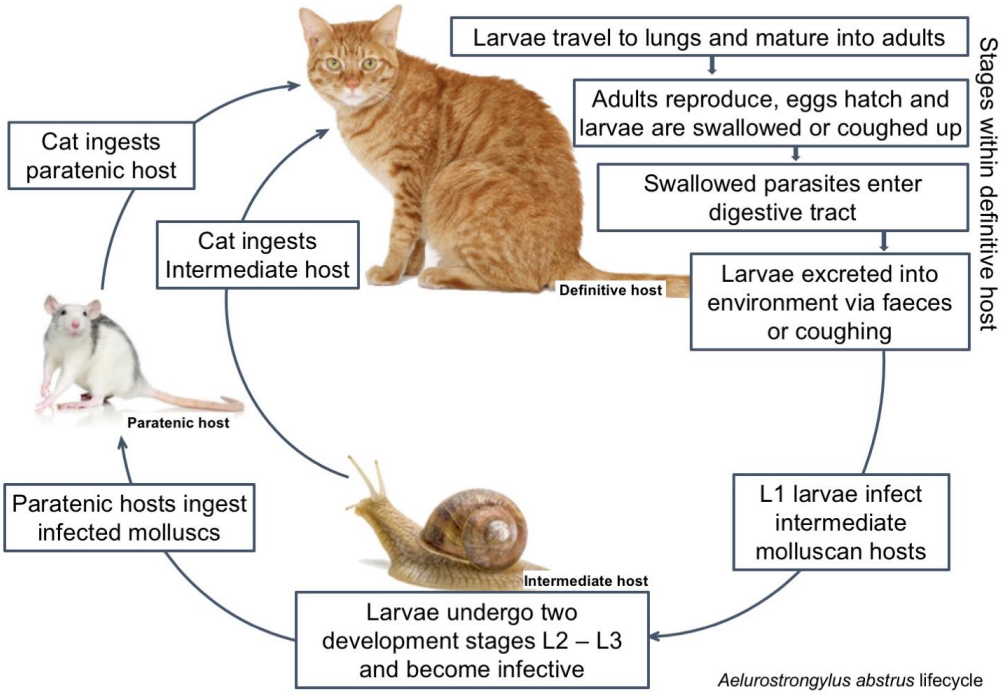


Figure 7 *Aleurostrongylus abstrusus* life cycle (original adapted from ESCCAP, 2011)

3.5.2 Pathogenesis and clinical signs

A. abstrusus infections are infrequently diagnosed (Zajac & Conboy, 2012) and unremarkable which may be due to the worm's low pathogenicity (Taylor et al., 2016). Consequently, most infections are unremarkable with the majority only diagnosed incidentally during post-mortem examination. Significantly, Foster & Martin (2011) noted that lower respiratory tract infections – LRTI's caused by *A. abstrusus* may go undiagnosed due to the self-limiting nature of the disease and lack of symptoms which when present mimic feline asthma responding well to symptomatic treatment for feline asthma.

Depending on the worm burden, symptoms can vary significantly from asymptomatic to severe. In mild infections, the animal may exhibit a mild chronic cough while resting and during exercise coughing, sneezing, nasal discharge and slight dyspnoea may be observed (Taylor et al., 2016). Severe manifestations include mild to chronic cough, sneezing, dyspnoea, nasal discharge and anorexia and may lead to death (Foster & Martin, 2011; Nabais et al., 2014).

3.5.3 Morphology

First-stage larvae measure 360–400 × 15–20µm (Zajac & Conboy, 2012; Elsheikha et al., 2016) and have a rounded head with a terminal oral opening (Traversa & Di Cesare, 2016). According to Elsheikha et al. (2016) the tail is kinked (S-shaped) and has small finger-like projections at the tip with cuticular spines and a deep dorsal and ventral incision. Adult stages within nodules in the respiratory system measure 5-12mm in length and 54-80µm wide.

3.5.4 Diagnosis

Clinical diagnosis of respiratory parasitosis in cats can be difficult as a variety of other conditions may share similar signs (Traversa & Di Cesare, 2016). According to Foster & Martin (2011) bacteria, fungi and viruses cause lower respiratory tract infections, which complicates diagnosis. Fine-needle-aspiration cytology, broncho alveolar lavage as well as other faecal examination techniques such as centrifugal flotation have been used for diagnosis. Still, the Baermann technique using macerated lung tissue (as a post mortem test) or faeces (as the most common *in vivo* test) remains the most sensitive method for *A. abstrusus* detection (Foster & Martin, 2011).

Current ESCCAP (2010) guidelines recommend that samples suspected for lungworms, where L1 larvae may be present, be examined using either the Baermann technique or microscopic detection of bronchial lavage fluid. For coprological methods at least 4g of fresh faeces are required, which should not be collected from kennel floors or runs (ESCCAP, 2010a). In the presence of compatible symptoms, practitioners should remain vigilant for the presence of lungworms especially *A. abstrusus*, even in territories traditionally considered free from these parasites, as they can easily be introduced into new areas due to definitive and intermediate hosts mobility (Traversa & Di Cesare, 2016).

3.5.5 Treatment and control

In most cases clinical signs can be resolved with anthelmintics (Elsheikha et al., 2016), these include different formulations of fenbendazole, benzimidazole and several macrocyclic lactones (Böhm et al., 2015). As it is often difficult to administer oral medication to cats, spot-on preparations are a good alternative and due to their proven effectiveness several have licenced in different countries. These include a spot-on formulations of emodepside 2.1%/praziquantel 8.6% given two weeks apart (Böhm et al., 2015) and imidocloprid 10%/moxidectin 1% (Traversa et al., 2009). A novel topical combination of Fipronil, (S)-methoprene, Eprinomectin and Praziquantel (Broadline ®, Merial) has also been shown to be very effective and safe for the treatment of *A. abstrusus* infections (Giannelli et al., 2015) this is a significant development as the combination is also effective against ectoparasites, cestodes, hookworms and roundworms.

Since infection involves the ingestion of intermediate or paratenic hosts, restricting access to potentially contaminated areas may be effective at reducing the risk. Nonetheless the predatory nature of the species means that the risk cannot be eliminated especially in endemic areas.

3.6 Cestodes

Cestodes otherwise known as tapeworms are flattened, acoelomate animals with no digestive tract. Their bodies are divided into three parts: scolex (anterior part that attaches to the host via suckers and hooks), neck (germinal region where strobili emerge) and strobili. The strobili are a complete chain of proglottid segments, each proglottid has reproductive organs and both sexes are present in the same individual. Maturation of the proglottids occurs from anterior to the posterior end of the tapeworm. As they have no digestive system, nutrients are absorbed through the body wall. The main Cestodes affecting domestic animals can be divided into two groups: Cyclophyllidea and Diphyllobothriidea. The Taeniidae and Dipylidiidae families comprise the main cestodes found in pets and are included in the first group (Bowman, 2014).

Table 5 Characteristics of tapeworms found in Europe. Adapted from ESCCAP (2017

Species	Pre-patent period	Patent period	Infective stages and route of infection	Distribution in Europe	Final hosts
Taenia spp.	4 - 10 weeks	Months up to several years	Ingestion of larval stages in intermediate host (cysticercus or coenurus)	Everywhere	Dogs and foxes (and cats)
Mesocestoides spp.	4 - 10 weeks	Several years	Ingestion of larval stages in meat or tissues from prey	Everywhere	Dogs, cats and foxes
Dipylidium caninum	3 weeks	Several months	Ingestion of larval stages in fleas or lice	Everywhere	Dogs, cats and foxes
Echinococcus multiocularis	28 days	Several months	Ingestion of larval stages in intermediate hosts (rodents)	France, central and eastern Europe	Dogs, racoon dogs, cats and foxes
Echinococcus granulosis	45 days	Several months	Ingestion of larval stages in fleas or lice	Central, Southern and Eastern Europe	Dogs (foxes)

3.7 Taeniidae

Taenia spp. and *Echinococcus* spp. belong to Phylum Plathelmynthes, Class Cestoda, Order Cyclophyllidea, Family Taeniidae (Taylor et al., 2016). These parasitic tapeworms inhabit the small intestines of their definitive hosts, which include dogs, cats and some wild carnivores (Ballweber, 2001). Species known to infect domestic animals (Table 6) include *T. taeniaeformis* that affects cats as primary definitive hosts and occasionally dogs, *T. serialis* that affects mostly dogs and rarely affects cats and finally *T. pisiformis, T. hydatigena, T. multiceps* and *T. ovis* that mainly affect dogs (Zajac & Conboy, 2012). The main species that infect humans are *T. saginata* and *T. solum.* Cows are intermediate hosts to the former and pigs are to the latter. Although these species can have a significant effect on public health cases are rare in Europe and North America (Ballweber, 2001).

Table 6 Some Taeniidae tapeworm species affecting carnivores (adapted from Ballweber, 2001)

			Metacestode (larval form)	
Species	**Definitive hosts**	**intermediate hosts**	**Type**	**Location**
Taenia pisiformis	Dogs, uncommon in cats	Lagomorphs	*Cysticercus*	Abdominal cavity, liver
Taenia ovis	Dogs	Sheep, goats	*Cysticercus*	Skeletal and cardiac muscles
Taenia hydatigena	Dogs	Domestic and wild ruminants	*Cysticercus*	Abdominal cavity, liver
Taenia taeniaeformis	Cats	Rodents	Strobilocercus	Liver
Taenia multiceps	Dogs	Sheep and cattle	*Coenurus*	Nervous tissue
Taenia serialis	Dogs	Rabbits, humans and rarely cats	*Coenurus*	Musculature, subcutis

3.7.1 Life cycle

3.7.1.1 *Taenia* spp.

Taenia spp. life cycle is indirect requiring an intermediate host. Whereas the adult forms generally inhabit the small intestine of the definitive host, the metacestode (larval) stages can be found within the tissues of the intermediate host.

The cycle begins with the excretion of gravid taeniid segments through the definitive host's anus. The segments then move around the anal area or faecal mass emptying their eggs in the process. The intermediate host becomes infected though ingestion of the eggs or gravid proglottids (Zajac & Conboy, 2012). Once ingested by the intermediate host, the eggs hatch and the hexacanth embryo burrows into the intestinal wall and then migrates though the bloodstream to a specific organ which varies depending on the species (Table 6). Here it grows, encysts and differentiates into the second stage larval form that is infective to the definitive host (Bowman, 2014). Depending on the species this may be either cysticerci or coenuri.

Definitive hosts become infected with *Taenia* spp. when they ingest an intermediate host infected with an immature metacestode stage. In the case of *Taenia* spp. once a suitable definitive host has ingested the second stage taeniid tapeworm, the bladder is digested and the scolex emerges and adheres itself to the mucosa of the small intestine (Bowman, 2014). It then begins to bud off into segments which form the strobili and grows into an adult, able to reproduce and produce gravid proglottids. In

the case of *Taenia* spp. the pre-patent period is between 4 to 10 weeks, with patency lasting from months to years.

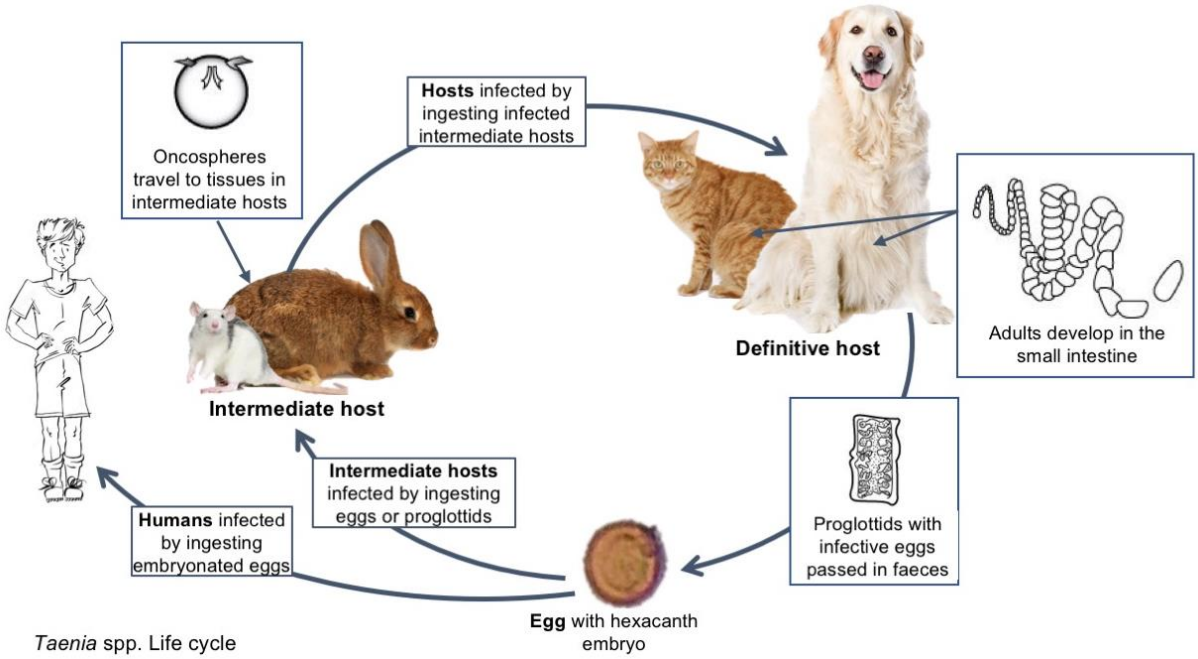


Figure 8 *Taenia* spp. life cycle (original illustration) adapted from ESCAAP (2017) - http://www.esccap.org/life-cycles/gl1/ (the egg in the illustration was detected in one of the samples).

3.7.1.2 *Echinococcus* spp.

The cycle is similar to that of *Taenia* spp. in what concerns *E. granulosus* regarding *E. multilocularis*, rather than becoming infected from the ingestion of metacestode contained within the tissues of intermediate hosts, as occurs in *Taenia* spp. definitive hosts become infected with *E. multilocularis* by ingesting an infected intermediate host (namely a rodent) containing a multiocular cyst (ESCCAP, 2010b) In the case of *E. multiocularis* pre-patency (28 days) and patency (several weeks) are significantly shorter than *Taenia* spp. (table 5).

3.7.2 Pathogenesis and clinical signs

Infections with *Taenia* spp. and *Echinococcus* spp. in adult dogs are generally subclinical and unremarkable (ESCCAP, 2010b; Taylor et al., 2016; Zajac & Conboy, 2012). In the case of *Taenia* spp. the only clue that the animal may be affected is when owners notice their pets rubbing their bottoms along the ground due to pruritus caused from the passage of mature segments from the anus (ESCCAP, 2010b).

3.7.3 Morphology

3.7.3.1 *Taenia* spp.

Depending on maturity and species, adult *Taenia* spp. tapeworms can vary greatly in length, from tens up to hundreds of centimetres (Bowman, 2014). The anterior end of the tapeworm that attaches to the host, is known as the scolex (Figure 9). This has four suckers and a nonretractable central portion known as a rostrum that is surrounded by hooks, but these may not be present in all species. The rest of the body is composed of segments known as proglottids which are generally rectangular. Each has a genital pore which alternates along the strobila. (Bowman, 2014). Careful attention to the number of rostrum hooks and morphology of mature segments in necessary to differentiate between species and genus.

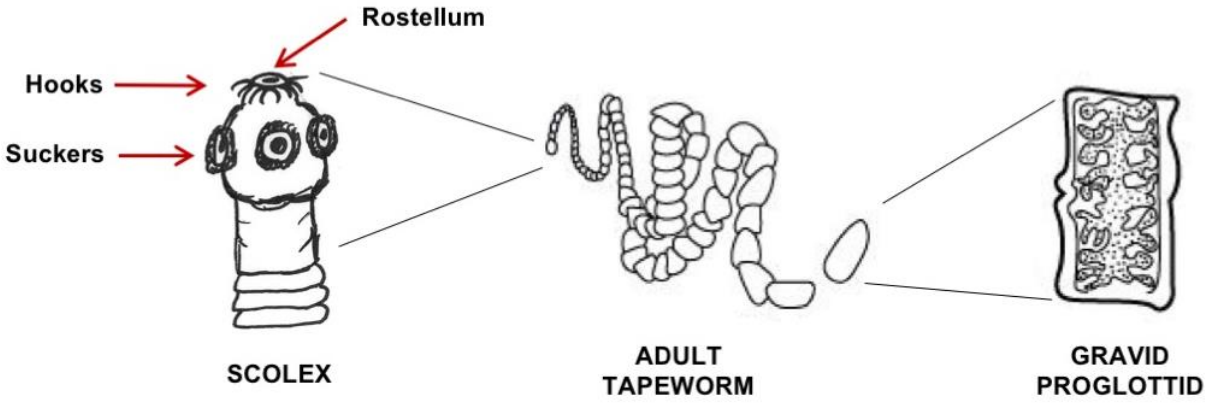


Figure 9 General characteristics of adult *Taenia* spp. (original illustration)

Eggs released from disintegrating gravid segments are surrounded by a fragile striated shell known as an embryophore containing an oncosphere with a hexacanth larva inside. Taeniid eggs are spherical and quite small measuring between 25 - 40µm in diameter (Zajac & Conboy, 2012).

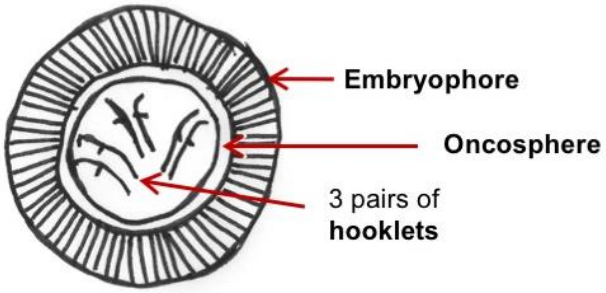


Figure 10 *Taeniidae* egg surrounded by embryophore (original illustration)

3.7.3.2 *Echinococcus* spp.

Adult *Echinococcus* spp. tapeworms differ significantly from *Taenia* spp. as they have smaller bodies (2 – 8mm) and are composed of only four or five segments (Bowman, 2014) compared to adult *Taenia* spp. that are significantly longer (up to meters in length) and may contain hundreds of segments. Unlike *Taenia* spp. which may have many gravid proglottids, in *Echinococcus* spp. adults only the terminal segment is gravid. (Bowman, 2014)

3.7.4 Diagnosis

Taeniid eggs measure between 25 and 40µm in diameter and can be detected when free from their proglottids using faecal flotation techniques (ESCCAP, 2010b; Zajac & Conboy, 2012). As it is not possible to differentiate *Taenia* spp. from *Echinococcus* spp. eggs microscopically (ESCCAP, 2010b; Zajac & Conboy, 2012) where possible diagnosis can be based on the examination of adults as these have species specific characteristics. When a definitive diagnosis is required, serodiagnostic tests can be used, namely based in the detection of coproantigens. Differential diagnosis for *Dipilidium caninum* can be made by observing egg packets containing up to 20 eggs in the animals faeces, with each egg containing an onchosphere with 3 pairs of hooks (Nolan, 2017).

3.7.5 Treatment and control

If a tapeworm infection is detected animals should be given an effective anthelmintic at appropriate intervals. (ESCCAP, 2010b). According to Taylor et al. (2016) effective cestocidal anthelmintics include: niclosamide, nitroscanate, praziquantel, epsiprantel or multiple benzimidazole doses of either febendazole or mebendazole. The most effective drugs on cestodes, are considered to be praziquantel and epsiprantel.

As eggs may remain viable in the environment for extended period of time, owners should restrict access or thoroughly clean and disinfect any areas that may be contaminated. They should also prevent their animals from gaining access to the various intermediate hosts, discourage hunting behaviour and not give raw meat or offal to their pets. (ESCCAP, 2010b). Thoroughly cooking meat given to pets or freezing it for 10 days at -20ºC are effective measures to kill cysticercoids.

3.7.6 Zoonotic risk

Species of *Taenia* spp. and *Echinococcus* spp. are known to infect humans and can cause serious disease. Similar to other animals, humans become intermediate hosts through the ingestion of eggs from definitive hosts which can generally be associated with poor hygiene practices

Humans can be either definitive or intermediate hosts to *Taenia* spp. species (Table 7). Once ingested, eggs hatch in the intestine and release their oncospheres which migrate through the blood to their target organs and develop into either coenuri or cysticercus depending on the species. Significant *Taenia* spp. affecting humans, with dogs as definitive hosts, include *T. multiceps* whose coenuri develop in the eyes and brain and *T. serialis* whose coenuri can usually found in subcutaneous tissue. In the CNS coenuri may cause headache, fever and vomiting and other serious neurological symptoms. In the eye they can cause inflammation, glaucoma and even lead to blindness. (CDC, 2017)

Table 7 Interactions between different *Taenia* spp. species that affect either cats or dogs and humans. (Table is not extensive with regard to all definitive or intermediate hosts a species may infect as emphasis was given to possible interactions between cats or dogs and humans) Data obtained from The Center for Food Security & Public Health (2005).

Species	Definitive hosts	Intermediate hosts	Metacestode
Taenia saginata	Humans	Cattle	*Cysticercus*
Taenia solium	Humans	Domestic & wild pigs, larvae occasionally found dogs and cats	*Cysticercus*
Taenia crassiceps	Foxes, found in dogs and wild canids	Wild rodents, humans can be infected	*Cysticercus*
Taenia hydatigena	Dogs, rarely cats	Sheep, goats, cattle, humans rarely infected.	*Cysticercus*
Taenia taeniaeformis	Cats,rarely dogs	Rodents, infection rare in humans	*Cysticercus*
Taenia multiceps	Dogs	Sheep, reported in humans	*Coenurus*
Taenia serialis	Dogs	Rabbits, reported in humans	*Coenurus*
Taenia brauni	Dogs	Rodents, reported in humans	*Coenurus*

Echinococcus spp. affecting humans include *E. granulosus* and *E. multiocularis.* Dogs are definitive hosts to both species and therefore the intimate relationship between dogs and humans can be a significant source of transmission when adequate hygiene practices are not taken. As a notifiable disease in Portugal, 646 cases of human hidatiosis by *E. granulosus* were reported between 1979 and 2008, significantly 69.2% pertained to patients who possessed dogs (David de Morais, 2010). The European

Centre for Disease Prevention and Control shows two further reported cases of *E. granulosus* in 2016 in the country.

According to CFSPH et al., (2011) symptoms in humans depend on size, number and location of the metacestode and are largely non remarkable until they become a mass lesion that is large enough to damage organs and tissues. In humans *E. granulosus* causes cystic echinoccosis and *E. granulosus* causes alveolar echinoccosis (Baneth et al., 2015).

3.8 Protozoa

3.8.1 *Cystoisospora*

Cystoisospora spp. are Protozoa (coccidia) that belong to Phylum Aplicomplexa, Class Conoidasida, Order Eucoccidiorida, Sub order Eimeriorina, Family Eimeriidae (Taylor et al., 2016). All species are obligate intracellular parasites that infect their intermediate and definitive hosts via the faecal oral route and are host-specific. The two species that affect cats are *Cystoisospora felis* and *Cystoisospora rivolta*, species affecting dogs include *Cystoisospora canis, Cystoisospora ohioensis and Cystoisospora burrowsi* (ESCCAP, 2011; Zajac & Conboy, 2012).

3.8.2 Life cycle

The majority of infections occur through the ingestion of sporulated oocysts from the environment but infection can also occur through the ingestion of infected paratenic hosts such as mice (Dubey, 2014). Paratenic hosts become infected when they ingest infective oocysts. Within the paratenic host, the sporozoites then invade the intestinal wall and become encysted as bradyzoites, which are then infective to cats who predate them (Bowman, Hendrix, Lindsay, & Barr, 2002).

Once an infective oocyst has been ingested the sporozoites encyst in distal portion of the small intestine, rarely the duodenum or jejunum. Development stages then occur within the enterocytes of the villi in the ileum (Bowman et al., 2002). After the development stages have occurred hosts excrete unsporulated oocysts into the environment that sporulated within a time frame depending on the temperature, but can vary from 40 hours at 20ºC to 8 hours at 38ºC (Bowman et al., 2002). According to Taylor et al. (2016), the prepatent period is 7 to 10 days and infections remain patent for 1 to 3 weeks.

3.8.3 Pathogenesis and clinical signs

Cystosporosis often occurs from the third to eighth week of life of a kitten or pup (ESCCAP, 2011) or in older debilitated pets and is often associated with diarrhoea (Zajac & Conboy, 2012) which usually occurs shortly before oocyst excretion. In severe cases faeces may contain blood (ESCCAP, 2011) which may cause anaemia (Zajac & Conboy, 2012). Other signs that may be present include, anorexia, weight loss and abdominal tenderness. It is often associated with other viral, bacterial and helminth co-infections (ESCCAP, 2011).

3.8.4 Morphology

Fresh oocysts are ovoid, to varying degrees depending on the species, and have smooth clear walls containing a single cell called a sporoblast (Zajac & Conboy, 2012). Sporulated cysts, the infective form, contain two structures known as sporocysts which in turn contain four sporozoites each. According to (Bowman et al., 2002) the sporocyst residuum is "granular and may contain refractile globules".

Oocysts affecting dogs and cats have thin colourless shells which may be tinted brown. Size depends on the species but the average *Cystoisospora felis* oocyst (45 x 33µm) is relatively larger than that of *Cystoisospora rivolta* (26 x 24µm). *C. felis* oocysts are also more ovoid than *C. rivolta* which are more rounded (Table 8). *C. felis* sporozoites are 10-15µm (Bowman et al., 2002).

Table 8 Characteristics of *Cystoisospora* oocysts that affect cats in Europe. (ESCCAP, 2011)

Cystoisospora	**Average size**	**Shape**	**Shell**
C. felis	45 x 33µm	Ovoid	Thin and colourless or brownish
C. rivolta	26 x 24µm	Round ovoid	Thin and colourless or brownish

Cystoisospora species affecting dogs are all round-oval shaped also vary in size from *C. canis* (39 x 32µm)*, C. ohioensis* (24 x 20µm) to *C. burrowsi* (21 x 18µm) (ESCCAP, 2011).

3.8.5 Diagnosis

Centrifugal or faecal flotation techniques can be used to detect oocysts (ESCCAP, 2011; Zajac & Conboy, 2012). Characteristics and dimensions for identification and differentiation can be found in Table 8 above.

3.8.6 Treatment and control

Due to the faecal oral route of transmission, and the fact that oocysts remain viable in the environment for several months (ESCCAP, 2011) hygiene is extremely important. Good litter tray hygiene and other measures are especially important in situations where there is a large density of cats as oocysts may build up due to their long viability in the environment. Controlling paratenic hosts such as insects is also vital.

At present Toltrazuril and Diclazuril are recomended for treating feline cystoisosporosis and in dogs Toltrazuril/Emodepside are approved for the simultaneous treatment of co-infections involving coccidia and roundworms. (ESCCAP, 2011)

CHAPTER IV - THE FIRST EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF CARDIOPULMONARY AND GASTROINTESTINAL PARASITES IN CATS AND DOGS FROM THE ALGARVE REGION OF PORTUGAL USING THE FLOTAC TECHNIQUE.

4.1 Objectives

- Estimate the prevalence of gastrointestinal and cardiopulmonary parasites in cat and dog populations from shelters in the Algarve using the FLOTAC technique.
- Compare mean eggs per gram (EPG) and oocysts per gram (OPG) between species to assess whether there is a difference in infection intensity.
- Evaluate whether any of the parasites found could be a potential risk to public health.

4.2 Materials and Methods

4.2.1 Study population

A total of 142 random stool samples were collected (n=66 dogs, n=76 cats) from 7 kennels and 6 catteries (Figure 13), located in various municipalities in the Algarve region of Southern Portugal, between February and April 2016. All of the samples were taken from adults >6 months old, no distinction was made between males and females. The facilities housing the animals belonged to either private associations or local municipalities. Vila Real de Santo António and Castro Marim - VRSACM are considered together as the location where the samples were collected from housed animals from both municipalities.

Municipality	Cats	Dogs
Albufeira	0	6
Aljezur	5	7
Lagoa	7	11
Lagos	30	27
Loulé	0	5
VRSACM	34	10
Total	76	66

Samples collected

Figure 11 Municipalities where samples were collected (original illustration).

4.2.2 Sample collection and preparation

All faecal samples were gathered in the morning and stored in numbered zip lock bags. To minimise environmental contamination, collections were performed on mornings preferentially after facilities had been cleaned the day before and care was taken to only collect the top layer of faeces so as to avoid contamination from the ground. As felines like to bury their faeces it wasn't entirely possible to isolate them from the sand or soil where they were buried, but an attempt was made in all cases to only collect parts not covered in sand.

Canine samples were preserved at a 1:4 ratio with formaldehyde 5% by placing two grams from each bag into a plastic container with 6ml of formaldehyde 5%, this was then homogenized using a disposable plastic spatula and the container was labelled and sealed airtight.

The feline samples were not fixed with formaldehyde but placed in a sealable container and refrigerated at 4ºC until being processed within three days. Although the distinction between preservation methods was made for logistical reasons both were in accordance with the experimental design protocol set out by the original authors (Cringoli et al., 2010).

Figure 12 My "assistant" pausing after collection!

Figure 13 Cats from one of the catteries

Figure 14 Samples ready for processing

4.2.3 Flotation solutions

Two different aqueous FS were chosen from the list suggested by Cringoli et al (2010) considering the parasites most commonly found in cats and dogs. The solutions chosen were: FS3 (zinc sulphate, s.g. 1.200) and FS7 (zinc sulphate, s.g. 1.350) (Table 9) Both solutions were prepared at room temperature on the same day in accordance with the methods outlined by Cringoli et al. (2010) summarized in (Table 9), and the densities were measured with a hydrometer at 20°C.

Table 9 Method for preparing each flotation solution (Cringoli et al, 2010).

FS	S.G.	Method
FS3	1.20	Dissolve 330g of zinc sulfate heptahydrate with 500ml of tap water using a magnetic stirrer then add water to reach a total volume o 1 liter.
FS7	1.35	Dissolve 685g of zinc sulfate heptahydrate with 685ml of tap water using a magnetic stirrer.

4.2.4 Procedure

The FLOTAC technique (Cringoli et al., 2010) is extremely sensitive and accurate to 2 EPG. The FLOTAC dual technique was chosen as it enables the use of two different flotation solutions (FS) with different specific gravities (SG), one for each chamber, in parallel for each sample making it possible to screen for a wider number of parasites from each sample and is recommended by Cringoli et al. (2010) for epidemiological surveys and routine diagnosis.

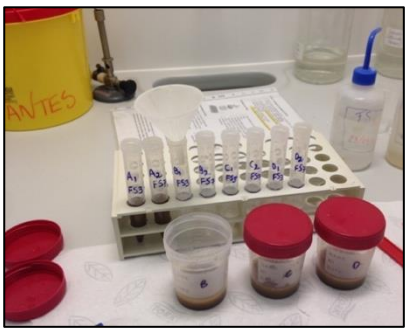


Figure 15 Filling each falcon tube with the contents from each homogenized container.

The procedure was conducted as follows: 12ml of tap water was added to each previously prepared plastic container containing 2g of faeces and 6ml of formaldehyde 5% (dilution ration 1:10). The whole container was then homogenized and filtered using a 500μm filter (Figure 15). A volume of 6ml was then placed into two falcon tubes and labelled tube 1 and 2. The falcon tubes were centrifuged for 3 minutes at 1500x to form a pellet. For feline samples the technique was the same except 18ml of water was added to 2g of refrigerated faeces (dilution ration 1:10), the rest of procedure was as above.

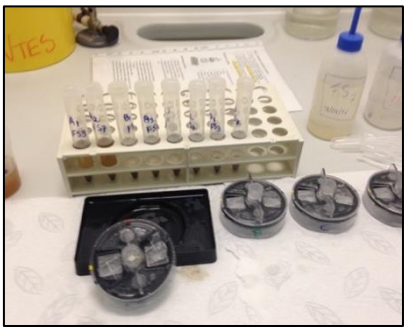


Figure 16 Filling the FLOTAC chambers from the falcon tubes.

The supernatant was removed from each previously centrifuged falcon tube leaving the pellet behind. Immediately before filling the FLOTAC chamber the respective flotation solution for each chamber (FS3 tube 1 and FS7 tube 2) was added to each tube up to the 6ml line. The FS solution was homogenized gently with the pellet so as to avoid air bubbles.

The screw was tightened to prevent air bubbles and the FLOTAC chamber was placed at an angle on the base (Figure 16) and each chamber was gently filled from the corresponding falcon tube (chamber volume = 5 ml).

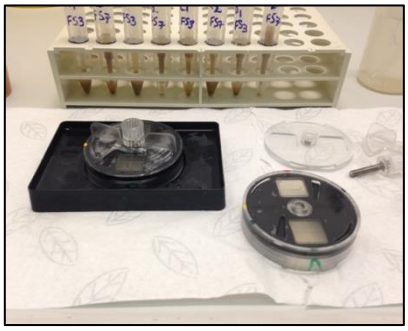


Figure 17 Removal of the screw, key and base.

The apparatus was then placed horizontally on the bench and further filled until a positive meniscus was formed (Figure 17). The screw was loosened and the translation disk rotated from green to red. The screw was then tightened and any excess liquid removed from the surface of the apparatus using Pasteur pipette and paper towel.

The apparatus was then centrifuged for 5 minutes at 1000x. Following centrifugation, the apparatus was placed horizontally on the counter and the top of each chamber was filled with the respective FS for each chamber. The screw was then loosened clockwise and the translation disk rotated from yellow to red. The screw was then removed and the base removed from the translation disk and viewed under the microscope (Figure 18).

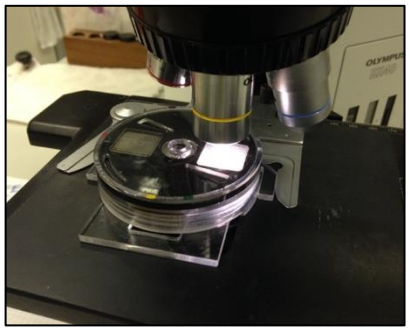


Figure 18 Reading the FLOTAC under a microscope

Once both chambers had been read, the apparatus was disassembled. The reading disk was loosened by rotating the disk from red to black. The disk, rubber separator and base were removed. All parts were then washed with soapy water, rinsed with clean water and left to air dry before the procedure was repeated.

4.2.5 Morphological characterization

All parasites were identified based on their morphology and size. They were then assigned to the lowest possible taxonomic rank that could be reliably determined without serologic testing.

Ancylostomatidae eggs were identified based on their oval shape, thin shells and sizes which range from 52–79 × 28–58 µm (Zajac & Conboy, 2012). As Ancylostomatidae are indistinguishable morphologically (Bowman et al., 2010) species identification wasn't possible.

Toxocara spp. eggs were detected based on their ascarid-like appearance, thick markedly pitted shells (Zajac & Conboy, 2012) and dimensions which were described by Fahrion (2011) as being (62.3 x 72.7µm) in the case of *T. cati* and (74.8 x 86 µm) for *T. canis*. Differentiation between *T. canis* and *T. cati*, was not attempted due to the similarities found between species, this was supported by a study performed by Uga et al. (2000) who found that although "*T. canis* eggs tended to be relatively larger than *T. cati* eggs", the "differences were not adequate for differentiation".

Trichuris spp. eggs were identified based on their lemon shape, brownish colour, thick smooth walls with characteristic symmetrical bi-polar plugs and size which for *T. vulpis* is stated to be 72–90 × 32–40µm (Márquez-Navarro et al., 2012; Zajac & Conboy, 2012). They were differentiated from *Capillaria aerophila* and *Capillaria boehmi*, two apparently similar parasites that can also affect dogs, based on the descriptions provided by Di Cesare et al. (2012) who describe *C. aerophila* & *C. boehmi* eggs as barrel shaped, with asymmetrical plugs containing no rings.

A. abstrusus L1 larvae were identified based on their size which was within the dimensions described by Zajac & Conboy (2012) and Elsheikha (2016) as being between 360–400 × 15–20µm, with a rounded head with terminal opening (Traversa & Di Cesare, 2016) and kinked (S-shaped) tail with small finger-like projections at the tip with cuticular spines and a deep dorsal and ventral incision (Elsheikha et al., 2016).

Taeniidae eggs were identified based on their morphological characteristics and sizes. which are 25 - 40µm (ESCCAP, 2010b; Zajac & Conboy, 2012). As it is not possible to differentiate *Taenia* spp. from *Echinococcus* eggs microscopically (ESCCAP, 2010b) any eggs found with characteristics belonging to either genus were considered as belonging to the Taeniidae family.

Cystoisospora spp. oocysts were identified based on their ovoid, thin colourless shells and size which in the case of *C. felis* is stated by ESCCAP (2011) to be (45 x 33µm).

4.2.6 Statistical analysis

Data was analysed using a χ^2 test or student's T-Test from within JASP (JASP Team., 2017). All calculations were made considering 95% confidence interval. If the probability for the null hypothesis was less than $P < 0.05$ the results were considered significant. The apparent prevalence for each parasite species detected was calculated

as: number of positive animals/number of animals in sample x 100 %. Confidence intervals were calculated with the binomial (Clopper-Pearson) method using the BinomLow and BinomHigh functions within Microsoft Excel® for Mac 2016 edition.

4.2.7 Note regarding safety procedures.

When handling and processing faecal samples, potentially infected with zoonotic agents, precautions must be taken to minimise the risk of infection. Protective clothing and disposable latex gloves were used at all stages from collection to processing and all samples were processed in a proper confined environment. After work, all surfaces, instruments and reusable materials used were cleaned thoroughly with Sodium Hypochlorite 2% solution and then rinsed with water. In order to eliminate the risk of inhalation or skin contact when using formaldehyde, gloves were used at all times and all steps were conducted in a laminar flow chamber. To protect the environment, all waste products were disposed of appropriately.

4.3 Results

4.3.1 Parasites detected using the FLOTAC technique

In order of decreasing taxonomic hierarchy, parasites classified at the family level included Taeniidae and Ancylostomatidae. Three were classified according to genera and were consequently *Toxocara* spp., *Trichuris* spp. *and Cystoisospora* spp. and one specie was determined to be *Aelurostrongylus abstrusus.*

4.3.1.1 Ancylostomatidae

Samples positive for Ancylostomatidae eggs were found in the municipalities of Lagoa 9,1% (1/11) and Lagos 3,7% (1/27) making up 3% (2/66) of the dog samples tested and the mean EPG was 1.5 ± 0.707.

Positive cat samples were found in three municipalities Aljezur 20% (1/5), VRSACM 5.9% (2/34) and a co-infection with Taeniidae was found in Lagoa 14.3% (1/7). Overall 5.26% (4/76) of cat samples were positive for Ancylostomatidae. In cats mean EPG was 4.75 ± 3.86. Figures 19, 20 and 21 show the morphology of some of the eggs found in this study.

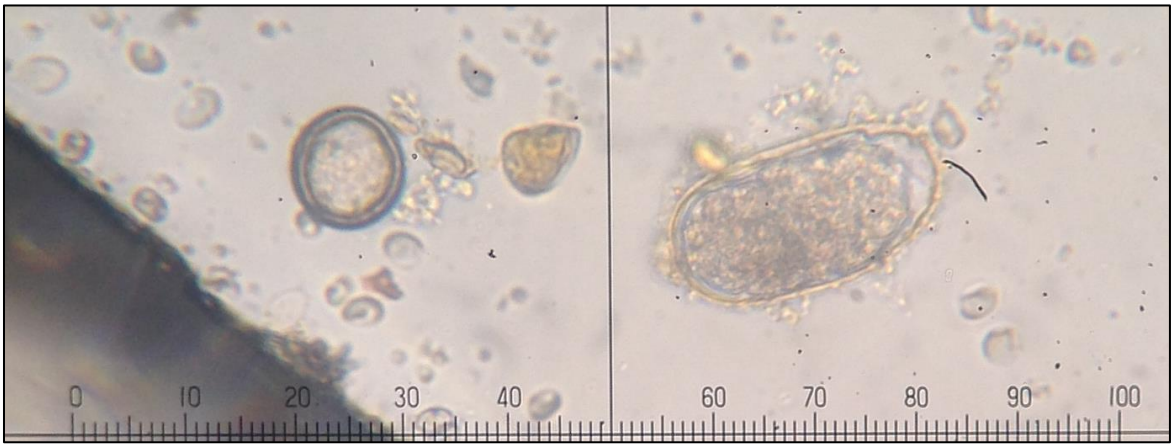


Figure 19 Ancylostomatidae and Taeniidae co-infection, found in cat faeces 400x (original)

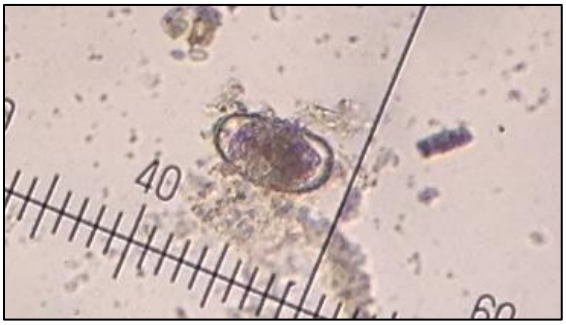


Figure 20 Ancylostomatidae eggs, found in dog faeces 100x (original)

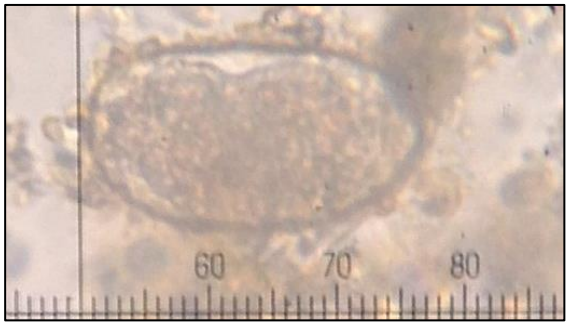


Figure 21 Ancylostomatidae eggs, found in cat faeces 400x (original)

4.3.1.2 *Toxocara* spp.

Toxocara spp. eggs were found in 13.64% (9/66) of the dog samples tested, locations where it was present included Lagoa 27.3% (3/11), Lagos 7.4% (2/27), VRSACM 20% (2/10) and two samples were co-infected with Taeniidae in Albufeira 33.3%(2/6). The mean EPG was 61.3 ± 74.64.

Overall 31.58% (24/76) of the cat samples were *Toxocara* spp. positive. Single infections were found in the municipalities of Aljezur 40% (2/5) and Lagos 53.3% (16/30) which accounted for 23.7% (18/76) of the cases. Co-infections made up the remaining 7.9% (6/76) and included two cases of *A. abstrusus* + *Toxocara* spp. 6.7% (2/30) and four cases of *Cystoisospora* + Taeniidae 13.3% (4/30) both in Lagos. The mean EPG in cats was 523 ± 688.75.

Mean egg sizes in this study for cats (n=20 eggs, 78.7 x 68.8µm) and dogs (n=20 eggs, 82.7 x 73µm) were close to the reference values. Although the majority of feline samples contained single celled embryos (Figure 22), some contained eggs in the morula stage (Figure 23) and some had larvae (Figure 24).

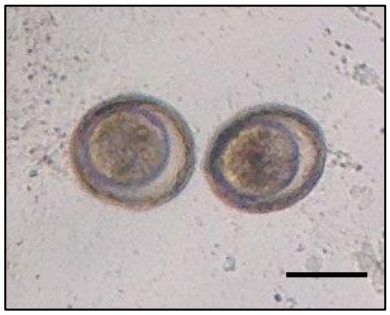


Figure 22 *Toxocara* spp. eggs with single celled embryos, found in cat faeces (original) Scale bar 50µm.

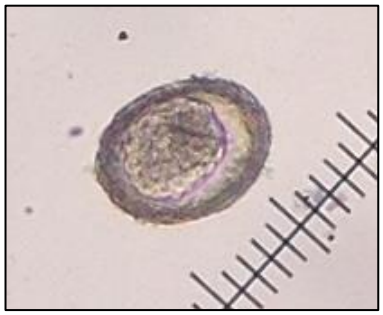


Figure 23 *Toxocara* spp. egg found in dog faeces (original) 100x

Figure 24 Sample co-infected with *Taeniidae* and *Toxocara* spp. found in dog faeces (original) 100x

4.3.1.3 *Trichuris* spp.

Trichuris spp. was only found in one dog sample, the sample which came from Lagos contained two eggs (Figures 25 & 26) and made up 3.7% (1/27) of the positive samples from that municipality and 1.5% (1/76) overall. No *Trichuris* spp. eggs were found in the cat samples. The two eggs measured (78 x 37µm; 81 x 37µm).

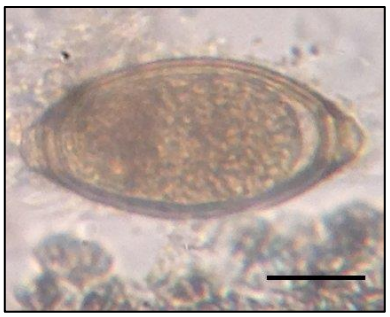


Figure 25 *Trichuris* spp. eggs, found in dog faeces (original) Scale bar 25µm.

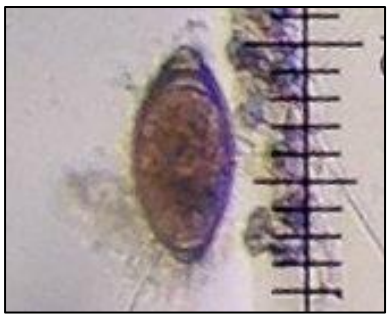


Figure 26 *Trichuris* spp. eggs, found in dog faeces (original) x100

4.3.1.4 Aelurostrongylus abstrusus

A. abstrusus was present in 3.95% (3/76) of the cat samples tested. A single infection was found in Aljezur 20% (1/5) and two positive samples from Lagos were co-infected with *Toxocara* spp. 6.7% (2/30). The co-infected sample is shown in Figure 27 and the characteristic (s-shaped) kinked tail can be seen in Figure 29.

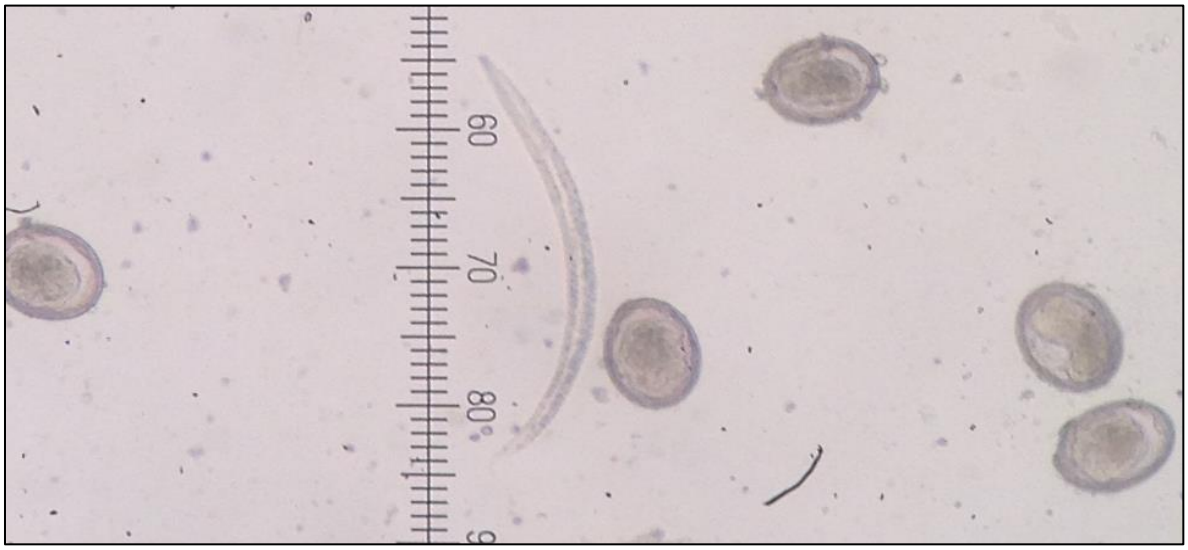


Figure 27 *A. abstrusus* sample co-infected with *Toxocara* spp. (Original) x100

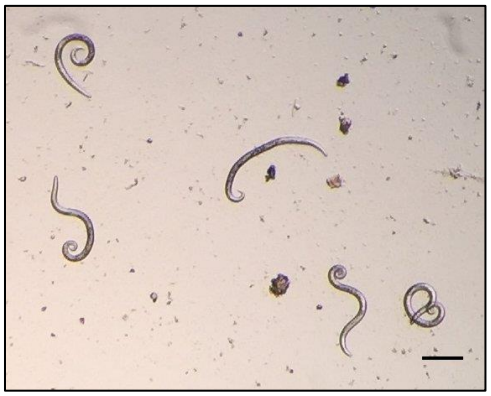


Figure 28 *A. abstrusus* L1 larvae (Original courtesy of Doctor Ana Margarida Alho) Scale bar 100µm.

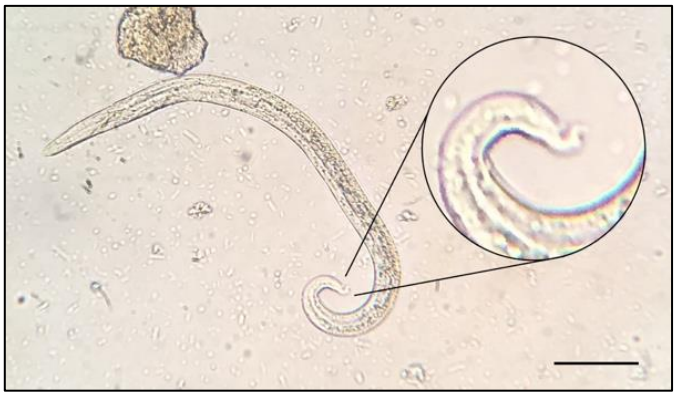


Figure 29 *A. abstrusus* L1 larvae (Original courtesy of Doctor Ana Margarida Alho) Scale bar 50µm.

4.3.1.5 Taeniidae

Four of the dog samples tested positive for Taeniidae 6% (4/66). Single and mixed infections were found in Albufeira and made up 16.7% (1/6) and 33.3% (2/6) respectively for that municipality. A single infected sample was also found in Lagos 3.7% (1/27). The mean EPG was 14.75 ± 15.06.

Taeniidae was only found in one cat sample, the sample which was co-infected with Ancylostomatidae came from Lagoa 14.3% (1/7) and had 73 EPG. Overall the prevalence for Taeniidae in cats was 1.32% (1/76).

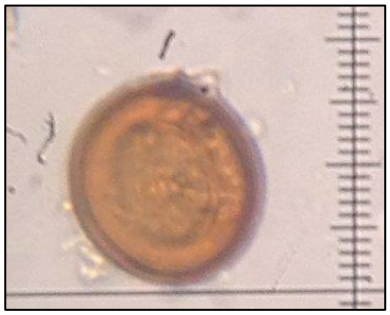


Figure 30 Taeniidae egg, found in dog faeces (original image) x400

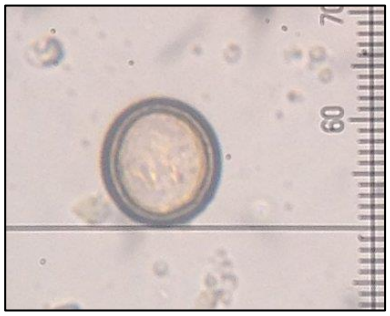


Figure 31 Taeniidae egg, found in cat faeces (original image) x400

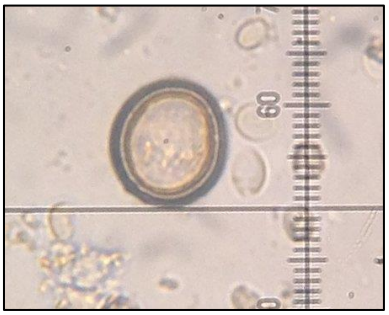


Figure 32 Taeniidae egg, found in cat faeces (original image) x400

Eggs were measured and mean length and width were calculated (Cats n=10 eggs, 30.6 x 28.8µm; Dogs n=20 eggs, 36.8 x 34.6µm), the values fell within the ranges expected for the *Taeniidae* family. Hexacanth larvae were also visible in most of the eggs visualized (Figures 31 & 32).

4.3.1.6 *Cystoisospora* sp.

Cystoisospora sp. was detected in 6.6% (5/76) of the cat samples. All positive samples came from Lagos, of these one was a single infection 3.3% (1/3) and four 13.3% (4/30) were co-infected with *Toxocara* spp. The mean OPG was found to be 437.6 ± 924.9. No dogs tested positive for this parasite. Some of the oocysts detected in this study can be seen in Figures 33 and 34.

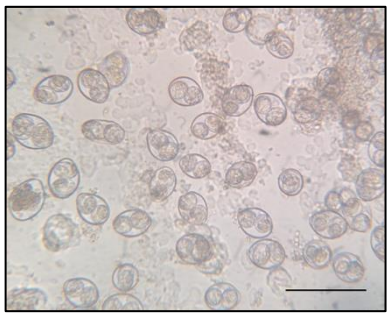


Figure 33 Sporulated *Cystoisospora felis* oocysts (original image) scale bar 100µm

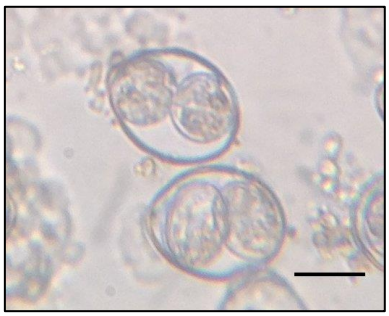


Figure 34 Sporulated *Cystoisospora felis* oocysts (original image) scale bar 25µm

Twenty oocysts were measured and the average was calculated (42.8 x 32.9µm) which was close to the average size for *C. felis*.

4.3.2 Dogs

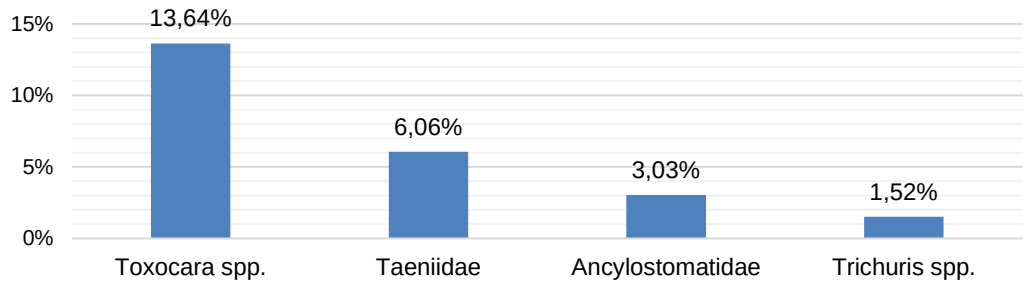
A total of 66 canine samples were tested, of these 21.2% (14/66) were positive for at least one parasite and 3% (2/66) of the samples tested were from an animal with a mixed infection. Species from Phylum Nematoda were present in the largest number of positive samples 18.2% (12/66) with one family (Ancylostomatidae) and two genera (*Toxocara* spp. and *Trichuris* spp) detected. Phylum Platyhelminthes accounted for 6.1% (4/66). No protozoa were found in the studied areas.

Table 10 Positive canine faecal samples by municipality and parasite(s) detected in each sample. [Absolute frequency is presented, followed by the % infected underneath (CI 95%)]

	Municipality						
	Albufeira (n = 6)	**Aljezur (n=7)**	**Lagoa (n=11)**	**Lagos (n=27)**	**Loulé (n = 5)**	**VRSACM (n=10)**	**Total (n=66)**
Ancylostoma-tidae			1	1			2
			9.1%(0.2 - 41.3)	3.7%(0.1 - 19.0)			3% (0.4 - 10.5)
Toxocara spp.			3	2		2	7
			27.3%(6.0-61.0)	7.4% (0.9 - 24.3)		20 (2.5 - 55.6)	10.6% (4.4 - 20.6)
Trichuris spp.				1			1
				3.7 (0.1 - 19.0)			1.5% (0.0 - 8.2)
Taeniidae	1			1			2
	16.7% (0.4 - 64.1)			3.7 (0.1 - 19.0)			3% (0.4 - 10.5)
Taeniidae + *Toxocara* spp.	2						2
	33.3% (4.3 - 77.7)						3% (0.4 - 10.5)
Positive samples	3		4	5		2	14
	50% (11 -88.2)		36.4%(10.9-9.2)	18.5% (6.3 - 38.1)		20%(2.5- 55.6)	21.2% (12.1-33.0)

4.3.2.1 Parasite prevalence in dogs across the region

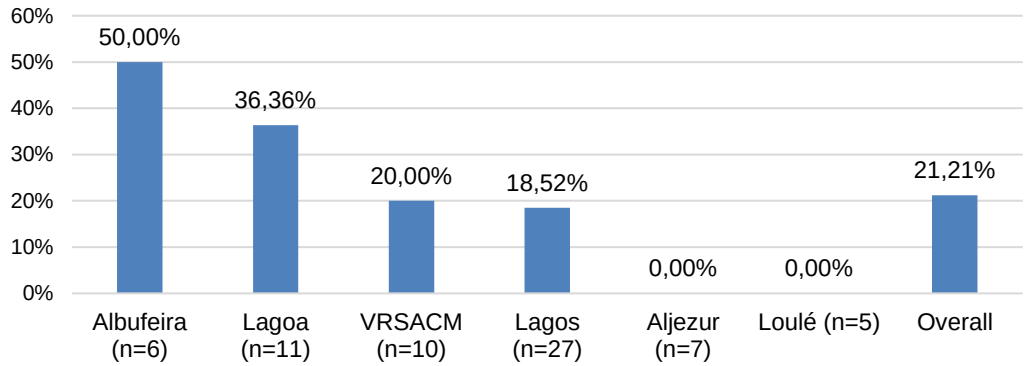
Across the region, nematodes were present in the largest number of positive samples 18.2% (12/66). *Toxocara* spp. was the most frequently detected nematode and also the most frequently detected parasite, 13.6% (9/66). Taeniidae accounted for 6% (4/66) of the positive samples followed by Ancylostomatidae 3% (2/66) and there was one case of *Trichuris* spp. 2% (1/66). The different apparent prevalences by parasite can be seen in Graphic 1. GRAFICO ALTERADO



Graphic 1 Prevalence in dogs across the region by parasite

4.3.2.2 Prevalence of positive dog samples by municipality

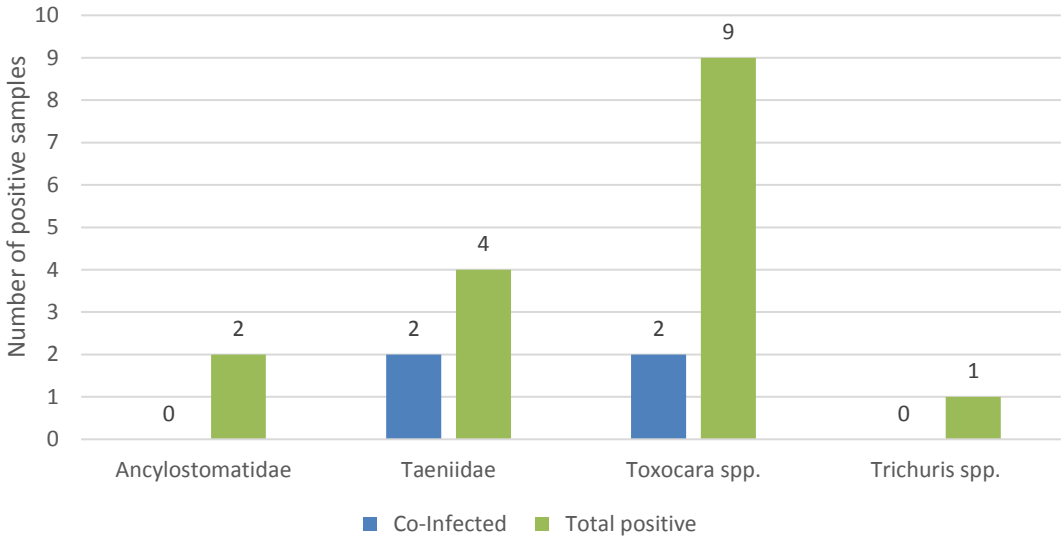
Positive samples were found in four of the six municipalities surveyed with the highest number in Albufeira 50% (3/6), followed by Lagoa 36% (4/11), VRSACM 20% (2/10) and Lagos 18.5% (5/27) with Aljezur and Loulé registering no positive samples. Graphic 2 shows the apparent prevalences for dogs by municipality.



Graphic 2 Positive dog samples by municipality GRAFICO ALTERADO

4.3.2.3 Co-infections

Only two dog samples harboured more than one parasite species, this was *Toxocara* spp. + *Taeniidae* 3% (2/66). In this particular case 50% (2/4) of the samples infected with *Taeniidae* were co-infected with *Toxocara* spp. The different types of co-infections detected are represented by Graphic 3. GRAFICO ALTERADO



Graphic 3 Co-infections in dogs by parasite

4.3.3 Cats

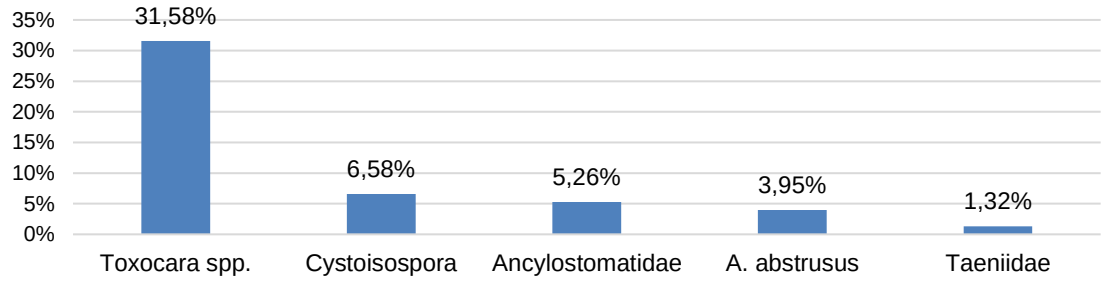
A higher proportion of the cat samples tested were found to be infected with at least one parasite 39.5% (30/76) when compared to the dog samples and 9.2% (7/76) had more than one parasitic infection. The most frequent phylum was Nematoda present in 40.5% (31/76) of the positive samples, followed by Protozoa 6.5% (5/76) and finally Platyhelminthes (Cestoda) 1.3% (1/76).

Table 11 Positive feline faecal samples by municipality and parasite(s) detected in each sample. [Absolute frequency is presented, followed by the % infected underneath (CI 95%)]

	Municipality				
	Aljezur (n = 5)	**Lagoa (n=7)**	**Lagos (n=30)**	**VRSACM (n=34)**	**Total (n=76)**
A. abstrusus	1				1
	20% (0.5 - 71.6)				1.3% (0 - 7.1)
Ancylostomatidae	1			2	3
	20% (0.5 - 71.6)			5.9% (0.7 - 19.7)	3.9% (0.8 - 11.1)
Toxocara spp.	2		16		18
	40% (5.3 - 85.3)		53.3% (34.3 - 71.7)		23.7% (14.7 - 34.8)
Taeniidae					
Cystoisospora sp.			1		1
			3.3% (0.1 - 17.2)		1.3% (0 - 7.1)
A. abstrusus + *Toxocara* spp.			2		2
			6.7% (0.8 - 22.1)		2.6% (0.3 - 9.2)
Ancylostomatidae + Taeniidae		1			1
		14.3% (0.4 - 57.9)			1.3% (0 - 7.1)
Cystoisospora sp. + *Toxocara* spp.			4		4
			13.3% (3.8 - 30.7)		5.3% (1.5 - 12.9)
Total positive samples	4	1	23	2	30
	80% (28.4 - 99.5)	14.3% (0.4 - 57.9)	76.7% (57.7- 90.1)	5.9% (0.7 - 19.7)	39.5% (28.4 - 51.4)

4.3.3.1 Parasite prevalence in cats across the region

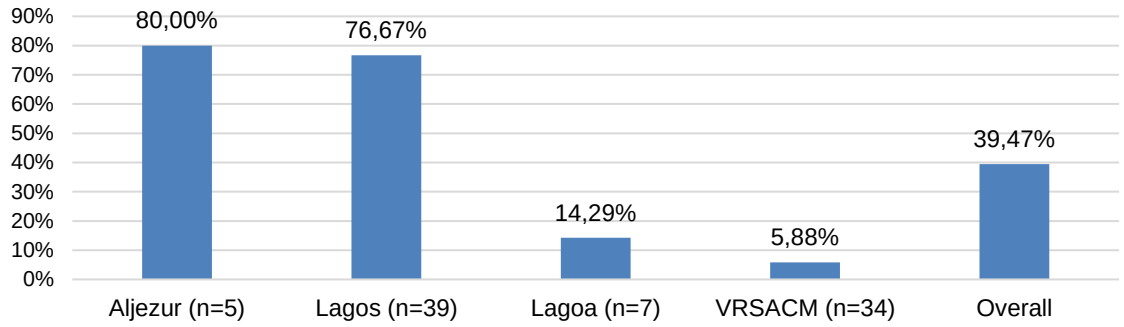
Toxocara spp. was the most common parasite detected 31.6% (24/76), followed by *Cystoisospora* sp. 6.6% (5/76), Ancylostomatidae 5.3% (4/76), *Aelurostrongylus abstrusus* 4% (3/76) and Taeniidae *1.3%* (1/76). The different apparent prevalences in cats for each parasite are shown in Graphic 4. GRAFICO ALTERADO



Graphic 4 Prevalence in cats across the region by parasite

4.3.3.2 Prevalence of positive cat samples by municipality

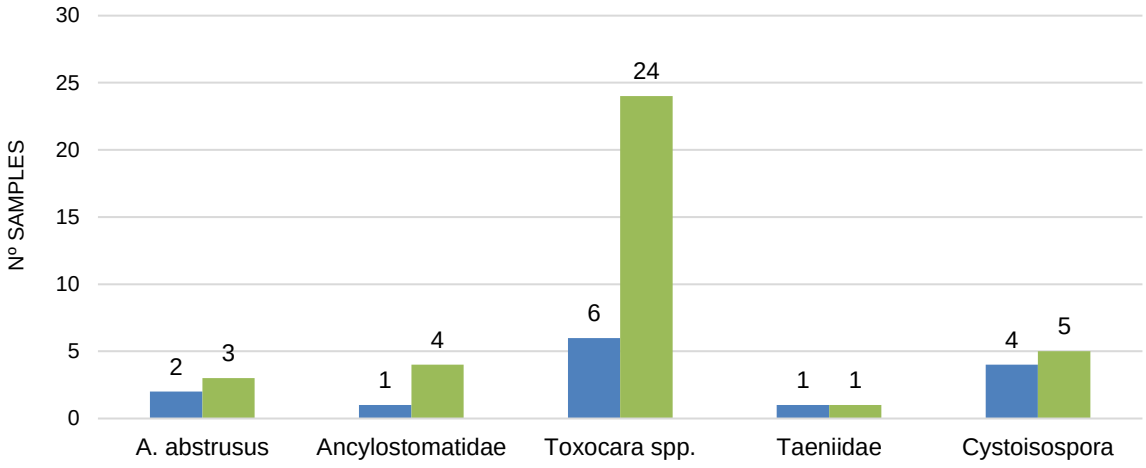
All four municipalities surveyed registered positive samples (Graphic 5). The highest percentage of positive samples was found in Aljezur 80% (4/5), followed by Lagos 76.7% (23/30), Lagoa 14.3% (1/7) and VRSACM 5.9% (2/34). GRAFICO ALTERADO



Graphic 5 Positive cat samples by municipality

4.3.3.3 Co-infections

Co-infections, involving two different parasites, were found in 9.2% (7/76) of the samples tested. The most frequent type was between *Cystoisospora* + *Toxocara* spp 5.3% (4/76) followed by *A. Abstrusus* + *Toxocara* spp. 2.6% (2/76) and there was one case of Ancylostomatidae + Taeniidae 1.3% (1/76*).* The percentage of co-infected samples by parasite was 80% (4/5) for *Cytoisospora* spp., 66.6% (2/3) for *A. abstrusus,* 25% (6/24) for *Toxocara* spp. and 25% (1/4) for Ancylostomatidae. The only positive sample for *Taeniidae* was also co-infected with Ancylostomatidae representing 100%, as demonstrated in Graphic 6. GRAFICO ALTERADO



Graphic 6 Co-infections in cats by parasite.

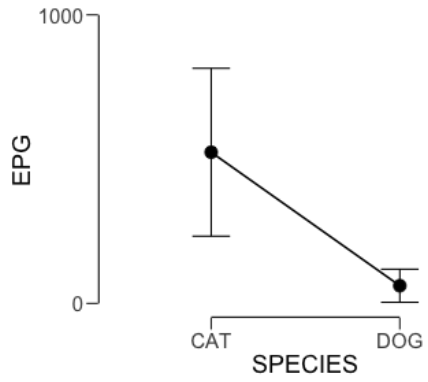
4.3.4 Quantitative EPG and OPG

The total number of EPG or OPG in each sample was counted (Table 12) and the intensity of infection for each parasite species in each host was calculated. The mean EPG count in dogs was 38.44 ± 61.30 and in cats the mean combined EPG and OPG was 436.88 ± 653.89, the difference in the mean numbers of eggs collected between species was statistically significant using one way anova (F = 5,37, df = 1, p=0.0248).

	Dogs		**Cats**	
	n positive	Mean (EPG) ± SD	n positive	Mean (EPG or OPG) ± SD
Toxocara spp.	9	61,3 ± 74,65	24	523,92 ± 688,75
Ancylostomatidae	2	1,5 ± 0,707	4	4,75 ± 3,86
Taeniidae	4	14,75 ± 15,06	1	73
Cystoisospora	0	0	5	437,60 ± 924,88
Trichuris spp.	1	2	0	0
	16	38,44 ± 61,30	34	436,88 ± 682,99

Table 12 Intensity of infections found by species, n positive = nº samples positive for the parasite (CI 95%)

Toxocara spp. was by far the most prevalent parasite affecting both species and therefore further analysis of EPG counts was warranted. The mean infection intensity for *Toxocara* spp. in dogs was 61,33 ± 74,65 EPG compared to cats which was 523,92 ± 688,75 EPG. Independent samples T-test was used to compare the infection intensity between species (t= 1,991, df = 31, p=0,055), the difference was not significant when considering a 95% confidence interval, but this parasite EPG showed in this sample to over eight times greater in cats than in dogs.



Graphic 7 Descriptive plot comparing mean *Toxocara* spp. EPG count in dogs and cats.

Due to the limited numbers of positive cases of Ancylostomatidae and Taeniidae, the other two parasite families affecting both groups, no statistical analysis was performed comparing EPG between species.

In dogs, the EPG count in all Ancylostomatidae, *Trichuris* spp. and Taeniidae positive samples was <100, the majority of *Toxocara* spp. positive samples 77,7% (7/9) also had <100 EPG. In cats the results showed that only 37.5% (9/24) of the *Toxocara* spp. positive samples had an EpG <100, also confirming the higher EPG counts in cats than in dogs. The only sample positive for Taeniidae in cats had an EPG <100 and out of the five cases of *Cystoisospora* sp. detected, 80% (4/5) had an OPG <100.

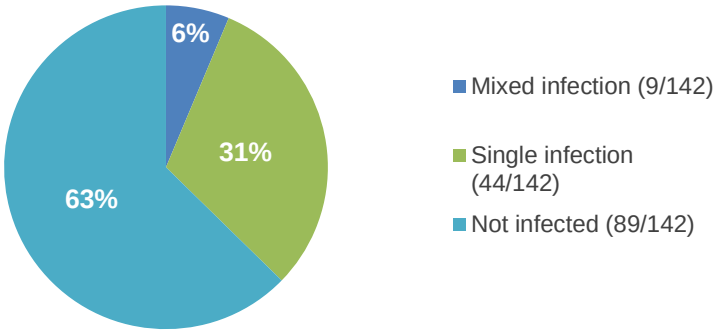
Table 13 Numbers of EPG or EPG < or > than 100 by species and parasite.

	Dogs			Cats		
	< 100	> 100	LH*	< 100	> 100	LH*
Toxocara spp.	77.7% (7/9)	22.2% (2/9)	201	37.5% (9/24)	62.5% (15/24)	2576
Ancylostomatidae	100% (2/2)	0%	0	100% (4/4)	0%	1
Trichuris spp.	100% (1/1)	0%	1	0%	0%	0
Taeniidae	100% (4/4)	0%	36	100% (1/1)	0%	73
Cystoisospora sp.	0%	0%	0	80% (4/5)	20% (1/5)	2080

* LH = Lowest to highest count - difference between the lowest and highest EPG or OPG found for each pair.

4.3.5 Co-infections in both species

Across the region, the results obtained demonstrate that 31% [CI 95%: 24–39] (44/142) of the samples tested, cats and dogs, were positive for at least one parasite while 6% [CI 95%: 2.9–11.6] (9/142) had mixed infections. Graphic 8 shows the different infection statuses out of all the samples tested.



Graphic 8 Infection prevalence status, out of the total number of samples tested

All mixed infections observed included two different parasitic species. Cases were found involving all three parasitic classes detected in the study: Nematoda, Protozoa and Cestoda. *Toxocara* spp. was the most frequent parasite detected in association with a second agent, accounting for 89% (8/9) of the cases. The most frequent mixed infections were found in cats and were between *Cystoisospora* sp. + *Toxocara spp*. 44% (4/9), followed by *A. abstrusus* + *Toxocara spp.* 22% (2/9) and one case of

Anycylostomatidae + *Taeniidae* 11% (1/9). In dogs there were two cases of Taeniidae + *Toxocara spp. 22%* (2/9). Mixed infections were found in 9.2% (7/76) of the cat samples and 3% (2/66) of the dogs. No triple infections were found in either species.

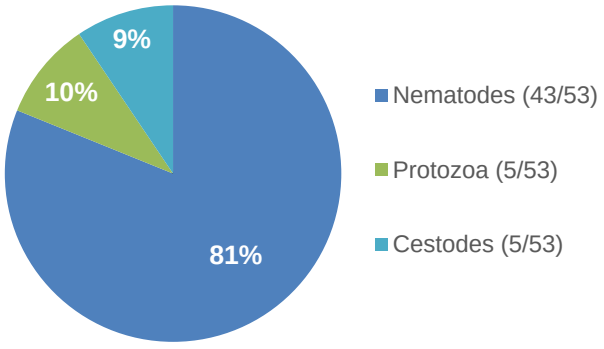
Table 14 Representation of the types of infection detected by species.

Species		Positive			Total
		Negative	Single	Mixed	
Cat	Count	46	23	7	76
	% within row	60.5 %	30.3 %	9.2 %	100.0 %
Dog	Count	52	12	2	66
	% within row	78.8 %	18.2 %	3.0 %	100.0 %
Total	Count	98	35	9	142
	% within row	69.0 %	24.6 %	6.3 %	100.0 %

Chi square test was performed to assess whether there was a relationship between the type of infection (negative, single or mixed) and species (χ^2 = 5.927, df = 2, P = 0.052), since the P-value (0.052) is higher than the significance level (0.05), the null hypothesis was accepted, concluding that in this study there was no relationship. When comparing negative samples to positive samples (single or mixed infection) between species, the difference was statistically significant (χ^2 = 5.509, df = 1, P = 0.019) indicating that cat samples were more likely to be positive than dog samples.

4.3.6 Parasite families in both species

Nematodes were by far the most commonly detected parasites with one family (Ancylostomatidae) and three species (*Toxocara* sp., *Trichuris* sp. and *A. abstrusus*) present, representing 81% (43/53) of the positive samples and 30% (43/142) of all the samples tested. Cestodes (Taeniidae) and Protozoa (*Cystoisospora* spp.) only accounted for 9.4% (5/53) each of the positive samples and 3.5% (5/142) respectively of the total tested. Graphic 9 shows the different proportions for each parasite class.



Graphic 9 Representation of proportion of positive cases for each class

4.3.7 Municipal prevalence for both species

The overall number of positive samples varied significantly by municipality with Albufeira registering the highest proportion of infected animals with 50% (3/6), followed by Lagos, 49,1% (28/57), Aljezur, 33,3% (4/12), Lagoa, 27,8% (5/18) and VRSACM, 9,1% (4/44), with Loulé registering no positive cases.

Table 15 Sample status by municipality

Location		Status		Total
		Negative	Positive	
Albufeira	Count	3	3	6
	% within row	50.0 %	50.0 %	100.0 %
Aljezur	Count	8	4	12
	% within row	66.7 %	33.3 %	100.0 %
Lagoa	Count	13	5	18
	% within row	72.2 %	27.8 %	100.0 %
Lagos	Count	29	28	57
	% within row	50.9 %	49.1 %	100.0 %
Loulé	Count	5	0	5
	% within row	100.0 %	0.0 %	100.0 %
VRSACM	Count	40	4	44
	% within row	90.9 %	9.1 %	100.0 %
Total	Count	98	44	142
	% within row	69.0 %	31.0 %	100.0 %

Chi-square test was performed on all samples tested and revealed that the proportion of negative to positive animals by municipality doesn't follow the expected distribution and therefore it is possible to conclude statistically that the ratio of positive to negative samples varies significantly by municipality (χ^2 22,01, df 5, $p < 0,001$). Due to the small sample sizes from some municipalities no statistical analysis was performed comparing the prevalence between them.

4.3.8 *Toxocara* prevalence for both species

Toxocara spp. was detected in 23.2% (33/142) of all the samples tested. All municipalities surveyed, with the exception of Loulé, registered infections in either dogs, cats or both. Lagos had the highest prevalence at 42.1% (24/57), followed by Albufeira 33.3% (2/6), Lagoa 16.7% (3/18), Aljezur 16.7% (2/12) and finally VRSACM 4.6% (2/44). The prevalence for *Toxocara* spp. in the region was 23.2% (33/142) out of 31% (44/142) positive samples. *Toxocara* spp. accounted for 75% (33/44) of all the positive samples.

In all municipalities where it was present, the ratio of *Toxocara* spp. infected animals to those infected with other agents, not including *Toxocara* spp*.,* was either equal or over 50%. The highest proportion of *Toxocara* spp*.* to other infections was found in Lagos 85.7% (24/28), followed by Albufeira 66% (2/3), Lagoa 60% (3/5), Aljezur 50% (2/4) and 50% VRSACM (2/4).

The Chi-square test was used to compare *Toxocara* spp. infection status with location, the results (χ^2 22,58, df 5, p < 0,001) demonstrate that there is a significant difference between the number of positive and negative samples by location.

Table 16 *Toxocara* spp. prevalence by municipality

		Toxocara spp.		
Location		**Negative**	**Positive**	**Total**
Albufeira	Count	4.00	2.00	6.00
	% within row	66.7 %	33.3 %	100.0 %
Aljezur	Count	10.00	2.00	12.00
	% within row	83.3 %	16.7 %	100.0 %
Lagoa	Count	15.00	3.00	18.00
	% within row	83.3 %	16.7 %	100.0 %
Lagos	Count	33.00	24.00	57.00
	% within row	57.9 %	42.1 %	100.0 %
Loule	Count	5.00	0.00	5.00
	% within row	100.0 %	0.0 %	100.0 %
VRSACM	Count	42.00	2.00	44.00
	% within row	95.5 %	4.5 %	100.0 %
Total	Count	109.00	33.00	142.00
	% within row	76.8 %	23.2 %	100.0 %

Toxocara spp. was present in 31,6% (24/76) of the cat samples and 13,6% (9/66) of the dogs. The chi-squared test (χ^2 6,375, df 1, p < 0,012) was calculated to compare infection status between species and showed that the difference was statistically significant (p <0.012).

Table 17 *Toxocara* spp. prevalence by species

Species		Toxocara spp.		Total
		Negative	Positive	
Cat	Count	52	24	76
	Expected count	58.34	17.66	76.00
	% within row	68.4 %	31.6 %	100.0 %
Dog	Count	57	9	66
	Expected count	50.66	15.34	66.00
	% within row	86.4 %	13.6 %	100.0 %

4.4 DISCUSSION

This is the first study conducted in the Algarve assessing the prevalence and infection intensity of gastrointestinal and cardiopulmonary parasites in dogs and cats using the highly sensitive FLOTAC technique. As all of the animals in the study were housed in shelters, with many having been able to roam free before being rescued, their medical history was unknown and there is a distinct possibility that many had not been treated with antiparasitic drugs for long periods of time. Out of the 142 faecal samples tested, (66 dogs, 76 cats) between February and April 2015, the overall prevalence of intestinal and/or lung parasites in the region was 31% (44/142). Although few studies are available on cats and dogs in Portugal, the overall prevalence of positive samples for both species is similar to that found in Évora, 39.2% (Ferreira et al., 2011) and Western Portugal, 24.7% (Melo, 2017).

4.4.1 Dogs

Across the region 21.2% (14/66) of the dogs were positive for at least one parasite. The regional prevalence observed is similar to previous studies in kennels in the country using other flotation methods including a study on dogs from 7 districts of the country by Félix (2010) that revealed a prevalence of 25% and Lebre (2011) which found that 24.6% of dogs from Lisbon had at least one parasitic infection. A study in Oporto that divided animals into healthy and those exhibiting gastrointestinal (GI) symptoms yielded a prevalence of 20.6% in healthy animals, similar to this study, and a slightly higher one of 33.7% in dogs with GI disease (Neves, Lobo, Simões, & Cardoso, 2014). In central Italy, similar prevalence levels were recorded, with 31% of dogs studied found to be infected with at least one parasite (Riggio, Mannella, Ariti, & Perrucci, 2013). Studies demonstrating significantly lower prevalence include 5% found in Vila Franca de Xira (Morgado, 2016) and 9.4% in Lancashire, UK using the

FLOTAC technique (Wright, Stafford, & Coles, 2016). Although the results from different studies aren't directly comparable, it is possible to conclude that the overall prevalence in dogs in this study is high which is concerning when considering the zoonotic nature of some of the species found and the fact the standard anthelmintic prophylaxis could have significantly reduced the number of positive samples or eliminated the infections altogether.

Nematodes were present in 100% (14/14) of the positive dog samples, two of which 14% (2/14) also contained cestodes. These findings are consistent with Bowman (2014) and ESCCAP (2010a) that nematodes are the most prevalent class of parasites in domestic canids. Other studies in Portugal have shown a similar tendency (Félix, 2015; Melo, 2017). Co-infections were present in only 2.6% (2/66) of the samples tested, both were from the municipality of Albufeira and were infected with Taeniidae + *Toxocara* spp., accounting for 14.2% (2/14) of all the positive samples. The prevalence of co-infections in this study is lower than other studies including: 8.1% in Évora (Ferreira et al., 2011) and 14.8% in central Italy (Riggio et al., 2013), but is similar to 4% found in a nationwide study in Portugal (Félix, 2015) and 5.4% in a study from Lodz, Poland (Zając et al., 2017).

4.4.2 Cats

Microscopic analysis revealed that 39.5% (30/76) of the faecal samples were positive for at least one endoparasite. The overall prevalence in this study is higher than a study in Finland using faecal flotation methods which found intestinal worms eggs in 7.1% of the samples tested (Näreaho et al., 2012) but is close to other large epidemiological studies in Europe which found prevalence levels of 35.1% (Beugnet et al., 2014) and 37.19% (Rehbein et al., 2014) and also regional studies in Pisa Italy 35% (Riggio et al., 2013), stray cats in Northern Italy 50.4% (Spada et al., 2013) and Lisbon and Setubal 43,5% (Teixeira Carvalho, 2017). It is significantly lower than 90.7% observed by Waap, Gomes, & Nunes, (2014) by means of parasitological necropsy of 162 stray cats from Lisbon, 63.9% observed in Braga and Viana do Castelo (B. Matos, 2016) and 65.31% found in north-western Brazil using the FLOTAC technique (Monteiro et al., 2016). Although similar results have been found in other studies, the results aren't directly comparable due to differences in methods used, locations where the animals came from, animal ages and even environmental conditions which vary according to the time of year the samples are collected.

Nematodes were present in 40.5% of the samples (31/76) and recorded a much higher prevalence than protozoans 6.5% (5/76) and cestodes 1.3% (1/76). Similar results were found in a study on stray cats in Italy (Spada et al., 2013) which found nematodes in 47.5% of the samples and a similar proportion of Protozoa 7.2%. Co-infections were found in 9.2% (7/76) of the cat samples, which is significantly lower than the frequency reported in other studies: 17.2% in seven European countries (Rehbein et al., 2014), 14.3% of stray cats in Northern Italy (Spada et al., 2013), 28.5% in Central Italy (Riggio et al., 2013) and 46.01% in Northern Brazil (Monteiro et al., 2016). It is close the 8.8% prevalence found by Zając et al. (2017) in Lodz, Poland. The most common mixed infection was *Cystoisospora* sp. + *Toxocara* spp. present in 5.3% (4/76) of the samples tested and representing 57% (5/7) of all co-infections in cats and is close the prevalence of this type of co-infection found by Melo (2017) 2.9% in Western Portugal. This particular association is perhaps not surprising when considering that *Toxocara* spp. was also the most common single parasite infection found in this study and also in 86% of the co-infected samples, its association with *Cystoisospora* appears to be supported by ESCCAP (2011) that the clinical presentation of *Cystoisospora* is "often associated with viral, helminth or bacterial co-infections". The other co-infections found were *A. abstrusus* + *Toxocara* spp. 2.6% (2/76) and Ancylostomatidae + Taeniidae 1.3% (1/76).

4.4.3 General observations

The study demonstrated that the prevalence of positive samples, with at least one parasite, varied significantly between species with 21.2% (14/66) of dog samples registering at least one parasite compared to 39.5% (30/76) of the cat samples, the difference was statistically significant ($p<0.05$) ($t=2.377$, $df=140$ $p=0.019$). Other studies show a similar trend in the difference in prevalence between species, these include Melo (2017) in Western Portugal (15% dogs vs 39% cats), Wright et al. (2016) in Lancashire (UK) using the FLOTAC technique (9.4% dogs vs 32.8% cats), Riggio et al. (2013) in central Italy (31% of dogs vs 35% cats) and Zając et al. (2017) in Lodz, Poland (29.5 of dogs vs 48.5% cats).

Mean EPG and OPG collected from all the samples from each species were found to be 38.44 ± 61.30 in dogs and 436.88 ± 682.99 in cats and the difference was found to be significant ($p = 0.025$). Reasons for the difference in the proportion of positive

samples between species and apparent infection intensity may be related to population density. The dogs were kept in individual enclosures with small numbers of animals <10, compared to the cats which were mainly free to roam about in large groups within large enclosures or facilities. Population density has previously been described as a contributing factor toward higher rates of *Cystoisospora* spp. in cats (Knaus et al., 2014) and it is also commonly accepted that transmissible pathogens are acquired more readily when higher numbers of individuals populate the same space compared to smaller numbers, especially for parasites showing direct life cycles like the ones mentioned before.

More general reasons may be that cats overall are harder to handle and administer drugs than dogs (Lefebvre & Reynolds, 2007) which may suggest that they are less likely to be treated than dogs. Cats are also considered to be more predatory than dogs which may be a factor especially when we consider that *Toxocara* spp., which can be transmitted via paratenic hosts, was by far the most prevalent parasite species detected in both species, and therefore contributed greatly to the overall prevalence in cats. Perhaps the most significant reason may lie in the results of a survey conducted by Matos et al., (2015) on dog and cat owners visiting a University of Lisbon's Veterinary Hospital that found that 89.7% of dog owners treated their dogs with endoparasitic drugs compared to only 63.6% of cat owners, significantly the study found that only 11.8% of the dogs were treated with the recommended treatment regimen (minimum quarterly) compared to only 5.5% of the cats. It is possible that this phenomenon of dogs receiving better antiparasitic prophylaxis compared to cats is also partly at play here.

Overall prevalence for both species varied significantly by municipality with the highest proportion of positive samples found in Albufeira, 50% (3/6), followed by Lagos, 49.1% (28/57), Aljezur, 33.3% (4/12), Lagoa, 27.8% (5/18) and VRSACM, 9.1% (4/44) with Loulé (n=5) registering no positive samples. In order to get a better idea of the parasites present in the region, the priority in this study was to spread out the sample collection across different municipalities in the region and therefore sample sizes from some municipality are relatively small making it difficult to compare results between them. However, the overall rate of positive samples did vary between municipalities and was found to be statistically significant ($p < 0.001$), these variations probably reflect the different prophylactic measures the animals were under and also the different housing

conditions including population density, hygiene practices and access to intermediate hosts in the case of cats.

Statistical analysis was performed to assess whether co-infections were more likely in dogs or cats, having been found in 3% and 9.2% of the samples respectively. No statistical relationship was proven (p=0.052) which may be related to the small sample sizes, nevertheless other studies on both species have shown a higher proportion of co-infected cats compared to dogs (14.8% dogs and 28.5%; Riggio et al., 2013).

4.4.4 Gastrointestinal nematodes

4.4.4.1 Ancylostomatidae

Ancylostomatidae eggs were found in 3% (2/66) of the dog samples, all samples contained <100 EPG. Other studies in the country revealing significantly higher prevalence include: 9.5% in Kennel dogs from Lisbon (Lebre, 2011), 11% of kennel dogs from Continental Portugal (Félix, 2015) and 31.28% of farm dogs and 70.3% of hunting dogs from Ponte de Lima (Mateus, Castro, Ribeiro, & Vieira-Pinto, 2014). Studies showing lower prevalence similar to this study include 1.3% in Vila Franca de Xira (Morgado, 2016) and 0.6% using the FLOTAC technique in Lancashire, UK (Wright et al., 2016).

A slightly higher proportion of cat samples 5.3% (4/76) revealed Ancylostomatidae eggs compared to the dogs, mean egg counts were <100 EPG in all cases. The results are similar to those found in Braga and Viana do Castelo 5.9% (B. Matos, 2016), Lancashire, UK, 3.1% (Wright et al., 2016) and slightly higher than those found in Europe, 1.4% (Beugnet et al., 2014) but lower than cats from catteries in Lisbon and Setubal, 10.4% (Teixeira Carvalho, 2017) and North-western Brazil also using the FLOTAC technique, 67.2% (Monteiro et al., 2016).

Species identification wasn't possible but it is probable that the dogs were infected with *A. caninum* and the cats with *A. tubaeforme* due to their predominance in central and southern Europe and continental Europe, respectively (ESCCAP, 2010a). *U. stenocephala* exists throughout Europe and infect cats and dogs, but occurs mainly in colder climates (ESCCAP, 2010a) which would reduce the probability of it being encountered in this study taking into account the temperate climate encountered in the Algarve.

4.4.4.2 Toxocara spp.

Toxocara spp. was the most dominant parasite detected in this study and was found in 13.6% of samples (9/66) of the dog and 31.6% (24/76) of the cat samples. Previous studies in the country with lower *T. canis* prevalence include 5% in Continental Portugal (Félix, 2015), 2.8% in kennels in Lisbon (Lebre, 2011) and 5.1% of healthy dogs and 7.8% of dogs with GI disease in Oporto (Neves et al., 2014). A lower prevalence was also found in Lancashire, UK using the FLOTAC technique 5.3% (Wright et al., 2016) and the Netherlands 4.6% (Nijsse, Ploeger, Wagenaar, & Mughini-Gras, 2014). Significantly higher prevalence were found in Pisa, Italy, 32.3% (Riggio et al., 2013) and kennelled sled dogs in Poland 36% (Bajer, Bednarska, & Rodo, 2011). Dogs of any age can become infected with *Toxocara* spp. (Awoke, Bogale, & Chanie, 2011; Zając et al., 2017) which may explain why high prevalence levels were found in this study even though all the animals were > 6 months old. A study by Nijsse, Mughini-Gras, Wagenaar, & Ploeger (2014) found that coprophagia was a widespread behaviour among household dogs and that due to this, coproscopical examination may overestimate patent *Toxocara* spp. infections by up to 50%. Although no information relating to this behaviour in the animals studied was available, it is possible that the results here are also overestimated because of this factor.

The *Toxocara* spp. prevalence of 31.6% in cats in this study is close to the one found by Waap et al., (2014) which found *T. cati* in 38.3% of the stray cats in Lisbon, lower than those found by B. Matos (2016) in Braga and Viana do Castelo 45.9% and slightly higher than that found by Teixeira Carvalho (2017) which found a prevalence of 18.1% in Lisbon and Setubal. Studies using the FLOTAC technique show varying prevalence, ranging from 26% in Lancashire, UK (Wright et al., 2016) to 40.7% in North-western Brazil (Monteiro et al., 2016) which when considering the 95% confidence interval are close to the results found here. Other reports from Europe indicate varying prevalence and these include 7.2% in the Netherlands (Nijsse, Ploeger, Wagenaar, & Mughini-Gras, 2016), 19.7% in owned cats from seven European countries (Beugnet et al., 2014), 22.2% in Italy (Riggio et al., 2013), 33% in stray cats from Northern Italy (Spada et al., 2013), 48% in Tirana, Albania (Knaus et al., 2014) and an extremely high prevalence of 84.8% in Denmark (Takeuchi-Storm et al., 2015). The high prevalence found here may be due to free roaming, that the cats had done before entering the shelter, which has been previously described as a significant factor contributing to the

acquisition of patent *Toxocara* spp. infections (Nijsse et al., 2016). It is generally believed that younger cats are at a higher risk for *T. cati* compared to older animals, however a recent study by Beugnet et al. (2014) found that although cats > 24 months old were at a lower risk for *Toxocara* spp. than those < 6 months old, cats > 6 years old could also become infected which demonstrated that immunity was not absolute. This may explain why such a large proportion of the cat samples from the mature population in this study were positive. The study by Beugnet et al. (2014) also found that population density +3 cats in the same house was a risk factor, when considering the elevated population density found in this study it is clear that this risk factor was also present here thereby contributing to the overall prevalence detected.

The χ^2 test showed that cats were significantly more likely to be infected with *Toxocara* spp. than dogs ($p = 0.012$) which is in accordance with previous studies where higher prevalence levels were detected in cats rather than dogs. (Lucio-Forster, Barbecho, Mohammed, Kornreich, & Bowman, 2016; Wright et al., 2016). *Toxocara* spp. average egg outputs were 61.3 ± 74.65 in dogs compared to 523.92 ± 688.75 in cats with 77.7% (7/9) of the former's samples containing <100 EPG compared to 37.5% (9/24) of the latter's. A similar tendency was observed by Wright et al. (2016) in Lancashire, UK also using the FLOTAC technique, which found that 55.6% of the dogs studied had <100 EPG compared to only 17.6% of the cats. Lower mean egg outputs from dogs (246.2 ± 45.1) compared to cats > 12 months old (549.4 ± 237.1) were also observed in Lodz, Poland (Zając et al., 2017). Although the average egg density in this study appears to differ greatly between species, statistical analysis revealed that it wasn't statistically significant ($p=0.055$). It is possible that the reduced egg output in dogs compared to cats may be in part due to coprophagy and not the result of patent infections (Nijsse, Mughini-Gras, et al., 2014). As *Toxocara* species weren't identified it wasn't possible to determine whether some of the eggs excreted by the dogs were in fact *T. cati*. Although none of the dogs were in direct contact with cats or appeared to have contact with their faeces at the shelters visited, many were taken on walks and could have ingested cat faeces then. The low counts (some as low as 3 EPG) which were observed may indeed support the idea that some of the positive samples were due to the ingestion of infected faeces rather than patent infections. One co-infected sample from Albufeira containing Taeniidae + *Toxocara* spp. revealed embryonated *Toxocara* spp. eggs (Figure 24). Seen as *Toxocara* spp. are not excreted from the host

embryonated, this would appear to indicate that the host wasn't patently infected, and that the eggs were passively passing through.

Cats are generally not believed to be coprophagic (Lucio-forster et al., 2016), it is therefore likely that the majority of the results found represent patent infections, nonetheless it is possible that some of the 17.6% of samples with less than 100 EPG were indeed due to sample contamination with *Toxocara spp*. within the sand from which the samples were collected. The increased *Toxocara* spp. prevalence and egg output found in this study in cats compared to dogs, suggests that untreated cats may play a greater role in contaminating the environment with *Toxocara* spp. eggs than dogs. Free roaming in cats is believed to contribute significantly to the acquisition of patent *Toxocara spp.* infections and consequently to environmental contamination (Nijsse et al., 2016). The extent of this contamination has previously been described by Otero et al. (2016) in parks in Lisbon, which found that 85.5% of samples from sandpits and 34.4% from parks in the municipality were contaminated with *T. cati* eggs posing an elevated risk to other cats and a zoonotic risk especially to young children playing in sandpits.

It is vital to emphasise that *Toxocara* spp., a parasite with a significant zoonotic risk, was by far the most frequently detected parasite in both species in this study. Due to ability for roundworms to continually re-infect dogs and cats regular anthelmintic treatment in young and adult animals is essential (Lee et al., 2010). Current ESCCAP guidelines recommend this be done at least four times a year to reduce environmental contamination and monthly to treat patent infections (ESCCAP, 2010a).

4.4.4.3 Trichuris spp.

Out of all the samples tested only one dog sample was positive for *Trichuris* spp., the sample containing two eggs came from Lagos and represented 2% (1/66) of the dog samples. *Trichuris* spp. appears to have a relatively low prevalence in dogs in Portugal with other studies showing similar results to ours, these include: 1.1% in Lisbon kennels (Lebre, 2011), 1.1% in healthy dogs and 2.6% in those with GI disease in Oporto (Neves et al., 2014) and an overall prevalence of 1.5% in continental Portugal (Félix, 2015). As some of the animals tested in the Lagos municipality were housed outside near land where animals graze there is also the possibility that the *Trichuris* spp. eggs found are the result of the dog in question eating mice or sheep faeces

(Nijsse, Mughini-Gras, et al., 2014). However this hipothesis is not likely here as *Trichuris ovis* eggs are darker than the ones found.

4.4.5 Pulmonary nematodes

Out of the 66 dog samples tested, none were positive for pulmonary nematodes. This is surprising considering that *Angiostrongylus vasorum* is endemic in the country (ESCCAP, 2010b) as confirmed by a large study of 906 shelter dogs from Northern to Southern mainland Portugal using the ELISA method which found that 1.32% of the animals tested were antibody positive and 0.66% were positive for both antibody and antigen (Alho et al., 2016). A 2% prevalence was also recorded in Lisbon by Nabais et al., (2014).

As it is possible to obtain a much higher *A. vasorum* average number of larvae per gram using the FLOTAC technique with the FS3 flotation solution compared to McMaster, flotation in tube and the Baermann-Wetzel technique (Schnyder et al., 2011), and both the FLOTAC technique and the most effective FS (FS3) solution were used in this study. It is possible that either the animals tested were negative or as with other copromicroscopic techniques, sensitivity and specificity were affected due to prepatency, intermittent larval excretion or the occurrence of mixed lungworm infections (Alho et al., 2016). Even though no positive samples were detected in this study, due to the confirmed endemicity in the country, vigilance is vital so that prompt and early detection of cases can be made and targeted therapy given at an early stage which generally results in a good prognosis for this potentially fatal parasite.

Across the region, 3,95% (3/76) of the cats were infected with pulmonary nematodes. *Aelurostrongylus abstrusus* L1 larvae were detected in three cats, two of which, 2.6% (2/76), were also co-infected with *Toxocara* spp. Positive samples came from catteries in Aljezur (n=1) and Lagos (n=2). The 3.95% prevalence encountered in the region is lower than the values observed in other studies from Lisbon (i.e. 12% and 12.4%) (Nabais et al., 2014; Waap et al., 2014), Northwestern Portugal (22.4%)(B. Matos, 2016) and Oporto (17.4%) (Payo-Puente et al., 2008) and closer to the prevalence levels encountered in the three regions of Portugal (i.e. Centre and South 1.7%, Lisbon 11.7% and North 2.5%) sampled by Giannelli et al. (2016), Pisa Italy (1.2%) (Riggio et al., 2013) and very close the overall results (4.1%) of the study conducted in 7

European countries by Beugnet et al. (2014) and 5.4% recorded by Teixeira Carvalho, (2017) in Lisbon and Setubal.

The FLOTAC technique has been compared to other standard copromicroscopic techniques including the Baermann, McMaster and the Wisconsin techniques and was shown to be significantly more sensitive at detecting *A. abstrusus.* (Gaglio, Cringoli, Rinaldi, Brianti, & Giannetto, 2008). As none of the abovementioned studies used the FLOTAC technique direct comparisons with this study aren't possible, nonetheless when compared to other studies in the country using less efficient copromicroscopic techniques a higher prevalence would have been expected. It is possible that some of the results in this study were false negatives, one reason for this is that infected cats don’t shed L1 constantly which causes significant variations in larval shedding. To avoid this and increase sensitivity, samples should have been collected over three consecutive days (Traversa et al., 2010) which wasn't possible in this case.

Cats infected with the feline lungworm may exhibit symptoms such as a chronic mild cough, dyspnoea following exercise as well as diarrhoea, anorexia and weight loss in more severe cases (Taylor et al., 2016) all clinical signs which may occur with other diseases. It is therefore essential that a differential diagnosis for *A. abstrusus* be included when diagnosing any cats exhibiting these clinical signs especially in endemic areas, so that effective anthelmintic therapy can be given ensuring a complete clinical and parasitological recovery (Crisi et al., 2016).

4.4.6 Gastrointestinal cestodes

Taeniidae eggs were present in 6% (4/66) of the dog samples, 50% (2/4) of which were co-infected with *Toxocara* spp. The prevalence found for dogs is slightly higher than other studies in the country: 0.51% in farm dogs and 1.98% in hunting dogs from Ponte de Lima (Mateus et al., 2014), 0.6% in kennels in Lisbon (Lebre, 2011) and the overall results from a study in mainland Portugal 3.5% (Félix, 2015).

Taeniidae was only found in one cat sample 1.3% (1/76), which came from Lagoa, the sample was also co-infected with Ancylostomatidae. The prevalence for cats in this study is very close to the results from other studies in Europe using flotation techniques: 1.3% out of 1519 from 7 European countries (Beugnet et al., 2014), 1.2% in Lisboa and Setubal (Teixeira Carvalho, 2017), 2% in Tirana, Albania (Knaus et al., 2014) and 1.5% in Finland (Näreaho et al., 2012).

As it is not possible to differentiate *Taenia* sp.eggs from *Echinococcus* sp. eggs microscopically (ESCCAP, 2010b), species identification wasn't possible. The low Taeniidae prevalence levels found in this study and those cited for both cats and dogs may be underestimated due to the parasite's biology, namely the irregular detachment of proglottids and consequent egg excretion (Takeuchi-Storm et al., 2015) making detection by coprological techniques less sensitive and intermittent. *Taenia* spp. and E*chinococcus* spp. eggs are also shed into the environment within proglottids and therefore even when they are excreted only a small number may be free in the faeces which could originate false negatives using faecal flotation techniques. (Näreaho et al., 2012). Each positive sample in this study had EPG <100 which seems to support the previous statement.

4.4.7 Gastrointestinal protozoa

No protozoa were detected in any dog samples. As the FLOTAC technique has been proven to detect intestinal protozoa infections in humans with a high sensitivity (Becker et al., 2011) it may be assumed that protozoa would be detected here if they were present, especially as one of the most effective FS for their detection (FS7) used in the previous study was used here. Moreover, the presence of Protozoa (*Cystoisospora* spp.) in the cat samples in this study would appear to confirm the effectiveness of the technique. The lack of positive samples is surprising considering other studies in Portugal that show high prevalence rates, including Félix (2015) with a prevalence of 8.5% in dogs from Western Portugal, Ferreira et al. (2011) which found that 47% of kennel dogs from Évora were infected with *Giardia* spp. and 6.1% were infected with *Cystoisospora* spp., as well as a study by Neves et al. (2014) in Oporto that found prevalence of 20% for *Cystoisospora canis* and 7.4% for *Giardia* spp. in healthy animals and 13.5% and 15.5% respectively in animals with GI disease. It is relevant to mention that the study in Oporto found that age $\leq$ 6 months was a risk factor in healthy animals for *Cystoisospora sp.* and $\leq$ 6 months was a risk factor for *Giardia* spp. in dogs with GI disease. The dogs in this study were all >6 months old and apparently healthy and therefore didn't present the abovementioned risk factors. Gates & Nolan, (2009) studied dogs over the age of one with a history of coccidiosis or giardiosis and found none were positive on subsequent faecal examination, suggesting life-long immunity. If we consider the population studied here were all >6 months old with many possibly

having not received adequate veterinary care when younger, it is possible that many had been infected with protozoa and consequently become immunized.

Cystoisospora spp. was detected in 6.6% (5/76) of the cat samples, four of which were also co-infected with *Toxocara* spp. The prevalence observed is lower than other studies in Portugal: *C. felis* in 14.2% of stray cats in Lisbon (Waap et al., 2014) and *C. felis* in 16.9% of cats from catteries in Lisbon & Setubal (Teixeira Carvalho, 2017). Studies in Europe also reveal higher prevalence: 9.7% in 7 European countries studied (Beugnet et al., 2014) and 17% in Tirana, Albania in adult cats <9 months old (Knaus et al., 2014). Infections by *Cystoisospora* spp. in cats are frequent and most commonly observed in dense populations or associated with unsanitary conditions (Knaus et al., 2014) and occur most frequently in juvenile animals (Gates & Nolan, 2009). The fact that kittens weren't included in this study may have contributed to the low prevalence encountered in this case.

4.4.8 Zoonotic risk factors

Up to 60 parasitic species affecting dogs and cats are known to be zoonotic to humans (Macpherson, 2005) including *Echinoccoccus* spp. tapeworms and *Toxocara* spp. roundworms both of which still persist in Europe despite the widespread availability of effective anthelmintics (Deplazes, van Knapen, Schweiger, & Overgaauw, 2011). Out of the six different parasitic species identified in this study four (*Ancylostomatidae*, *Toxocara* spp., *Trichuris* spp. and Taeniidae) have zoonotic potential.

Ancylostomatidae eggs were detected in both species which is significant as the larvae are able to invade human hosts percutaneously causing CLM (Bauerfeind et al., 2015) and also eosinophilic enteritis (Macpherson, 2005). Although species identification wasn't possible in this study, *A. caninum* (dogs), *A. tubaeforme* (cats) as well as *Uncinaria stenocephala* (dogs and cats) have all been implicated in this disease

The fact *Toxocara* spp. was by far the most prevalent parasite detected in both species, is concerning considering the risk it can pose to humans due to its ability to cause VLM (Overgaauw & Knapen, 2008; Bauerfeind et al., 2015), OLM and covert toxocarosis (Macpherson, 2005; Overgaauw & Knapen, 2008). Although a higher EPG count was found in cats compared to dogs, when taking into account the ability for embryonated eggs to remain viable in the environment for up to a year (Deplazes et al., 2011; Otero et al., 2016), it is clear from the high prevalence found that the animals studied pose a

significant risk to human health if left untreated especially when considering their potential to contaminate the environment (Otero et al., 2016). As cats like to bury their faeces, children playing in sandpits are particularly at risk of this zoonosis.

Although *Trichuris* spp. was only found in one sample it is still important as the relevance of *T. vulpis* infections in humans is not yet clear (Bauerfeind et al., 2015). Cases of human infection with *T. vulpis* have been reported (Dunn et al., 2002; Márquez-Navarro et al., 2012) and although infrequent there is a possibility that many infections are misidentified as *Trichuris trichiura* by human practitioners due to morphological similarities between species (Dunn et al., 2002).

Taeniidae were detected in both species, no PCR sequencing was performed so species identification wasn't possible. Still many zoonotic *Taenia* spp. as well as *E. granulosus* have been reported in Europe and exist in Portugal (ESCCAP, 2010a; Guerra et al., 2013). Their potential presence here is particularly concerning considering the fact that unlike nematode eggs which need to embryonate in the environment these are directly infective following excretion and able to cause hydatid disease or cystic echinococcosis in the case of *E. granulosus.* Humans can act as intermediate hosts to several *Taenia* spp. for which dogs are definitive hosts, including *T. multiceps* whose coenuri develop in the eyes and brain and *T. serialis* whose coenuri can usually found in subcutaneous tissue. In the CNS coenuri may cause headache, fever and vomiting and other serious neurological symptoms. In the eye they can cause inflammation, glaucoma and even lead to blindness. (CDC, 2017). When considering a study from China that found that the mean number of *Taenia solium* eggs in each mature proglottid was 28.332 (Ma et al., 2002), although *T. solium* doesn't affect cats or dogs the large number of eggs per proglottid from a species of the same family gives us an idea of the potential for environmental contamination from a single proglottid and consequent risk to human and animal health.

4.4.9 Study limitations

The use of different detection methods (coprological/necropsy), different coprological techniques, sampling collection methods and different epidemiological environments make prevalence comparisons between studies difficult. These results may in fact underestimate the true prevalence of certain parasites in the region that exhibit intermittent excretory patterns or overestimate others, like *Toxocara* spp. in dogs, due

to coprophagy (Nijsse, Mughini-Gras, et al., 2014). The collection of individual samples from the same individuals over a period of three days and preventing them from eating faeces could have addressed these inconsistencies, but wasn't possible. As intestinal parasites may not be evenly distributed within the faecal mass, homogenization of the entire mass prior to sampling would also have given a more accurate representation (Cringoli et al., 2010). Although all efforts were made to collect samples corresponding to individual animals multiple sampling from the same individual cannot be discarded.

CHAPTER V - CONCLUSION

The results from this study demonstrate that gastrointestinal and cardiopulmonary parasites are common in adult shelter dogs and cats in the Algarve with *Toxocara* spp. the dominant parasite in both species. The proportion of positive samples for at least one parasite as well as the average numbers of eggs and oocysts collected was high in both cases, but significantly higher in cats than dogs, demonstrating that the former pose a greater threat to human health and potential for environmental contamination in the region than the latter. The high prevalence of clinically important parasites observed also indicates that a large proportion of the animals surveyed don't receive regular anthelmintic prophylaxis or are not treated within the recommended intervals which is concerning considering both species were found to harbour zoonotic parasites including *Toxocara* spp., Ancylostomatidae and Taeniidae.

The presence of *A. abstrusus* in the region is important and highlights the need for veterinary practitioners to include this parasite in the differential diagnosis of feline patients exhibiting respiratory problems.

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APPENDIX I

Dog sample results

		FLOTAC		EPG			
Location	**ID**	**Chamber 1**	**Chamber 2**	*Ancylostomatidae*	*Toxocara spp.*	*Trichuris spp.*	*Taeniidae*
Loulé	7	-	-	-	-	-	-
	10	-	-	-	-	-	-
	12	-	-	-	-	-	-
	13	-	-	-	-	-	-
	14	-	-	-	-	-	-
Albufeira	39	*Toxocara* spp., *Taeniidae*	*Toxocara* spp., *Taeniidae*	-	9	-	5
	40	-	-	-	-	-	-
	42	-	-	-	-	-	-
	46	-	-	-	-	-	-
	47	*Taeniidae*	*Taeniidae*	-	-	-	6
	49	*Toxocara spp.*, *Taeniidae*	*Toxocara* spp., *Taeniidae*	-	4	-	11
Lagoa	56	*Ancylostomatidae*	*Ancylostomatidae*	2	-	-	-
	57	-	-	-	-	-	-
	59	-	-	-	-	-	-
	62	-	-	-	-	-	-
	64	*Toxocara* spp.	*Toxocara* spp.	-	18	-	-
	66	*Toxocara* spp.	*Toxocara* spp.	-	50	-	-
	68	-	-	-	-	-	-
	72	-	-	-	-	-	-
	78	-	-	-	-	-	-
	81	*Toxocara* spp.	*Toxocara* spp.	-	162	-	-
	86	-	-	-	-	-	-
VRSACM	107	-	-	-	-	-	-
	108	-	-	-	-	-	-
	109	*Toxocara* spp.	*Toxocara* spp.	-	3	-	-
	115	-	-	-	-	-	-
	131	*Toxocara* spp.	*Toxocara* spp.	-	86	-	-
	136	-	-	-	-	-	-
	138	-	-	-	-	-	-
	145	-	-	-	-	-	-
	146	-	-	-	-	-	-
	147	-	-	-	-	-	-
Aljezur	169	-	-	-	-	-	-
	181	-	-	-	-	-	-
	182	-	-	-	-	-	-
	183	-	-	-	-	-	-
	186	-	-	-	-	-	-

Appendix I continued

Dog sample results (continued)

Location	ID	FLOTAC Chamber 1	FLOTAC Chamber 2	EPG *Ancylostomatidae*	EPG *Toxocara spp.*	EPG *Trichuris spp.*	EPG *Taeniidae*
Aljezur	189	-	-	-	-	-	-
	191	-	-	-	-	-	-
Lagos	195	-	-	-	-	-	-
	197	-	-	-	-	-	-
	201	-	-	-	-	-	-
	202	-	-	-	-	-	-
	203	-	-	-	-	-	-
	205	-	-	-	-	-	-
	207	*Trichuris* spp.	*Trichuris* spp.	-	-	2	-
	208	-	-	-	-	-	-
	213	-	-	-	-	-	-
	214	-	-	-	-	-	-
	215	-	-	-	-	-	-
	218	*Toxocara* spp.	*Toxocara* spp.	-	16	-	-
	219	-	-	-	-	-	-
	220	-	-	-	-	-	-
	222	*Ancylostomatidae*	*Ancylostomatidae*	1	-	-	-
	223	-	-	-	-	-	-
	224	-	-	-	-	-	-
	228	-	-	-	-	-	-
	230	-	-	-	-	-	-
	235	-	-	-	-	-	-
	237	-	-	-	-	-	-
	239	-	-	-	-	-	-
	240	-	-	-	-	-	-
	241	*Taeniidae*	*Taeniidae*	-	-	-	37
	242	*Toxocara* spp.	*Toxocara* spp.	-	204	-	-
	246	-	-	-	-	-	-
	248	-	-	-	-	-	-

Appendix II

Cat sample results

Location	ID	FLOTAC Chamber 1	FLOTAC Chamber 2	EPG or OPG *Ancylostomatidae*	EPG or OPG *Toxocara spp.*	EPG or OPG *Taeniidae*	EPG or OPG *Cystoisospora*
Lagoa	69	Taeniiae, *Ancy.*	Taeniiae, *Ancy.*	2	-	73	-
	70	-	-	-	-	-	-
	71	-	-	-	-	-	-
	76	-	-	-	-	-	-
	78	-	-	-	-	-	-
	79	-	-	-	-	-	-
	80	-	-	-	-	-	-
VRSACM	89	-	-	-	-	-	-
	90	-	-	-	-	-	-
	91	-	-	-	-	-	-
	92	-	-	-	-	-	-
	93	-	-	-	-	-	-
	94	-	-	-	-	-	-
	95	-	-	-	-	-	-
	97	-	-	-	-	-	-
	98	-	-	-	-	-	-
	99	*Ancylostomatidae*	*Ancylostomatidae*	7	-	-	-
	100	-	-	-	-	-	-
	101	-	-	-	-	-	-
	102	*Ancylostomatidae*	*Ancylostomatidae*	9	-	-	-
	103	-	-	-	-	-	-
	105	-	-	-	-	-	-
	106	-	-	-	-	-	-
	107	-	-	-	-	-	-
	108	-	-	-	-	-	-
	109	-	-	-	-	-	-
	110	-	-	-	-	-	-
	111	-	-	-	-	-	-
	123	-	-	-	-	-	-
	125	-	-	-	-	-	-
	126	-	-	-	-	-	-
	132	-	-	-	-	-	-
	135	-	-	-	-	-	-
	136	-	-	-	-	-	-
	138	-	-	-	-	-	-
	139	-	-	-	-	-	-
	143	-	-	-	-	-	-

Note: ***A. abs*** - *Aelurostrongylus abstrusus,* ***Cystoiso*** - *Cystoisospora,* ***Ancy*** - *Ancylostomatidae*

Appendix II continued

Cat sample results (continued)

Location	ID	FLOTAC Chamber 1	FLOTAC Chamber 2	EPG or OPG *Ancylostomatidae*	EPG or OPG *Toxocara spp.*	EPG or OPG *Taeniidae*	EPG or OPG *Cystoisospora*
VRSACM	144	-	-	-	-	-	-
	145	-	-	-	-	-	-
	146	-	-	-	-	-	-
	147	-	-	-	-	-	-
Aljezur	186	-	-	-	-	-	-
	188	*A. abs.*	*A. abs.*	-	-	-	-
	189	*Ancylostomatidae*	*Ancylostomatidae*	1	-	-	-
	190	*Toxocara* spp.	*Toxocara* spp.	-	120	-	-
	191	*Toxocara* spp.	*Toxocara* spp.	-	10	-	-
Lagos	192	*Toxocara* spp., *Cystoiso*.	*Toxocara* spp., *Cystoiso*.	-	100	-	34
	193	*Toxocara* spp.	*Toxocara* spp.	-	284	-	-
	194	*Toxocara* spp., *Cystoiso*.	*Toxocara* spp., *Cystoiso*.	-	40	-	12
	195	*Toxocara* spp.	*Toxocara* spp.	-	7	-	-
	196	*Toxocara* spp., *Cystoiso*.	*Toxocara* spp., *Cystoiso*.	-	23	-	20
	197	*Toxocara* spp.	*Toxocara* spp.	-	1308	-	-
	198	-	-	-	-	-	-
	199	*Toxocara* spp.	*Toxocara* spp.	-	324	-	-
	201	*Toxocara* spp.	*Toxocara* spp.	-	135	-	-
	202	-	-	-	-	-	-
	203	*Toxocara* spp.	*Toxocara* spp.	-	38	-	-
	204	-	-	-	-	-	-
	205	*Toxocara* spp., *Cystoiso*.	*Toxocara* spp., *Cystoiso*.	-	604	-	30
	206	*Toxocara* spp.	*Toxocara* spp.	-	732	-	-
	207	*Toxocara* spp.	*Toxocara* spp.	-	77	-	-
	208	-	-	-	-	-	-
	209	*Toxocara* spp.	*Toxocara* spp.	-	6	-	-
	210	*Toxocara* spp.	*Toxocara* spp.	-	20	-	-
	211	*Toxocara* spp.	*Toxocara* spp.	-	4	-	-
	212	*Toxocara* spp.	*Toxocara* spp.	-	1224	-	-
	213	-	-	-	-	-	-
	214	*Toxocara* spp.	*Toxocara* spp.	-	1512	-	-
	215	*A. abs.*, *Toxocara* spp.	*A. abs.*, *Toxocara* spp.	-	760	-	-
	216	*A. abs.*, *Toxocara* spp.	*A. abs.*, *Toxocara* spp.	-	478	-	-
	217	-	-	-	-	-	-
	218	*Cystoiso*.	*Cystoiso*.	-	-	-	>2000
	219	*Toxocara* spp.	*Toxocara* spp.	-	2580	-	-
	220	*Toxocara* spp.	*Toxocara* spp.	-	1804	-	-
	221	*Toxocara* spp.	*Toxocara* spp.	-	384	-	-
	227	-	-	-	-	-	-

Note: ***A. abs*** - *Aelurostrongylus abstrusus,* ***Cystoiso*** - *Cystoisospora,* ***Ancy*** - *Ancylostomatidae*

EPG counts in dog samples

Toxocara spp.	
n	9
Mean	61,33
Std Dev	74,65
SE	24,88
t	2,31
Margin of Error	57,38
Lower Limit	3,95
Upper Limit	118,72

Ancylostomatidae	
n	2
Mean	1,500
Std Dev	0,707
SE	0,500
t	12,706
Margin of Error	6,353
Lower Limit	-4,853
Upper Limit	7,853

Taeniidae	
n	4
Mean	14,75
Std Dev	15,06
SE	7,53
t	3,18
Margin of Error	23,97
Lower Limit	-9,22
Upper Limit	38,72

EpG and OPG counts in cat samples

Toxocara spp.	
n	24
Mean	523,92
Std Dev	688,75
SE	140,59
t	2,07
Margin of Error	290,84
Lower Limit	233,08
Upper Limit	814,75

Ancylostomatidae	
n	4
Mean	4,750
Std Dev	3,862
SE	1,931
t	3,182
Margin of Error	6,146
Lower Limit	-1,396
Upper Limit	10,896

Cystoisospora	
n	5
Mean	437,60
Std Dev	924,88
SE	413,62
t	2,78
Margin of Error	1148,39
Lower Limit	-710,79
Upper Limit	1585,99

Confidence Level **95%**

EPG in dogs and cats*

EPG Dogs[1]	
n	16
Mean	38,50
Std Dev	61,26
SE	15,31
t	2,13
Margin of Error	32,64
Lower Limit	5,86
Upper Limit	71,14

EPG Cats[2]	
n	29
Mean	436,76
Std Dev	653,89
SE	121,42
t	2,05
Margin of Error	248,73
Lower Limit	188,03
Upper Limit	685,49

*No oocysts or A. abstrusus were included in the calculations

[1]Dog EPG include the sample positive for *Trichuris* spp.

[2]The Taeniidae sample is included.

SUMMARY STATISTICS

POSITIVE SAMPLES		
	Cats	**Dogs**
n samples	76	66
nº positive samples	30	14
	Cats	**Dogs**
Sample Proportions	0,395	0,212
Risk Difference (RD)	0,183	
SE (RD)	0,075	
Z	1,96	
Margin of Error	0,148	
CI for Risk Difference		
Lower Limit	0,035	
Upper Limit	0,33	
Relative Risk (RR)	1,861	
CI for Relative Risk		
Lower Limit	1,082	
Upper Limit	3,199	
Odds Ratio (OR)	2,422	
CI for Odds Ratio		
Lower Limit	1,146	
Upper Limit	5,119	
One sided p-value	0,008	
Two sided p-value	0,015	
Confidence Level	95%	

TOXOCARA POSITIVE		
	Cats	**Dogs**
n samples	76	66
nº positive samples	24	9
	Cats	**Dogs**
Sample Proportions	0,316	0,136
Risk Difference (RD)	0,179	
SE (RD)	0,068	
Z	1,960	
Margin of Error	0,133	
CI for Risk Difference		
Lower Limit	0,046	
Upper Limit	0,313	
Relative Risk (RR)	2,316	
CI for Relative Risk		
Lower Limit	1,160	
Upper Limit	4,624	
Odds Ratio (OR)	2,923	
CI for Odds Ratio		
Lower Limit	1,245	
Upper Limit	6,862	
One sided p-value	0,004	
Two sided p-value	0,008	
Confidence Level	95%	

PRIMEIRO RASTREIO DE PARASITAS CARDIOPULMONARES E GASTROINTESTINAIS EM CÃES E GATOS DA REGIÃO DO ALGARVE UTILIZANDO A TÉCNICA DE FLOTAC

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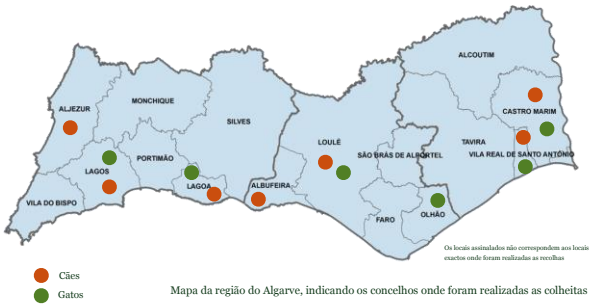
Introdução e objectivos

Felídeos e canídeos são hospedeiros de vários parasitas, alguns dos quais com potencial zoonótico. O contacto íntimo que muitos donos têm com os seus animais, associado a uma desparasitação por vezes irregular e ineficaz, aumenta o risco de transmissão destes agentes aos humanos. Actualmente, poucos dados estão disponíveis sobre a situação parasitária em cães e gatos no Algarve. Por esta razão foi realizado um estudo epidemiológico da fauna parasitológica gastrointestinal e cardiopulmonar em cães e gatos desta região.

Métodos

Entre Fevereiro e Abril de 2016, **140 amostras fecais** (n=64 de canídeos, n=76 de felídeos) foram colhidas aleatoriamente em 7 canis e 6 gatis da região. As amostras foram suspensas em água, filtradas e centrifugadas. Uma solução de sulfato de zinco foi adicionada ao sedimento, e 5ml de solução foram transferidos para cada uma das câmaras do FLOTAC, procedendo-se em seguida à sua centrifugação. O disco de leitura foi removido e colocado num microscópio para visualização e análise.



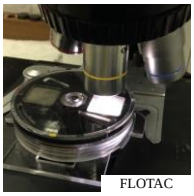
Mapa da região do Algarve, indicando os concelhos onde foram realizadas as colheitas

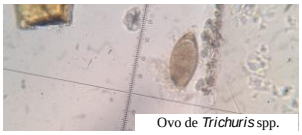
Resultados

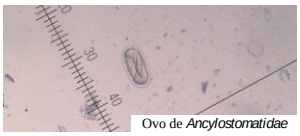
No total, 22% (14/64) dos cães estavam parasitados, 3% (2/64) com infeções mistas. ***Toxocara* spp.** foi o parasita mais frequente com 14% (9/64), seguido por ***Taeniidae*** 5% (3/64), ***Ancylostomatidae*** 3% (2/64) e ***Trichuris* spp**. 2% (1/64). Nos felinos 41% (31/76) das amostras estavam parasitadas, registando-se em 9% (7/76) casos de infeções mistas. *Toxocara* spp. foi o parasita mais frequente com 32% (24/76), seguido por ***Cystoisospora*** 7% (5/76), *Ancylostomatidae* 5% (4/76), ***Aelurostrongylus abstrusus*** 4% (3/76) e *Taeniidae* 1% (1/76).

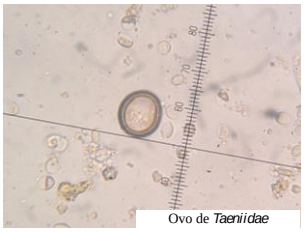

Recolha


Processamento das amostras

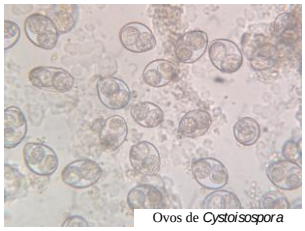

FLOTAC

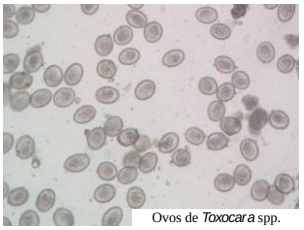

Ovo de *Trichuris* spp.


Ovo de *Ancylostomatidae*


Ovo de *Taeniidae*


Aelurostrongylus abstrusus


Ovos de *Cystoisospora*


Ovos de *Toxocara* spp.

Conclusões

Estes resultados demonstram um maior grau de parasitismo nos felinos estudados comparativamente com os cães. Em ambos os grupos observou-se a presença de **parasitas com potencial zoonótico**, tais como ***Toxocara* spp.** e ***Ancylostoma*.** A elevada prevalência de *Toxocara* spp. nas duas espécies é particularmente preocupante, representando um risco para o bem-estar animal e um problema de saúde pública, alertando para a necessidade de uma profilaxia regular. A maior prevalência observada nos felinos pode ser em parte justificada por questões de maneio e maior proximidade dos animais mantidos em gatis, características ideais para a infecção continuada por parasitas com ciclo de vida directo. Em ambos os casos a dependência de doações financeiras para a implementação de medidas profiláticas regulares torna difícil a erradicação destes agentes.

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APPENDIX V - Certificate relating to the presentation of the poster at EFOMV congress, 2016

CERTIFICADO

A Ordem dos Médicos Veterinários certifica que **Sinclair Owen, Ana Margarida Alho e Luís Madeira De Carvalho** foram os autores do póster **Primeiro Rastreio de Parasitas Cardiopulmonares e Gastrointestinais em Cães e Gatos da Região do Algarve utilizando a Técnica de Flotac**, que foi apresentado no 7º EFOMV, nos dias 26 e 27 de novembro de 2016, no Centro de Congressos de Lisboa, ao qual foi atribuído o 2º prémio.

Lisboa, 15 de março de 2017

O Bastonário da OMV

Dr. Jorge Cid