Universidade de Aveiro Departamento de Biologia
Ano 2015

Lucia da Silva Soares Oliveira

Hospedeiros, parasitas e ambiente – O caso particular dos ungulados domésticos e silvestres no centro de Portugal

Hosts, parasites and environment – The particular case of domestic and wild ungulates of central Portugal

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Hosts, parasites and environment – The particular case of domestic and wild ungulates of Central Portugal

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia Aplicada, realizada sob a orientação científica do Doutor Carlos Manuel Martins Santos Fonseca, Professor Associado com Agregação do Departamento de Biologia da Universidade de Aveiro; da Doutora Rita Maria Tinoco da Silva Torres, Investigadora de Pós-doutoramento da Universidade de Aveiro; e Doutor Luís Manuel Madeira de Carvalho, Professor Associado com Agregação da Faculdade de Medicina Veterinária da Universidade de Lisboa.

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palavras-chave Helmintes, gado doméstico, sazonalidade, intertransmissibilidade, ungulados silvestres

resumo

As helmintoses que acometem os ruminantes representam globalmente uma grande limitação à produção de gado. Visto que a pecuária tem um papel central na sustentabilidade da economia rural local e na subsistência das suas populações, é necessária a implementação de uma adequada gestão sanitária. Tal só é possível através da monitorização dos parasitas presentes a uma escala regional. O foco deste trabalho são os ungulados domésticos, nomeadamente o gado bovino (*Bos taurus*), caprino (*Capra hircus*) e ovino (*Ovis aries*), pela sua importância económica e social na área de estudo, as Serras de Montemuro, Arada e Freita. Entre Novembro de 2014 e Agosto de 2015, foram recolhidas 96 amostras fecais, de forma aleatória e em vários concelhos. De forma a determinar a presença de helmintes, foram realizadas técnicas coprológicas que permitiram identificar ovos de nemátodes gastrointestinais (*Nematodirus* sp., *Strongyloides papilosus*, *Trichuris ovis* e estrongilídeos gastrointestinais), larvas L1 de nemátodes pulmonares (*Dictyocaulus viviparus*, *Muellerius capillaris* e *Protostrongylus* sp.), ovos de céstodes (*Moniezia benedeni*) e ovos de tremátodes (*Fascicola hepatica*). A classe Nematoda foi a que apresentou uma maior prevalência (n=70; 72,9%), e dentro dessa classe, os estrongilídeos gastrointestinais (EGI) predominaram quer nos bovinos (n=20; 52,6%) quer nos pequenos ruminantes (n=44; 75,9%). A única espécie de tremátode encontrada, *Fasciola hepatica*, é um agente zoonótico que apenas se observou nos meses mais quentes. As infeções mistas foram uma constante ao longo do ano, com uma prevalência de 78% nos pequenos ruminantes e de 55% nos bovinos. Os EGI foram observados ao longo de todo o ano, apresentando uma maior prevalência de Maio a Julho, após um mês particularmente chuvoso, coincidindo com um aumento na temperatura e com baixos valores de precipitação e humidade relativa. Observou-se nemátodes pulmonares de pequenos ruminantes ao longo do ano coincidindo o aumento da sua prevalência, entre Janeiro e Março, com meses de temperaturas baixas e baixa precipitação. Os resultados obtidos no método de contagem de ovos coincidiram com os métodos qualitativos: as maiores contagens verificaram-se nos meses mais quentes, onde todos os hospedeiros apresentaram níveis de infeção altos, fugindo à norma anual. Foi também feita uma revisão bibliográfica que aborda o papel dos parasitas ao nível das comunidades, tendo como base a transmissão de helmintes entre ruminantes domésticos e silvestres. Esta permitiu concluir que os nemátodes, pelo seu caráter generalista e ubíquo, apresentam espécies comuns a ruminantes silvestres e domésticos. Assim pode-se inferir que a área de estudo poderá ser propensa à transmissão entre espécies, atendendo que alguns dos parasitas encontrados estão referenciados como parasitas interespecíficos, quer em ungulados domésticos quer em silvestres. Este estudo demonstra a importância do conhecimento e do controlo das infeções parasitárias na interface doméstico-silvestre, sendo necessário que biólogos, veterinários, médicos e produtores de gado atuem em conjunto de modo a prevenir doenças infeciosas que afetem não só os ruminantes domésticos mas também os silvestres e o Homem.

keywords Helminths, livestock, seasonality, cross-transmission, wild ruminants

abstract

The helminthosis affecting ruminants represent a major constraint to livestock production globally. Since livestock plays a central role in the sustainability of local rural economy and in the livelihood of their populations, the implementation of an adequate health management is vital. This is only possible through surveillance of the resident parasites at a regional scale. The focus of this work are domestic ruminants, namely cattle (*Bos taurus*), goats (*Capra hircus*) and sheep (*Ovis aries*), due to its economic and social relevance in the study area, Montemuro, Arada and Freita mountains. Between November 2014 and August 2015, 96 faecal samples were collected randomly in several counties. To determine the presence of helminths, were performed some coprological techniques that allow us to identify gastrointestinal nematodes eggs (*Nematodirus* spp., *Strongyloides papillosus, Trichuris ovis* and gastrointestinal strongyles), L1 larvae of pulmonary nematodes (*Dictyocaulus viviparus, Muellerius capillaris* and *Protostrongylus* spp.), tapeworms eggs (*Moniezia benedeni*) and eggs of liver fluke *Fascicola hepatica*. Nematoda was the most prevalent class (n=70; 72,9%) in the hosts under study. Within this class, gastrointestinal strongyles predominated both in cattle (n=20; 52,6%) and in small ruminants (n=44; 75,9%). The only species of trematode found was *Fasciola hepatica*, a zoonotic parasite, which was only observed in the warmer months. Mixed infections were constant throughout the year, showing a gradual increase, with 78% of prevalence in small ruminants and 55% in cattle. Strongyles were observed throughout the year, with a higher prevalence in May-July, after a particularly rainy month, coinciding with an increase in temperature and low rainfall and relative humidity. L1 larvae of pulmonary nematodes, of small ruminants, was also present throughout the year, coinciding the increased of its prevalence with months of low temperatures and rainfall, between January and March. The results obtained in the faecal egg count method (FEC) coincide with the qualitative methods: the highest burdens were seen in the warmer months, where all hosts showed high infection levels, contrary to annual norm. Additionally, a literature review was also done, addressing the role of parasites at community level, based on the cross-transmission of helminths among domestic and wild ruminants. This allowed to conclude that nematodes, for its general and ubiquitous character, exhibit the most common parasites of wild and domestic ruminants. One can infer that the study area is prone to transmission between different species given that some of the found parasites are referred as interspecific parasites. This study demonstrates the importance of background and control of parasitic infections in domestic-wild interface, requiring a multi-disciplinary team of biologists, veterinarians, human doctors and livestock farmers in order to prevent possible epidemics affecting not only domestic ruminants, but also wild ones and the human being.

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CHAPTER I

Introduction

1. GENERAL INTRODUCTION

1.1. Parasites in the ecosystem

Parasites are ubiquitous and have dramatic effects on their hosts (Marcogliese, 2005; Mitchell *et al*., 2005). Parasitism can be defined as an ecological association in which a smaller organism, the parasite, lives on/in the body of another organism, the host. This relationship is obligatory for the parasite and have deleterious effects on the host. Thus, to classify a species as parasitic the following conditions need to be fulfilled: i) use of the host as a habitat; ii) nutritional dependence on its host; and iii) causing negative effects on its host (Anderson & May, 1978; Bowman, 2014). So, parasites should be seen as part of the biology of the host: by causing harm effects they can jeopardize host survival, fecundity and behaviour, perturbing the normal functioning of a community by regulating the host population sizes and genetic structure, likewise occurs in competition and predation (Anderson & May, 1978; Dobson & Hudson, 1986; Mitchell *et al*., 2005; Rose *et al*., 2014).

Environmental conditions have a major impact on parasite populations determining the spread and geographical distribution of parasites, most importantly the free living larval stages that occur on the pasture (Stromberg, 1997; Berenguer, 2007). Each species of parasite requires certain conditions, both biotic and abiotic, so that their persistence and spread are possible (Berenguer, 2007). Within the abiotic factors, the ones that have the greatest importance are climate (temperature, humidity, rainfall, wind and solar radiation), soil and water resources (Berenguer, 2007). For example, Mitchell *et al*. (2005) showed that temperature can change the nature of the interaction between host and parasite. In general, temperature and water-related variables influence transmission dynamics of helminths (Mas-Coma *et al*., 2008; Morgan & Wall, 2009). Thus, the understanding of the host-parasite interaction is inextricably linked to the environmental conditions that may vary seasonally and annually (Stromberg, 1997; Dobson *et al*., 2003).

Concluding, parasites are important as components of ecological systems (Dobson & Hudson, 1986; Marcogliese, 2005).

1.2. Parasites of ruminants: particularly in cattle, sheep and goats

Endoparasites, parasites that lives inside the host, are always relevant in livestock farms, particularly for ruminants in extensive farming because they are more likely to infection due to grazing in open areas (Kumar *et al*., 2013). A parasite within a host is meant to cause an infection, i.e., the presence of a microparasite (bacteria, virus and protozoan) or a macroparasite (helminth) within at least one member of the host population (Scott, 1988). Among the endoparasites, helminths have a tremendous importance and are of veterinary interest because of the possible serious damages they can impose on animals, which is then translated in severe production losses (Mas-Coma *et al*., 2009). This direct or indirect losses were translated into numbers, such is the case of liver fluke disease in Switzerland that has been estimated at 52 million € costs per year in cattle alone (Schweizer *et al*., 2005); or the cost of nematode parasitism of sheep estimated to be on the order of 99 million € per year, in Great Britain (Morgan *et al*., 2013); also data from UK Food Standards Agency (FSA) for 2011 showed that 22% of cattle livers and 6% of sheep livers were rejected due to fluke infection, costing the UK beef and sheep industry £13-15 million (IFAH, 2015).

Within the overall known helminths, the typical parasitic worms of vertebrates belong to the phyla Platyhelminthes (flatworms, flukes and tapeworms), Nematoda (roundworms), Acanthocephala (thorny-headed worms), and occasionally larvae of the group known as the Nematomorpha (Gordian worms) (Bowman, 2014). Like other living things, the parasites are also organized in Kingdom, Phylum, Class, Order, Family, Genus and Species. We will present parasites of the major domestic ruminant species (Zajac & Conboy, 2012) that are likely to appear in faeces and which distribution area encompasses Europe (Table 1).

1.2.1. Class Nematoda

Nematodes, also called roundworms, are relatively small, dioecious wormlike organisms. In Figure 1 is possible to analyse the general life cycle common to the majority of nematodes. Among them the body form is remarkably constant, a fact that makes identification and taxonomic classification difficult (Shapiro, 2010; Bowman, 2014). Further will be presented the orders that comprises the nematodes that affect the respiratory and gastrointestinal systems of domestic ruminants: **Strongylida**, **Rhabditida**, **Ascaridida** and **Enoplida**. The order Strongylida is composed of four superfamilies: Metastrongyloidea, Strongyloidea, Trichostrongyloidea and Ancylostomatidea. In order Rhabditida the superfamily of interest is Rhabditoidea. And in Enoplida the focus are the superfamily Trichinelloidea.

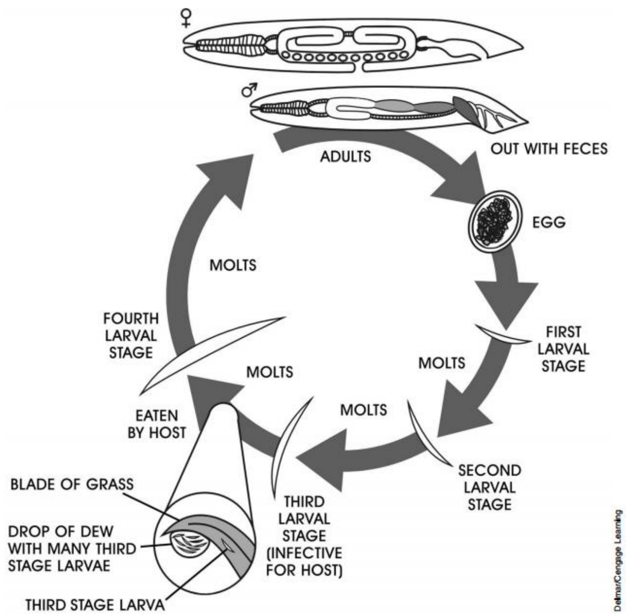


Figure 1 – Nematodes general life cycle (Source: Shapiro, 2010).

Table 1 – Taxonomy of some important parasites that affect domestic ruminants (adapted from Foreyt, 2001 and Bowman, 2014).

Kingdom	Animalia								
Phylum	Nematoda								
		Order	Strongylida						
				Superfamily	Metrastrongyloidea				
						Family	Protostrongylidae		
								Species	*Muellerius capillaris*
									Protostrongylus spp.
				Superfamily	Trichostrongyloidea				
						Family	Trichostrongylidae		
						Family	Dictyocaulidae		
								Species	*Dictyocaulus* spp.
						Family	Molineidae		
								Species	*Nematodirus* spp.
				Superfamily	Strongyloidea				
				Superfamily	Ancylostomatoidea				
						Family	Ancylostomatidae		
						Subfamily	Bunostominae		
						Genus	*Bunostomum*		
		Order	Rhabditida						
				Superfamily	Rhabditoidea				
						Family	Strongyloididae		
								Species	*Strongyloides papillosus*
		Order	Ascaridida						
						Genus	*Toxocara*		
								Species	*Toxocara vitolorum*
		Order	Enoplida						
				Superfamily	Trichinelloidea				
								Species	*Trichuris ovis*
								Species	*Capillaria* spp.
Phylum	Platyhelminthes								
Class	Cestoidea								
		Order	Cyclophyllidea			**Family**	Anoplocephalidae		
								Species	*Moniezia* spp.
Class	Trematoda								
		Subclass	Digenea						
						Family	Fasciolidae		
								Species	*Fasciola hepatica*
						Family	Paramphistomatidae		
								Species	*Paramphistomum* spp.
						Family	Dicrocoeliidae		
								Species	*Dicrocoelium dendriticum*

Order Strongylida

Within this order, **Metastrongyloidea** comprises the lungworms from the family **Protostrongylidae** that affects the domestic ruminants. One of the most important genera of nematodes that live in the lungs *(Dictyocaulus)*, hence lungworms, falls within the Trichostrongyloidea rather than the Metastrongyloidea superfamily (Zajac & Conboy, 2012; Bowman, 2014). They are parasites of the respiratory, vascular and nervous systems of mammals and are found in lung parenchyma of sheep, goats, and some deer (Zajac & Conboy, 2012). The oviparous protostrongylid females deposit unsegmented eggs in the surrounding lung, vascular or neural tissues (Bowman, 2014). Those eggs are swallowed and develop into first-stage larvae before they appear in faeces. Once in faeces, are then ingested by a snail, where occurs larvae development into infective L3. Infection of small ruminants is followed by ingestion of these infected snail while grazing (Zajac & Conboy, 2012; Bowman, 2014). Most cases are asymptomatic but heavy infections may cause clinical disease, especially in goats infected with ***Muellerius*** (Zajac & Conboy, 2012). The superfamilies **Strongyloidea** and **Trichostrongyloidea** (as sub-family **Bunostominae**) are responsible for strongylid infections of ruminants. Numerous genera belong to this group, including *Ostertagia*, *Haemonchus*, *Cooperia*, *Trichostrongylus*, *Teladorsagia*, *Mecistocirrus*, *Oesophagostomum*, *Bunostomum*, *Chabertia*, *Camelostrongylus* and *Lamanema*. The adult females lay typical strongyle eggs, the term commonly used, or strongylid eggs, the properly term. Development and survival of the infective stage depend on prevailing conditions of temperature and moisture (which vary among species). The maturation of fourth-stage larvae may be held temporarily in abeyance before some unknown stimulus restarts their final development (Zajac & Conboy, 2012; Bowman, 2014), known as hypobiosis. They are found in gastrointestinal tract of ruminant and camelid hosts. The typical strongylid life history is generally applicable to members of the superfamilies Trichostrongyloidea and Strongyloidea. Adult worms in the gastrointestinal tract produce eggs that develop in faecal material in the environment (free-living L1 and L2) then infective larvae (L3) are released onto pasture, where they infect grazing hosts. *Nematodirus* slightly differ because the larvae development of the infective third stage occurs within the egg, then ruminants are infected when they ingest the hatched L3 (Zajac & Conboy, 2012; Bowman, 2014). Theoretically all grazing animals could be infected with strongylid parasites, therefore these parasites are of greatest importance and vary with host and region. Despite many infections being asymptomatic, trichostrongyloid nematodes are especially common and pathogenic in grazing ruminants. Young, nonimmune animals are the most susceptible to subclinical and clinical disease, which may include diarrhoea, anaemia, hypoproteinaemia, reduced growth, and death in severe cases (Zajac & Conboy, 2012; Bowman, 2014). It should be noted that *Trichostrongylus* spp. is a zoonotic agent that is transmitted to humans by the ingestion of larvae from contaminated environment and whose reservoir hosts are mainly cattle, sheep and goats (Foreyt, 2001; Bowman, 2014).

Within the superfamily **Ancylostomatoidea** there is family **Ancylostomatidae** (hookworms) parasites of the small intestine whose female lays typical strongylid eggs that appear in the faeces (Bowman, 2014) as it happens in the two superfamilies described above. **Bunostominae** are the subfamily that herbivorous hosts are only parasitized by (concretely for ruminants the genera ***Bunostomum***). Infection occurs through ingestion or skin penetration by infective larvae, which then undergo through the tissues of the host before developing into adult hookworms in the small intestine (Bowman, 2014).

Order Rhabditida

Rhabditoidea is the only superfamily from the respective order. From the only three genera that parasitize domestic animals, the **Strongyloididae** family is the one whose representatives parasitize gastrointestinal tract of ruminants, particularly the genus **Strongyloides** (Bowman, 2014). They are found in small intestine of ruminants and also camelids. Eggs shed in the faeces hatch, releasing fist-stage larvae. After a period of free-living development in the environment, infective third-stage larvae are able to infect the host by ingestion or penetration of the skin. Transmammary infection also occurs and is the major mode of transmission of *Strongyloides* species in mammals. After an initial infection has been established, additional larvae tend to migrate to deeper body tissues, from which they passed to offspring in the colostrum and milk (Zajac & Conboy, 2012; Bowman, 2014). This infection usually has no clinical significance, although when infection is heavy it can result in severe diarrhoea in young animals leading to dehydration, and may include inappetence, emaciation, weakness, cachexia, anaemia, respiratory distress and abnormal stools (Zajac & Conboy, 2012; Bowman, 2014).

Order Ascaridida

In order **Ascaridida** the relevant genus is ***Toxocara*** since it comprises parasites of the small intestine of various mammals. The species of interest is ***Toxocara vitolorum*** a parasite that appears in cattle faeces (Zajac & Conboy, 2012; Bowman, 2014). This species affect cattle and buffalo: calves are infected via transmammary (leading to impaction and death), and grazing animals by ingestion of embryonated eggs (in heavy infection can occur diarrhoea, weight loss and death) (Zajac & Conboy, 2012; Bowman, 2014). The eggs are remarkably resistant and the eggs remain infective in soil for many years (Bowman, 2014).

Order Enoplida

The nematodes in the order **Enoplida** differ morphologically speaking from all the other nematodes presented here, either in adult or larvae stages (*see* Bowman, 2014). The superfamily **Trichinelloidea** contains some very common parasites of domestic animals. In particular for ruminants: the genus ***Trichuris*** and ***Capillaria***. The *Thichuris* egg is recognized quite well for its lemon shape with a distinct bipolar plugs (Zajac & Conboy, 2012; Bowman, 2014). The adult parasites are found in caecum and colon of ruminants and camelids and the eggs produced, after passed in faeces, stay a minimum of three to four weeks in the environment in order to reach the infective stage, but does not hatch unless swallowed by a suitable host. Once eggs are ingested, all development occurs within the epithelium of the intestine (Zajac & Conboy, 2012; Bowman, 2014). The infective egg is highly resistant, so animals that graze in the same contaminated environments tend to become reinfected after treatment. This eggs are often found in ruminant faecal samples. Clinical signs are rare but diarrhoea cases are seen in heavy infection (Zajac & Conboy, 2012; Bowman, 2014).

The **capillarids** comprise a very large group of worms that parasitize all classes of vertebrates. In the case of ruminants they are affected by the intestinal capillariasis (Bowman, 2014). They are found in small intestine of ruminants and camelids (Zajac & Conboy, 2012). Parasite eggs are shed in faeces and infection follows ingestion of infective eggs in the environment, however capillarids are considered clinically insignificant (Zajac & Conboy, 2012; Bowman, 2014).

1.2.2. Class Cestoidea

Cestoidea is composed of hermaphrodite organisms known as tapeworms. An adult tapeworm is essentially a chain (strobila) of independent, progressively maturing reproductive units. The Class is divided into eighteen orders but only two are of veterinary significance: **Cyclophyllidea** and Diphyllobothriidea (Bowman, 2014). Parasites from Cyclophyllidea are found mainly in terrestrial vertebrates and within this order, with interest to domestic ruminants, there is the Family **Anoplocephalidae**, and more specifically ***Moniezia*** organisms. They are found in the small intestine of cattle, sheep and goats. The tapeworm life cycle has three stages: egg, larvae and adult. All tapeworms are endoparasites with two hosts in their life cycle: the intermediate host, where the larval tapeworm lives and may also cause pathology, and the definitive host, where the adult tapeworm lives. Ruminants get infected following ingestion of the intermediate host (free-living oribatid mites) containing infective cysticercoids of *Moniezia* species of sheep and cattle (Shapiro, 2010; Zajac & Conboy, 2012; Bowman, 2014) (Figure 2). Fortunately, adult tapeworms are relatively non-pathogenic. Species that invade the bile ducts cause condemnation of the liver at slaughter leading to economic loss (Bowman, 2014).

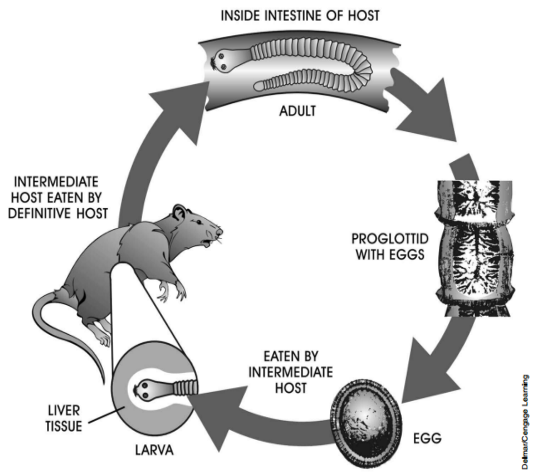


Figure 2 – Cestodes life cycle (Source: Shapiro, 2010).

1.2.3. Class Trematoda

All flukes infecting dogs, cats, ruminants, horses and swine are digeneans and are of veterinary interest (Bowman, 2014). The life cycle of *Fasciola hepatica* is typical of the Digenea: adult liver flukes produce fertile eggs that leave the host by way of the common bile duct and intestinal tract; if these eggs are carried to water, a ciliated miracidium develops within them over several weeks or months, yet depending on water temperature; on hatching, the miracidia seek for an appropriate lymnaeid snails, in which they develop; cercariae emerging from the snail encyst on vegetation and are ingested by host animals; larvae leave the gastrointestinal tract and migrate through the liver to reach the bile ducts (Zajac & Conboy, 2012; Bowman, 2014) (Figure 3). Digenean trematodes are very discriminating in their choice of snail hosts so their geographic distribution are highly dependent on that, on the other hand adult trematodes seem to be able to reach a broad range of definitive host species (Bowman, 2014).

Family Fasciolidae: *Fasciola hepatica* and *Fasciola gigantica* are parasites of the liver and bile ducts of herbivorous mammals and man, however *F. gigantica* is more restricted to the tropics. *Fascioloides magna* is a liver parasite of the white-tailed deer, but it can also infect other ruminants (Bowman, 2014). Fasciolosis can cause acute or chronic fluke disease. Acute fluke disease occurs during young flukes invasion of the liver by from recently ingested metacercariae, causing a consequent inflammatory reaction that result in highly fatal clinical illness characterized by abdominal pain with a disinclination to move. Chronic fluke disease is associated with the presence of adult trematodes in the bile ducts and is characterized by the classical clinical signs of liver fluke infection: gradual loss of condition, progressive weakness, anaemia, hypoproteinaemia and development of edematous subcutaneous swellings are noted, especially in the intermandibular space and over the abdomen (Bowman, 2014). The incidence of fasciolosis has been related to air temperature, rainfall and/or potential evapotranspiration (Mas-Coma *et al*., 2008), even Bowman (2014) notes that a fully development is highly related with summer temperatures.

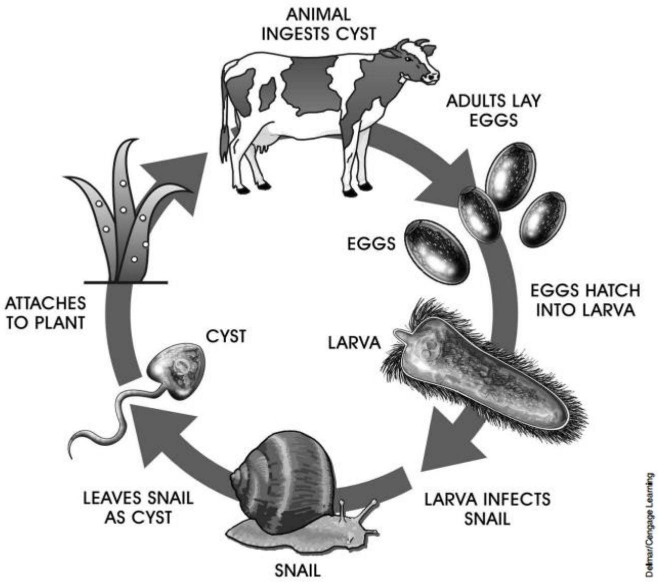


Figure 3 – *Fasciola hepatica* life cycle (Source: Shapiro, 2010).

Parasites of the Family **Paramphistomatidae** are found mainly in rumen and reticulum of cattle, sheep and other ruminants, and also in camelids. The extramammalian portion of the *Paramphistomum* fluke life cycle it is very similar to *Fasciola* spp. (Zajac & Conboy, 2012; Bowman, 2014). Larval paramphistomes in the duodenum and upper ileum are reported to cause enteritis which leads to diarrhoea, emaciation and death in severe cases (Zajac & Conboy, 2014).

Family Dicrocoeliidae are parasites of the gallbladder and bile and pancreatic ducts of mammals, birds and reptiles (Bowman, 2014). Within this family, ***Dicrocoelium dendriticum*** affects domestic and wild ruminants, pigs, dogs, horses and rabbits (Zajac & Conboy, 2012). Whereas most trematode life cycle involve water, this species is adapted to a sequence of hosts that attend dry habitats: embryonated eggs are ingested by the terrestrial snail *Cionella lubrica*, then an ant, *Formica fusca*, acts as the second intermediate host in which the cercariae encyst and the definitive host becomes infected by inadvertently ingesting infected ants (as well as can happen to humans) while grazing; the metacercariae encyst in the small intestine and migrate up to the common bile duct (Zajac & Conboy, 2012; Bowman, 2014). *D. dendriticum* causes no clinical illness in cattle, lambs or yearling sheep, but these trematodes are long-lived, and pathologic damages in the liver increase in severity and extent with the duration of the infection. Additionally heavy infections cause hepatic cirrhosis leading to anaemia, weight loss, lowered wool production, decreased lactation and premature aging (Zajac & Conboy, 2012; Bowman, 2014).

As helminthosis often involves multiple parasite species, in order to ensure that the appropriate treatment is applied, it is crucial to identify the etiologic agent responsible for the symptoms of disease and/or production losses (Morgan *et al.,* 2013), thus clinical diagnosis is inconclusive. Coprological examination, for diagnosis of helminthosis, is the most common laboratory procedure in veterinary practice. Faecal examination offer the advantage of being relatively inexpensive (it can be applied on large areas and in a large number of samples) and non-invasive and yet reveal the presence of parasites in several body systems (Morgan *et al.,* 2013). Parasites of the gastrointestinal system produce eggs, larvae or oocysts that leave the body of the host through faeces, even as larvae from the respiratory system that are coughed into the pharynx and swallowed, appearing later in faeces (Zajac & Conboy, 2012; Morgan *et al.,* 2013). The diagnosis is based on morphological characteristics associated to the host species. However, some parasites produce similar eggs, which it is not always possible to identify at species level, how is the case of Strongyle-type eggs from livestock (Zajac & Conboy, 2012). They just could be classified at species level through faecal culture, which consists in incubating the eggs in order to allow first-stage larvae to hatch and develop to infective-stage larvae, in a week to 10 days, and then differentiate them by morphology and morphometries, however the proportion of 'infective larvae/strongyle eggs abundance' is not so accurate and can be time-consuming and laborious (Roeber *et al*., 2013; Bowman, 2014). Immunologic (antigen/antibody detection tests like ELISA, IFA or Immunochromatographic tests) and molecular methods (nucleic acid extraction like PCR-based methods) are more advanced alternatives (Zajac & Conboy, 2012). Yet, the results of immunological tests can be biased by cross-reactivity in shared antigenic composition of closely related parasite species and by limited information about which antigens are actually responsible for immune responses in helminths (Roeber *et al*., 2013). Then, molecular techniques showed to be the most high sensitivity and specificity method even in cases of low parasitic burden (Tavares *et al*., 2011). However this techniques are very expensive, which is a limiting factor to become a routine screening procedure alternatively to faecal morphological examination (Tavares *et al*., 2011; Zajac & Conboy, 2012). As any methodology there are limitations, for example a negative result may indeed indicate a recent infection in which the parasites are not yet producing eggs or larvae. For example in a Faecal Egg Count (FEC) method, the EPG (eggs per gram of faeces) value does not reflect a real infection because a lower count does not necessarily mean that there are few worms in the digestive tract of the host. Even a small sample of the host population may not accurately reflect parasitism within that flock/herd (Scott, 1988; Ueno & Gonçalves, 1998; Zajac & Conboy, 2012). Animal health is directly related to levels of production and safe trade (Bruckner *et al*., 2002) which requires the implementation of prophylactic measures, such as pharmacological control. At the same time, the increase in disease levels and production losses is attributed to treatment failure. And, ironically, this becomes frequent due to the indiscriminate use of anthelmintic (Morgan *et al.,* 2013). Anthelmintic Resistance (AR) is the ability of parasites to adapt quickly to selection pressures imposed by control efforts on farms (Morgan & Wall, 2009). The development and implementation of innovative and refined approaches to worm control and targeted at the appropriate regional scale, is a prerequisite for reducing the enormous burden helminth parasitism imposes upon ruminant livestock production (Morgan *et al.,* 2013). Like optimise treatments such medicate those infected animals instead of indiscriminately treat all the flock/herd simultaneously (Greer *et al*., 2009; Morgan *et al.,* 2013). There are also non-pharmacological treatments that could be supplementary and/or alternative control strategies as the biological control by nematophagous fungi (*see* Madeira de Carvalho *et al.,* 2007; 2012; Riádigos *et al*., 2014).

1.3. Parasite transmission in domestic-wildlife interface

Livestock animals graze in areas shared with other wild animals, namely wild ungulates. Martin *et al*. (2011) mentioned that multiple publications dealing with wildlife diseases consider that they are close ecological and phylogenic with livestock. It is also known that for many pathogens more than one species can serve as a host so many pathogens are transmitted from host to host at least by one species of vector (Kessing, 2008). Host-specific parasites make up less than half of the nematode parasites infecting any of the ungulate host species (Walker & Morgan, 2014). Therefore, parasite transmission between wild and domestic animals in both directions might occur and recommendations concerning about parasite control are useful to managers (Morgan *et al*., 2004).

An evidence of in-field cross-transmission of parasitic gastrointestinal nematodes between wild and domestic ruminants comes from Italy: Cerutti *et al*. (2010) using molecular phylogenetic methods found that a single parasite population led to infection cycles between all hosts studied. Other research carried out by Chintoan-Uta *et al.* (2014), in United Kingdom, confirmed that nematode populations of wild deer successfully infected cattle and sheep. The same authors also indicated that deer (fallow deer, red deer and roe deer) could play a role in the spread of anthelmintic resistance. Another research from UK also suggested that roe deer, fallow deer and wild rabbit can represent reservoirs of the liver fluke, *Fasciola hepatica*, for cattle (Simpson, 2002). In addition, Pato *et al.* (2013) found that, in Iberian Peninsula, several species primarily parasites of livestock infected roe deer, suggesting that grazing in common areas might be a risk factor. In Portugal, Figueiredo (2011) made a parasitological survey of the caprine of the National Park Peneda-Gerês and showed that parasites of domestic and feral goats and the wild species *Capra pyrenaica* showed similarities, which indicates that helminths transmission between wild and domestic hosts can be common.

A work with two sympatric arctic ungulates, muskoxen (*Ovibos moschatus*) and Dall's sheep (*Ovis dalli*), showed that host switch may be a consequence of the introduction of a given host species into its historical habitat, leading to pathogen transmission and may result in emerging infections and diseases in both hosts (Kutz *et al*., 2004). For instance, roe deer (*Capreolus capreolus*) was recently reintroduced in central Portugal, as a conservation project for the endangered Iberian wolf (*Canis lupus signatus*) population. Such reintroduction can promote habitat and resources sharing which consequently supports closer contact with domestic populations (Böhm *et al*., 2007). Thus their exposure to livestock parasites will likely increase and roe deer are particularly susceptible to livestock-associated nematode species (Chintoan-Uta *et al*., 2014), besides wild animals can act as reservoirs of livestock infectious agents (Simpson, 2002). In fact, there is an opportunity for host switching, i.e., infection of alternative host species (Walker & Morgan, 2014). Thus, this can be a real problem in reintroduction projects and simultaneously is a great opportunity to understand domestic-wildlife interactions, at country level.

1.4. Portuguese framework

Some research has already been done with the internal parasites of livestock in Portugal. For instance, Guerreiro (2009) estimated the prevalence of gastrointestinal (GI) parasites in Alentejo and despite the advances in prophylaxis, GI infections caused by helminths and protozoa continue to be a common problem, in that area. Gomes (2012) drew attention to the contamination of pastures because «if we are investing in a deworming, but pastures remain contaminated, it is very difficult to achieve fasciolosis control only with drugs, even with those that eliminate early forms». Anastácio (2011) refers that in livestock medicine, nematodes infections deserves special attention particularly Gastrointestinal Strongyles (GIS) and coccidia of genus *Eimeria* spp.; in one of the farms studied in Ribatejo the adults were highly parasitized, of which *Trichostrongylus* sp. were the more frequent GIS genus. Lagares (2008) confirmed this by detecting GIS and *Eimeria* spp. infection in different age groups in the majority of Cova da Beira farms; also Cardoso (2010) that found GIS in 82% of samples collected in Odemira. Anastácio (2011) suggested that animals infected by protozoan *Eimeria* spp. that show no clinical symptoms act as reservoirs because they tend to excrete without manifesting signs of disease. Ramos (2013) studied a native cattle, Brava breed, and corroborates that in cattle extensive production parasitic gastroenteritis are caused mainly by GI nematodes of family Trichostrongylidae, supporting previous researches.

Most studies have been performed in South Portugal and to our knowledge no studies have been done in central Portugal. This is particularly important because i) livestock farming is central to the sustainability of rural communities and their income is highly dependent of this activity (Morgan *et al*., 2013) which is the case of central Portugal where the income source of small land size or landless famers rely solely on livestock and agriculture, as well as play an important role in protein intake (milk, cheese and meat) (Matos, 2000); ii) a recent roe deer reintroduction project started, therefore it is important to evaluate potential transmission, as previously mentioned; and iii) occurs a habitat overlap between the endangered Iberian wolf (*Canis lupus signatus*) and its primary prey, livestock (Torres *et al*., 2015). Be aware that livestock extensive grazing could promote transmission of pathogenic agents, especially those whose life cycle is based on predator-prey interactions (Guerra *et al*., 2013).

Hereupon the loss of individual animals has a greater economic impact so it is imperative to do a surveillance of the etiologic agents of this region, in order to conserve national animal genetic resources and contribute to the sustainability of the primary sector. It becomes even more important when we know that the major economic impact of parasitism is due to sub-clinical infections, i.e., when the animal do not show any signs of infection (Morgan *et al.,* 2013). Therefore, this study aims to fill up this research gap.

2. AIMS AND STRUCTURE OF THE THESIS

2.1. Thesis aims

This thesis has three key objectives:

i) Determine the diversity and prevalence of parasites in three domestic ungulates in central Portugal;
ii) Relate parasite prevalence with season;
iii) Provide a baseline for future works, particularly, for disease surveillance of livestock parasitic infections, but also to control cross-transmission diseases between livestock and roe deer.

2.2. Thesis structure

This thesis is organized in 7 chapters:

Chapter I – is a general introduction to the baseline topic of this work: the role of parasites in ecosystem, the parasites which affect livestock and a briefly taxonomic classification and characterization. We will also approach the cross-transmission issue and present the parasitic Portuguese framework. It also elucidates the thesis goals and structure.

Chapter II – describes the study area and also explain the field and laboratory methods as well as the statistical analysis applied to the research results.

Chapter III – is the result of the practical research and it is about the prevalence of helminth parasites along the seasons, under Arada, Freita and Montemuro mountains.

Chapter IV – is a literature review about interspecific transmission of parasites between wild and domestic ungulates. This allied to preliminary results obtained in the Chapter III, will be a baseline to understand if cross-transmission between roe deer and domestic ungulates are possible to occur under the study area.

Chapter V – contains the main conclusions of the thesis as well as a critical review of what was done in practical and in theoretical work, and also has brief recommendations for future studies.

Chapter VI – contains all the literature references used in the whole thesis.

Chapter VII – contains an annexe with illustrations and photographic images of parasites found in Chapter III.

CHAPTER II

Material and methods

2. MATERIAL AND METHODS

2.1. Characterization of the study area

The study was carried out in Montemuro (M), Arada and Freita (AF) Mountains. Administratively, they cover two regions of Continental Portugal, two districts and seven counties (Table 2). Montemuro Mountain reaches a maximum of 1381m and occupies an area of 38763 ha (Vieira, 2005; Almeida, 2009; ICNB, 2014b). In other hand, Arada and Freita covers 28659 ha and its maximum altitude is 1119m (Almeida, 2009; ICNB, 2014a).

Table 2 – Administrative framework of the study area (Source: ICNB).

Region	District	Counties	% M on county	% AF on county
North	Aveiro	Vale de Cambra	-	11%
		Arouca	3%	39%
	Viseu	Cinfães	35%	-
		Resende	17%	
		Lamego	14%	
Central		S. Pedro do Sul	-	50%
		Castro Daire	31%	1%

Montemuro, Arada and Freita Mountains belong to Natura 2000 Network[1] and are classified as Sites of Community Importance (SCIs) along with Paiva River - the only physical separation between them (Figure 4). According to Rocha *et al.* (2012), since they correspond to average mountain areas, this Sites shares similar characteristics such as geological formation, biogeographic classification, habitats and biodiversity, economic activities and demographic trends.

[1] "It is a network of nature protection areas established under the 1992 Habitats Directive. The aim of the network is to assure the long-term survival of Europe's most valuable and threatened species and habitats" (http://ec.europa.eu/environment/nature/natura2000/index_en.htm).

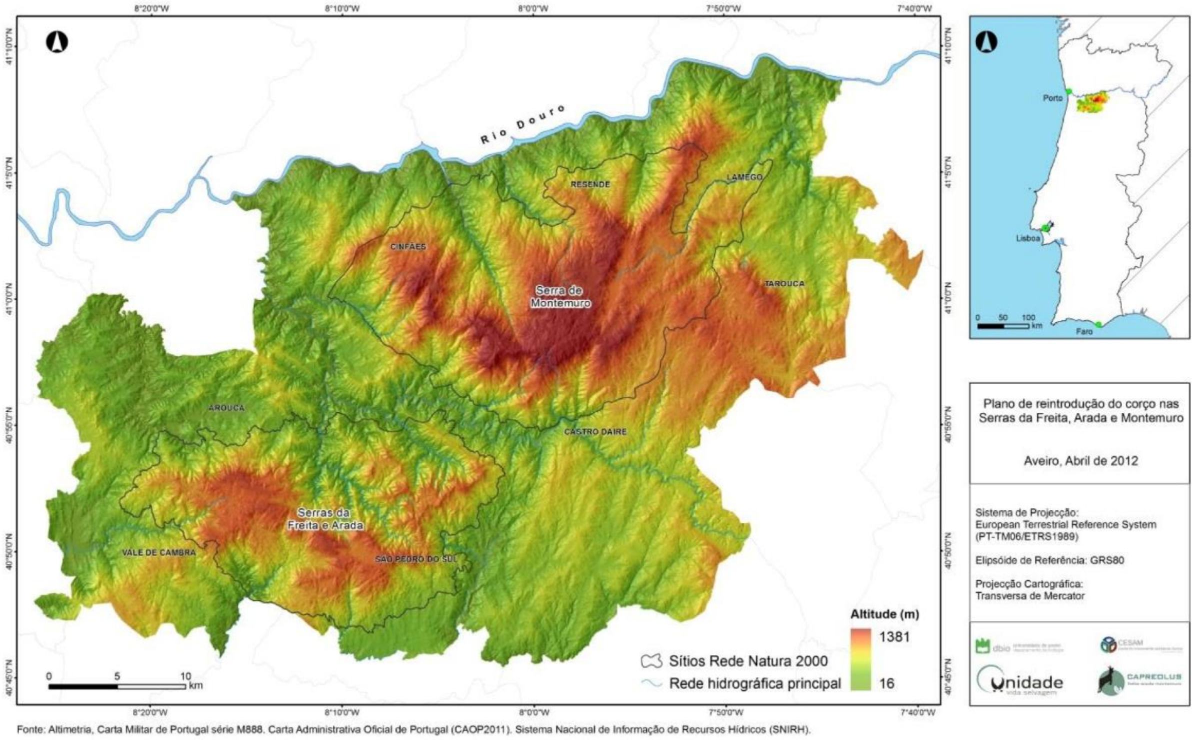


Figure 4 – Location of the study area.

In geological terms, and according to the peninsular major morphostructural units, the study area is located in the Hesperian Massif at the designated Central Iberian Zone (Ribeiro, 2006). The geodiversity of the territory is dominated by a thick sequence of shales and greywacke of ante-Ordovician and variscan granites. Highlighting the granite of Montemuro, which occupies almost the entire county of Cinfães, the granite of Castro Daire, granite of Serra da Freita and other smaller ones such as those from Alvarenga, Arouca, Regoufe and Castanheira. The latter corresponds to an international relevant geosite widely known by "Pedras Parideiras". Later, Alpine Orogeny was responsible for the occurrence of an important set of faults which increased a differential erosion, which, linked to the different hardness of the rocks, is responsible for the geomorphology of the area, where ridges, cliffs, plateaus and deep valleys create landscapes and unforgettable panoramas as the overview of "Portas de Montemuro" (Rocha *et al*., 2012).

Concerning to the biogeography, broadly speaking the study area belongs to the Mediterranean Region. However, while Montemuro integrates only this region, Arada and Freita are located in the transition zone between the Atlantic and Mediterranean Regions (Rocha *et al*., 2012). According to Costa *et al*. (1998) the Mediterranean Region is characterized by having a climate where rain is scarce in the summer and there is excess of precipitation in the remaining seasons. At high altitudes is recorded negative temperatures and frequent snowfall, in contrast to the valley areas where the weather is generally temperate. The average annual temperature is between 7,5ºC and 16ºC, ranging between 0°C (in winter) and 30°C (in summer) (ADRIMAG, n.d.).

These mountains are composed of a range of natural and semi-natural habitats, some of which are considered priorities by the sectoral plan of the Natura 2000 Network. The meadows are a typical semi-natural habitat sided by oak trees and streams which have a high importance for rural communities, constituting an indispensable source of food for livestock as grazing areas (Vieira, 2005). This is also an important territory for the conservation of the Iberian wolf (*Canis lupus signatus*), namely the subpopulation that occurs south of the Douro River. It can be also observed other species many of which are Iberian endemisms (ICNB, 2014a; 2014b).

The demographic and socioeconomic status of these areas present themselves as predominantly rural, where 63% to 94% of the Useful Agricultural Area (UAA) is intended for use in agriculture, forestry and grazing with emphasis on the extensive livestock farming of cattle, goats and sheep (ICNB, 2014a; 2014b). However, between 1999 and 2009, the number of farms decreased in all counties. These data is in line with the decrease, quite pronounced in some counties in 2013, of the number of primary sector workers (PORDATA, 2015). Along with this apparent abandon of farming activities there is also a decrease in population densities (average number of individuals per km^2), as well as the resident population, both at the county level (data from 2011 and 2014, respectively), which are discordant with the national trend that tends to increase. In fact, the resident population in the active range (between 15 and 64) was the one that decreased more. In the last 12 years, the number of births and the percentage of population under 15 years of age fall in every counties. Adding this to the unemployment rate, which also increased in every county in the same period, mainly affecting the range between 25 and 34 years (PORDATA, 2015), and to households' gross disposable income, that in the northern and central region are the lowest in the country (INE, 2011).

2.2. Field and laboratory procedures

The study included field work, with sample collection and subsequent faecal examination for the diagnosis of parasitism at Wildlife Research Unit (University of Aveiro) and Parasitology and Parasitic Diseases Laboratory at Veterinary Medicine College (University of Lisboa).

2.2.1. Data collection

From November 2014 till August 2015, 96 faecal samples were randomly collected from domestic ruminants, namely cattle (*Bos taurus*), mainly Arouquesa breed, goats (*Capra hircus*) and sheep (*Ovis aries*), in grazing areas and/or villages (Figure 5). The sampling was carried out on a monthly basis with a minimum sampling effort of 20 samples per season (Tayce *et al*., 2008). Fresh faeces were collected fresh, after defecation, individually wrapped with the respective species identification, date and place of sampling and placed in thermal bags at 4 °C to avoid degradation of the parasitic forms, until processing in the laboratory (Zajac & Conboy, 2012).

2.2.2. Coprological examination

The aim was to determine the presence of helminths therefore indirect methods were performed: the Baermann test, modified McMaster test, Floatation (Willis technique) and Faecal Sedimentation. We started by carrying out the egg-detection methods separately, however we made a small change to optimize them, i.e., McMaster, Willis and Sedimentation turned to a 3-in-1 method. The procedure was the following:

- Baermann test is a qualitative method which enables us to identify L1 larvae of lungworms nematodes. It consists in an amount of faeces involved in a porous material under tap water which will promote the larvae migration (that requires a period of 24 to 48 hours). After a waiting time the supernatant was extracted and the residual liquid was analysed microscopically, between slide and coverslip (Figure 5) (Zajac & Conboy, 2012; Alho *et al.*, 2014).

Figure 5 – Baermann test.

- 3-in-1 method consists in performing the Sedimentation technique following the Flotation ones (McMaster and Willis) based on gravity, in other words once you enhance the lighter eggs to float - as will explain later - it is expected that heavier ones will sediment, all in the same solution. McMaster and Willis' technique, the flotation methods, allows us to rescue nematode and cestodes eggs and protozoan oocysts. A liquid of higher density[2] than the eggs is added to faecal sample in order to make them float. In other hand, Sedimentation is a qualitative method employed to detect trematodes eggs because they are too large and heavy to float reliably, however they sink rapidly to the bottom of a faecal/water suspension and this is the basis of the faecal sedimentation technique (Figure 6).

  McMaster, the quantitative one, shows us the infection level by faecal egg count (FEC). Begins by homogenizing 2 grams of faeces and adding 28 ml of saturated sucrose solution. So the liquid obtained after filtration is placed in McMaster chamber and observed under a microscope. The total number of counted eggs is multiplied by 50 to obtain the final number of eggs which gives a rough estimate of the parasitic burden of the animal, as known as, EPG (eggs per gram of faeces) (Thienpont *et al.*, 1986; Zajac & Conboy, 2012).

[2] Saturated salt solution was replaced by sugar solution because causes less plasmolysis and distortion in eggs and oocysts. Some eggs float better than in salt solutions (Foreyt, 2001). Indeed, the change was reflected in better results.

Floatation or Simple Flotation (in case Willis technique), the qualitative one, determine the presence or absence of helminth eggs, namely from nematode and cestode worms. The liquid obtained in the previous preparation is placed in a 10 ml tube until it forms a meniscus to the surface. Immediately it is placed a coverslip and in order to eggs float and adhering to the coverslip we must wait at least 15 minutes. Then it is placed on a slide and observed microscopically to identify the eggs in the sample (Thienpont *et al*., 1986).

After performing the previous technique, as has been said above, it is expected that heavier eggs to sediment. So, still in the same preparation, the supernatant is removed and a portion of sample is placed and stained, with methylene blue[3], between slide and coverslip. The purpose of colouring is to facilitate the distinction between trematodes eggs and sediment waste (straw fragments, vegetal cells, minerals, etc.), under microscope examination (Zajac & Conboy, 2012).

Figure 6 – Techniques for helminth eggs detection: modified McMaster test, Willis technique and Sedimentation preparation.

2.3. Statistical analysis

A database was constructed in Microsoft Office Excel 2013. Prevalence was the parasitological parameter determined and is described as a quantitative descriptor, commonly expressed in percentage, which makes an estimate for the whole population based on an amount of samples (Bush *et al*., 1997). Prevalence allows us to divide hosts into two categories, infected and uninfected, without regard to the moment that host acquire the infection (Bush *et al*., 1997).

Results were summarized as Prevalence, and respective confidence interval, and means ± Standard Deviation. A Chi-square test at 0,05 of significance level was used to determine if there were significant differences in the prevalence of common parasites of the different hosts, also was used to determine if there were significant differences in overall prevalence of helminths per season and if there were significant differences in the prevalence of nematodes in faecal egg count method per season.

[3] The dye was not used from the very beginning, however we felt the need to employ in order to improve the examination of the sample since eggs do not stain.

CHAPTER III

Prevalence and seasonal variation of livestock helminths in central Portugal

3. PREVALENCE AND SEASONAL VARIATION OF HELMINTHS OF LIVESTOCK IN CENTRAL PORTUGAL

3.2. Abstract

Parasitic infections represent a major global constraint to livestock production. Considering the economic impact that livestock production has, especially in local rural economy, the present study was conducted to evaluate the diversity and prevalence of parasites on the most common livestock species, namely cattle (*Bos taurus*), goats (*Capra hircus*) and sheep (*Ovis aries*), in central Portugal between November 2014 and August 2015. A total of 96 faecal samples were randomly collected and were assessed by coprological techniques. Of the total faecal samples, 75 (78%) were found to be infected with at least one helminth species. The highest prevalence was found in small ruminants (93%), followed by cattle (55%). It was identified gastrointestinal nematodes eggs (*Nematodirus* sp., *Strongyloides papillosus, Trichuris ovis* and Strongyle type eggs), first-stage larvae of pulmonary nematodes (*Dictyocaulus viviparus, Muellerius capillaris* and *Protostrongylus* sp.), cestodes (*Moniezia benedeni*) and trematodes (*Fasciola hepatica*). Mixed infection was the rule, since 78% of small ruminants and 55% of cattle were infected by two or more helminth parasites. Gastrointestinal nematodes were the most overall prevalent type of helminth: among these Strongyle type eggs were the commonest (66,7%), that showed statistically significant differences between host species (χ^2=4,2; p≤0,05), followed by *Nematodirus* sp. (15,6%), *T. ovis* (11,5%) and *S. papillosus* (8,3%). Given the presence/absence of parasites throughout the year, there was no statistically significant differences in small ruminants over the seasons (χ^2=1,61; p>0,05), however it was observed in cattle (χ^2=108,2; p≤0,05). Mean faecal egg counts were generally moderate in small ruminants (550-2000 EPG) and low in cattle (0-100 epg) and the prevalence of gastrointestinal nematodes egg output was considered statistically significant, either in small ruminants (χ^2=146,87; p≤0,05), either in cattle (χ^2=203,79; p≤0,05), on a monthly basis. Our results show that helminth infections are highly prevalent in livestock from central Portugal, particularly in small ruminants, and our baseline information suggests that seasons, ruled by climate conditions, could represent a factor risk in helminth epidemiology.

Key words: Sheep, goats, cattle, helminth, EPG, prevalence, seasonality, Portugal.

3.2. Introduction

Infectious diseases have largely increased in the last decades (Daszak *et al*., 2000). The infections caused by helminth parasites are very common in grazing livestock worldwide and impose significant economic losses and welfare burden (Gorski *et al*., 2004; Martínez-Valladares *et al*., 2013; Morgan *et al*., 2013), including weight gain reduction through tissue damage and protein loss consequently affecting outputs of meat, milk and carcass quality and ultimately animal mortality (Perry & Randolph, 1999; Fitzpatrick, 2013; Sevimli, 2013).

Livestock systems are a significant global asset with a value of at least $1.4 trillion playing a significant role in economy and social conditions (Thornton, 2010; Choubisa & Jaroli, 2013), especially in developing countries where it is one of the fastest growing agricultural subsectors

(Thornton, 2010). In Portugal, agriculture generates about 2% of GDP of the total economy and in the northern and central interior regions employment is strongly supported by the primary sector (GPP, 2012). However in the last years there was a rural depopulation and consequently the abandon of traditional agricultural practices: according to the latest Agricultural Census, in 2009, were surveyed less 111000 farms than in 1999, which means that in a decade one in four farms ceased its activity, mostly the small sized farms (41%) (INE, 2009). Additionally, occurred a decrease in the number of livestock (cattle, sheep and goat) and a subsequent decrease in the production of derivatives (e.g., milk, cheese and butter) (IACA, 2014).

In order to promote a proper sanitary management it has been suggested that a diagnosis of livestock helminthosis at individual/herd level should be a priority (Morgan *et al.,* 2003).

At European level, helminth parasites have great representation as infectious agents (e.g. Chartier &Reche, 1992; Hoste *et al.,* 1999; Cringoli *et al.,* 2002; Pedreira *et al*., 2006; Zanzani *et al*., 2014). There are some reports in Mediterranean regions: in Turkey, gastrointestinal strongyle type eggs are widespread (Sevimili, 2013) and some studies showed a concomitant increase of parasitic prevalence and rainfall (Tinar *et al*., 2005; Umur & Yukari, 2005); in Greece, almost all classes of helminth have been reported by affecting cattle (Diakou & Papadopoulus, 2002), also *Toxocara* sp. was observed when climate conditions were specially rainy (Theodoropoulus *et al*., 2010), and in small ruminants, the month of the year had statistically significant effects on faecal egg counts (Papadopoulus *et al*., 2003; 2007); in Spain, sheep and goat gastrointestinal infections showed a similar pattern as environmental factors (Martínez-González *et al*., 1998; Valcárcel & Romero, 1998). In Portugal, several coprological studies (particularly in master's theses context) are made in order to survey helminths of cattle, sheep and goats, mainly in south region (e.g. Crespo & Jorge, 1999; Crespo *et al*., 2007; Lagares, 2008; Guerreiro, 2009; Cardoso, 2010; Anastácio, 2011; Pimenta et al., 2013; Ramos, 2013), but no study had as a goal the relation between their findings and climate variables.

In central Portugal, the resident population still subsists on agriculture and extensive livestock production of small ruminants and native cattle breed. The demographic and socioeconomic status of these areas present themselves as predominantly rural, where 63% to 94% of the Useful Agricultural Area (UAA) is intended for use in agriculture, forestry and grazing with emphasis on the extensive livestock farming of cattle, goats and sheep (ICNB, 2014a; 2014b). However, the number of farms also decreased along with the number of primary sector workers (PORDATA, 2015). As well as population density, especially the resident population in the active age (between 15 and 64 years old) was the one that decreased more (PORDATA, 2015). Thus, by playing important roles in decreasing the livestock production which is central to the sustainability of rural economy and to the settlement of these populations it is clear the importance of researching the parasitism level in these areas. To the authors best knowledge, there is no publication on the prevalence of livestock helminthosis in central Portugal, namely in the western part. Therefore, the main aim of this pilot study was to investigate the prevalence and diversity of helminth parasites genera/species of cattle, sheep and goats in central Portugal, between November 2014 and August 2015. Additionally, the geographical distribution of domestic ruminant helminths, even on a limited scale, is characterized by variations since its dynamics are influenced by environmental conditions, namely climate, soil and water resources (Berenguer, 2006), allowing the evaluation on how prevalence varied during the seasons, since the understanding of the environment-parasite-host interactions are the key for effective parasite control programs (Ueno & Gonçalves, 1998; Dobson *et al.*, 2003).

3.3. Methods

3.3.1. Study area

The study area is located at central Portugal, in the territory occupied by Montemuro, Arada and Freita Mountains, belonging to two sites of Nature 2000 network. The biogeographic Mediterranean region predominates, with some Atlantic influences (Rocha *et al.*, 2012), while at high altitudes negative temperatures and frequent snowfall are recorded, in contrast to the valley areas where the weather is generally temperate (ADRIMAG, n.d.). An important mammal species present in the study area is the endangered Iberian wolf (*Canis lupus signatus*). It is a rural area where agriculture, forestry and grazing, with emphasis on the extensive livestock farming of cattle, goat and sheep are the predominant economic activity. However, the number of farms decreased in all counties, as well as the number of primary sector workers. This apparent abandon of farming activities is clear along with population ageing (ICNB, 2014a; 2014b; PORDATA, 2015). Hence, it is so important to focus on the health of the livestock in order to minimize economic losses in such a fragile region.

3.3.2. Sample collection

From November 2014 to August 2015, 96 fresh faecal samples were randomly collected from cattle (n=38), goats and sheep (n=58), in grazing areas and/or villages. The sampling was carried out on a monthly basis with a minimum sampling effort of 20 samples per season (Tayce *et al.*, 2008), focused on herds naturally exposed to sources of infection. Faeces were collected fresh after defecation and placed in thermal bags at 4°C to avoid the degradation of the parasitic forms until processing (Zajac & Conboy, 2012).

3.3.3. Coprological examination

The diversity and prevalence of helminths was evaluated using the following indirect methods: i) Baermann test used to identify L1 larvae of lungworm nematodes by larvae migration (Zajac & Conboy, 2012; Alho *et al.*, 2013); ii) the modified McMaster test and Willis' flotation technique that allowed us to isolate gastrointestinal nematodes and cestodes eggs, but also protozoan oocysts, with the help of a saturated solution (Thienpont *et al.*, 1986; Zajac & Conboy, 2012); and iii) the Natural Sedimentation technique was used to detect trematode eggs (Zajac and Conboy, 2012). Firstly, we started by carrying out the egg-detection methods separately; however we made a small change to optimize them, i.e., McMaster, Willis and sedimentation turned to a three-fold method. It consisted in performing the sedimentation technique following the flotation techniques (McMaster and Willis) based on gravity, by doing this the lighter eggs are expected to float while the heavier ones to sediment, all in the same solution.

To the interpretation of Faecal Egg Count were used the following thresholds for classification of the infection level:

- Small ruminants – low=50-500, moderate=550-2000, high= >2000 (Tarazona, 1986);
- Cattle – low=0-100, moderate=100-500, high= >500 (Taylor, 2010).

3.3.4. Statistical analysis

Data were analysed using Microsoft Office Excel 2013 and the Confidence Interval Calculator (Herbert, 2013). Results were summarized as Prevalence (95% Confidence interval), an epidemiological quantitative descriptor, and arithmetic means ± SD. For common parasites, a Chi-test with 5% of significance level was applied.

3.3.5. Meteorological data

Meteorological data were collected from 'Weather Report for Agriculture' provided by IPMA, I. P. (Portuguese Institute for Ocean and Atmosphere). The monthly mean temperature, relative humidity and rainfall are used in this research.

3.4. Results

3.4.1. Prevalence

A total of 75 samples (78%), 54 from small ruminants (93%) and 21 from cattle (55%), were infected with at least one helminth species. The parasites found were: Strongyle type eggs (eggs from two superfamilies, Trichostrongyloidea and Strongyloidea that are not possible to be distinguished morphologically from one another), two genus (*Nematodirus* sp. and *Protostrongylus* sp.) and six species (*Dictyocaulus viviparus, Fasciola hepatica, Moniezia benedeni, Muellerius capillaris, Strongyloides papillosus* and *Trichuris ovis*) (Table 3). *Nematodirus* sp. eggs belong to Trichostrongyloidea superfamily, however compared with other family members it is easily differentiated from the remaining.

Overall, class Nematoda was the most prevalent helminth group, namely gastrointestinal nematodes (GIN) (n=70; 72,9% of prevalence), in which Strongyle eggs were the most prevalent (n=64; 66,7%) in both hosts (Table 4). Since April till August, i.e., in spring and summer time, it was possible to identify L1 larvae in nineteen samples (32%). Seventeen small ruminants were positive to *Muellerius capillaris* (49%) and ten to *Protostrongylus* sp. (29%). Only one cattle sample was parasitized (4%) with *Dyctiocaulus viviparus*. Additionally, we also identified coccidian oocysts, namely *Eimeria* spp. in 22,9% of samples, which had greater expressiveness in small ruminants than in cattle - although it does not count for the overall results because it is a protozoa parasite and not a helminth.

Table 3 – Presence (+) and absence (-) of parasites per host (GI: gastrointestinal).

Helminth	Small Ruminants	Cattle
GI Nematodes		
Strongyle	+	+
Nematodirus sp.	+	-
Strongyloides papillosus	+	+
Trichuris ovis	+	-
Pulmonary Nematodes	+	+
Cestodes *Moniezia benedeni*	+	+
Trematodes *Fasciola hepatica*	-	+

Table 4 – Number of positive samples and the prevalence (%) of parasites found in fresh faeces collected from small ruminants and cattle in central Portugal (CI: Confidence Interval).

	Small ruminants (n=58)			Cattle (n=38)			Total (n=96)		
	Positive samples	Prevalence	CI (95%)	Positive samples	Prevalence	CI (95%)	Positive samples	Prevalence	CI (95%)
GIN	**49**	**84,5**	**73,1 - 91,6**	**21**	**55,3**	**39,7 - 69,9**	**70**	**72,9**	**63,3 - 80,8**
Strongyle	44	75,9	63,5 - 85,0	20	52,6	37,3 - 67,5	64	66,7	56,8 - 75,3
Nematodirus sp.	15	25,9	16,4 - 38,4	0	0,0	0 - 9,2	15	15,6	9,7 - 24,2
Strongyloides papillosus	6	10,3	4,8 - 20,8	2	5,3	1,5 - 17,3	8	8,3	4,3 - 15,6
Trichuris ovis	11	19,0	10,9 - 30,9	0	0,0	0 - 9,2	11	11,5	6,5 - 19,4
Pulmonary Nematodes	**36**	**62,1**	**49,2 - 73,4**	**2**	**5,3**	**1,5 - 17,3**	**38**	**39,6**	**30,4 - 49,6**
Cestodes *Moniezia benedeni*	**3**	**5,2**	**1,8 - 14,1**	**1**	**2,6**	**0,5 - 13,5**	**4**	**4,2**	**1,6 - 10,2**
Trematodes *Fasciola hepatica*	**0**	**0,0**	**0 - 6,2**	**2**	**5,3**	**1,5 - 17,3**	**2**	**2,1**	**0,6 - 7,3**

Figure 7 illustrates a comparative overview of the overall prevalence (with the respective Confidence Interval) of parasites per host based in the data presented in Table 4.

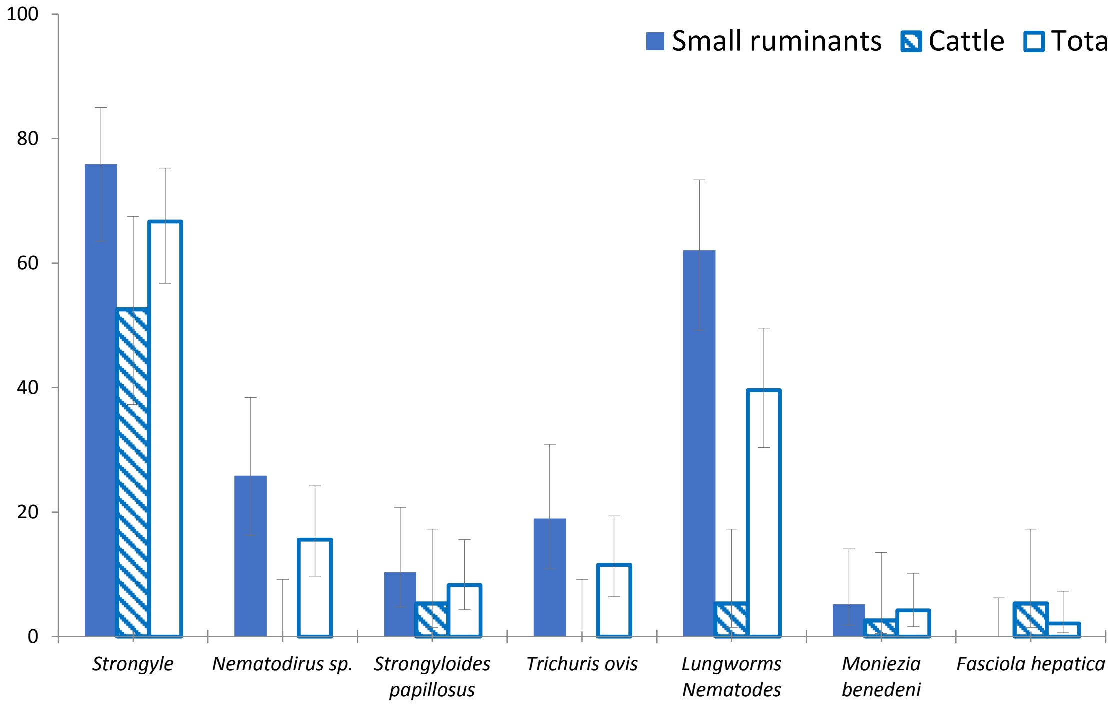


Figure 7 – Overall prevalence of parasites per host.

There are only three common helminth species in the sampled ruminants: Gastrointestinal Strongyles, *Strongyloides papillosus* and *Moniezia benedeni*. Only Strongyles showed statistically significant differences (χ^2=4,2; p≤0,05) in prevalence between the host species (Figure 8).

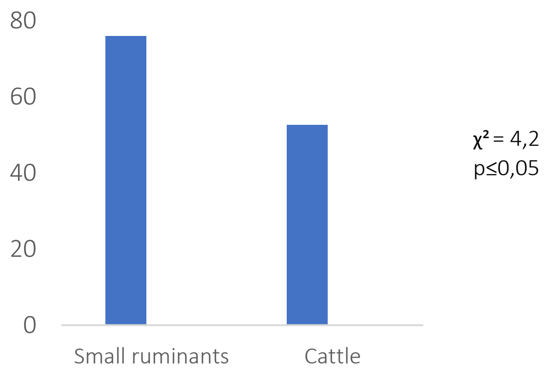


Figure 8 – Gastrointestinal Strongyles prevalence in the studied ruminants.

3.4.2. Seasonal patterns

In Figure 10, meteorological data measured during this research are presented: a) monthly mean temperature, b) monthly rainfall, c) monthly relative humidity and d) the overview of the three meteorological parameters. This figure will be the basis for the remaining Results section.

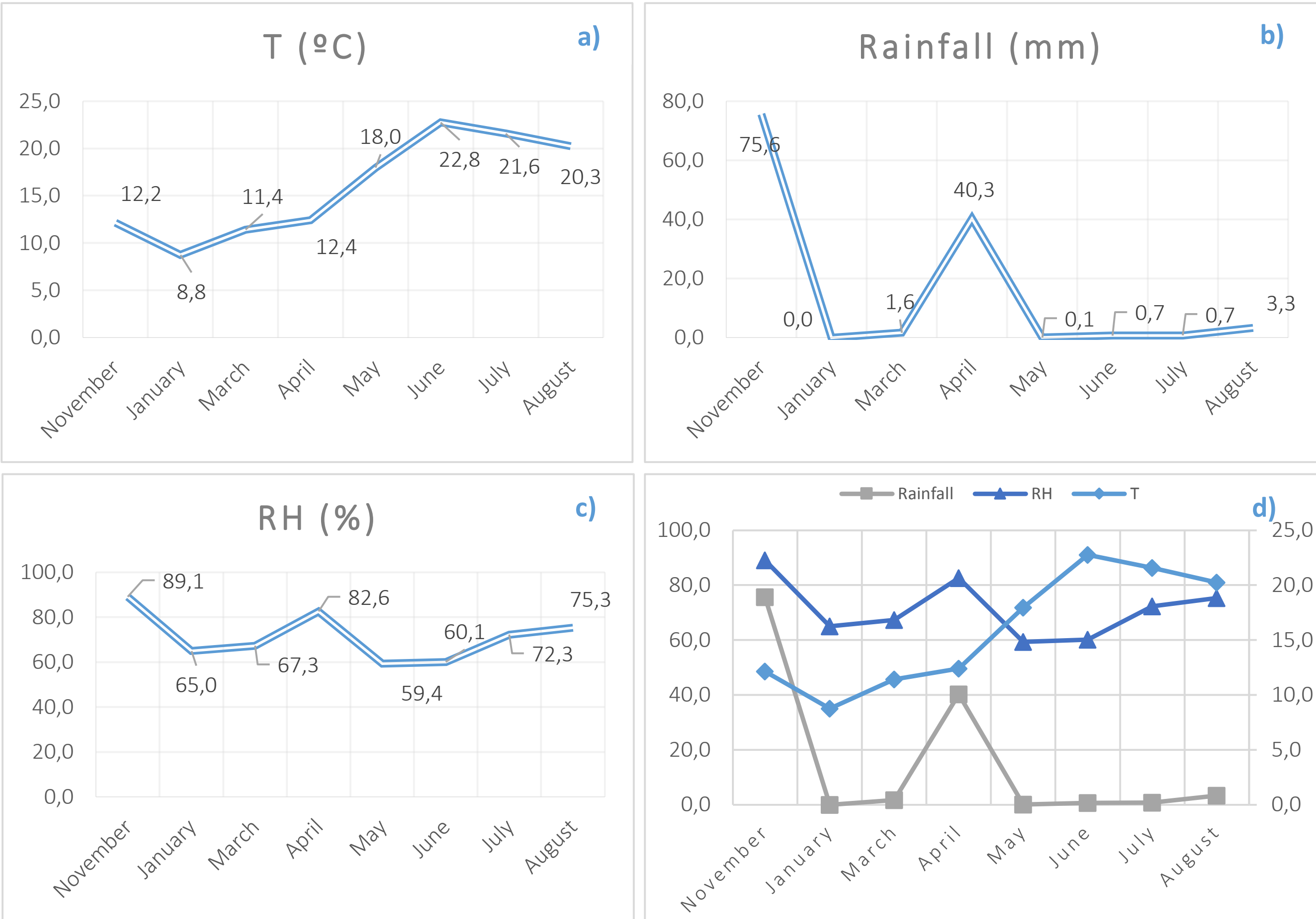


Figure 9 – a) Mean temperature values in study area during the research; b) Mean rainfall values in study area during the research; c) Mean relative humidity in study area during the research; d) Overview of the three meteorological parameters.

i) Overall prevalence

It was not found statistically significant differences in prevalence of helminths in small ruminants over the seasons (χ^2=1,61; p>0,05). In general, parasites found in small ruminants were present almost throughout the year, except for *Moniezia benedeni* and *Strongyloides papillosus* that showed a clear seasonality, being present only in June and July, in the first case, and in April, July and August, in the second. However, these last two species never exceeded the 35% of prevalence. Strongyle eggs were present all over the year, presenting higher prevalence in middle spring and early summer (May and June), when the temperatures were higher. The L1 larvae of lungworms nematodes were also observed over the year, with higher prevalence in winter (January and March), the coldest and humid months. Though with lower prevalence, *Nematodirus* sp. and *Trichuris ovis* were also observed throughout the study. All data is presented in Figure 10.

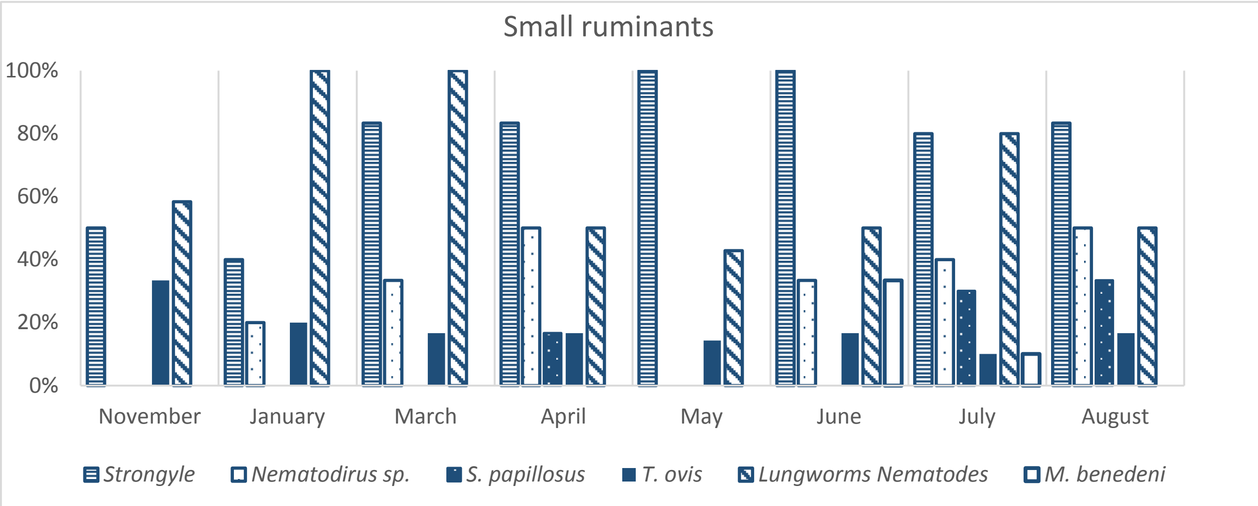


Figure 10 – Prevalence of total parasites found in small ruminants on a monthly basis.

In cattle, we found statistically significant differences in seasonality in the prevalence of helminths (χ^2=108,2; p≤0,05). Besides, there was no parasitic form observed, neither in November, nor in March. The only endoparasite eggs that occurred through the year was Gastrointestinal Strongyles, with higher prevalence in warmer months, like it was observed in small ruminants. Also *Moniezia benedeni* and *Fasciola hepatica* eggs were only observed in summer, namely in July. On the other hand *S. papillosus* eggs appeared in January (the coldest and rainless month), and L1 larvae of lungworms were only observed in April (the rainiest month). All data is presented in Figure 12.

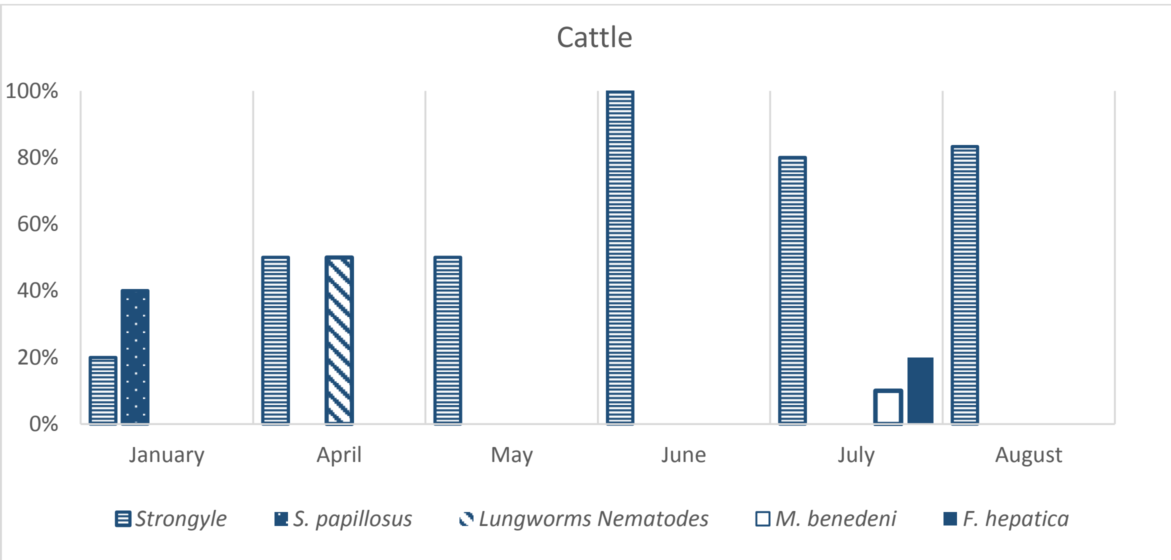


Figure 11 – Prevalence of total parasites found in cattle on a monthly basis.

ii) Mixed infections

Co-infection of gastrointestinal helminths was the rule in small ruminants, since only four animals (7%) were infected with only one endoparasite and 43 (78%) were infected with two or more. Only one bovine was infected with one species, while 21 (55%) were infected by two or more helminths. In the next figure it is displayed the overall look of co-infection, where it is clear a peak in the warmer months over the colder, with a maximum peak in June and a minimum peak in January (Figure 12).

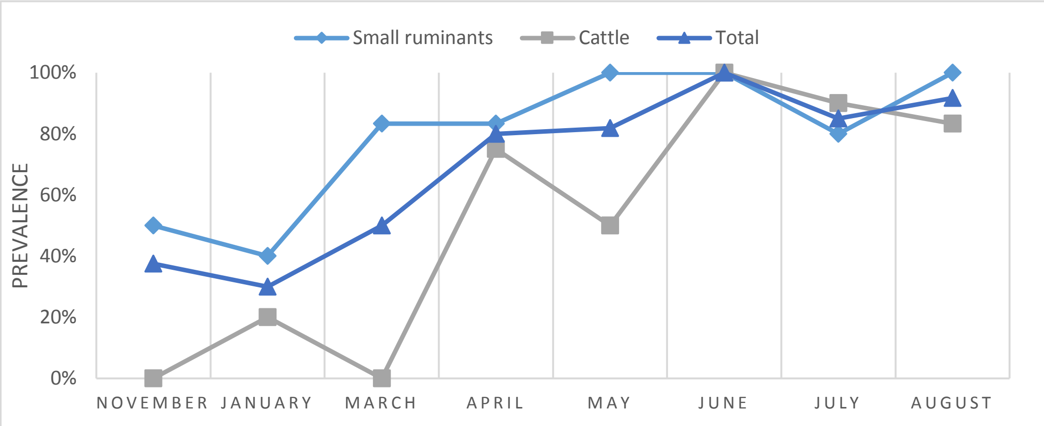


Figure 12 – Co-infection prevalence throughout the year.

iii) Faecal Egg Count

Forty-one of the total samples (42,7%) were positive for eggs of gastrointestinal nematodes worms. The monthly prevalence of GIN eggs output was considered statistically significant different either in small ruminants (χ^2=146,87; p≤0,05), as in cattle (χ^2=203,79; p≤0,05). In Table 5 it is possible to have an overview of the FEC results obtained during the year per season, month and host.

In cattle (n=15; 39,5% prevalence), the month with higher prevalence was July (70%), with a maximum of 550 EPG and a mean of 157 EPG (± 99,4). Despite it was recorded one case of high infection level (in summer), the number of eggs per sample were mostly low. However, the egg shedding presented two peaks: one in spring (April) and another in summer (July) (Figure 13).

Table 5 – Seasonal trends of nematode egg output per season, month and host (SR: small ruminant; CI: Confidence Interval; EPG: eggs per gram of faeces; T: total; SEM: Standard Error Mean).

Season	Month	Host	Total Samples	Positive Samples	Prevalence (%)	CI (95%)	T of eggs	EPG range	EPG Mean	SEM
Autumn	November	Cattle	4	0	0,0	0 - 49,0	0	0	0	± 4,4
		SR	12	3	25,0	8,9 - 53,2	75	0-45	25	
		Total	**16**	**3**	**18,8**	**6,6 - 43,0**	**75**	**0-45**	**25**	
Winter	January	Cattle	5	2	40,0	11,8 - 76,8	33	0-22	17	± 1,84
		SR	5	1	20,0	3,6 - 62,5	11	0-11	11	
	March	Cattle	6	0	0,0	0 - 39,0	0	0	0	
		SR	4	0	0,0	0 - 49,0	0	0	0	
		Total	**20**	**3**	**15,0**	**5,2 - 36,0**	**44**	**0-22**	**15**	
Spring	April	Cattle	4	1	25,0	4,6 - 69,9	200	0-200	200	± 158,2
		SR	6	5	83,3	43,7 - 97,0	2450	0-850	490	
	May	Cattle	4	1	25,0	4,6 - 69,9	50	0-50	50	
		SR	7	6	85,7	48,7 - 97,4	9900	0-7600	1650	
		Total	**21**	**13**	**61,9**	**40,9 - 79,3**	**12600**	**0-7600**	**969**	
Summer	June	Cattle	1	0	0,0	0 - 79,4	0	0	0	± 99,4
		SR	6	2	33,3	9,7 - 70,0	300	0-150	150	
	July	Cattle	10	7	70,0	39,7 - 89,2	1100	0-550	157	
		SR	10	6	60,0	31,3 - 83,2	3500	0-1450	583	
	August	Cattle	6	4	66,7	30,0 - 90,3	350	0-150	88	
		SR	6	3	50,0	18,8 - 81,2	4900	0-4650	1633	
		Total	**39**	**22**	**56,4**	**41,0 - 70,7**	**10150**	**0-4650**	**461**	

In small ruminants (n=26; 44,8% of prevalence), the most prevalent month was May (85,7%),with a maximum of 7600 EPG and a mean of 1650 EPG (± 158,2). The mean output of eggs also showed a two peaks pattern: one in spring (May), and another in summer (August), with a delay of two months compared with the cattle one (Figure 14).

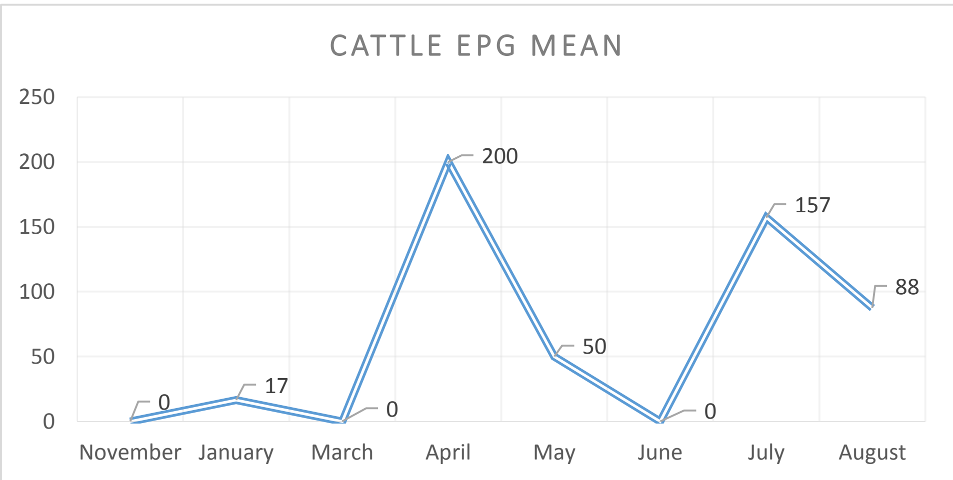


Figure 13 – Seasonal mean EPG of cattle throughout the year.

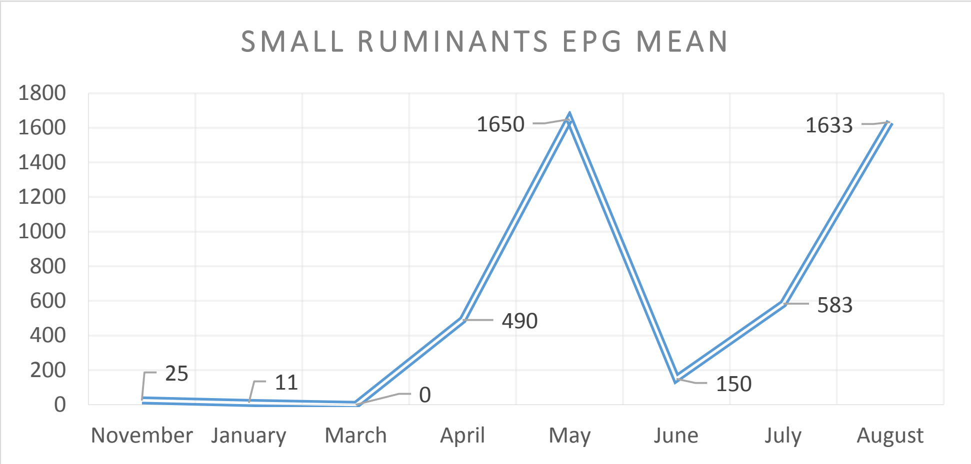


Figure 14 – Seasonal mean EPG of small ruminants throughout the year.

3.5. Discussion

To our knowledge, this is the first study evaluating the prevalence of livestock helminthfauna in Arada, Freita and Montemuro Mountains, in central Portugal. This research revealed that the majority of samples were positive and that helminth infection in ruminants occurs throughout the year. Thus parasitic infection is ubiquitous in grazing ruminants, being more prevalent in small ruminants than in cattle. It also showed that seasons could represent a risk factor in helminthosis epidemiological dynamics under the study area.

In **small ruminants**, 93% of samples were infected by helminths, namely, strongyle type eggs, *Nematodirus* sp., *Strongyloides papillosus*, *Trichuris ovis*, *Muellerius capillaris*, *Protostrongylus* sp. and *Moniezia benedeni*. Other studies carried out in Portugal also showed the presence of all of these gastrointestinal nematodes and cestodes (Crespo & Jorge, 1999; Lagares, 2008; Guerreiro, 2009; Duro, 2010; and Anastácio, 2011), except the pulmonary nematodes that are not widely discussed. Also in Greece, Kantzoura *et al*., (2012) found the same gastrointestinal nematodes, in the same order of prevalence, however the flocks were much less infected than ours. A lower prevalence was found in **cattle** (55%), in which slightly more than half of the population were infected with at least one type of helminth. Crespo *et al*. (2013) also showed similar results in beef and in Brava cattle breeds, 52,9% and 63,3%, respectively. In northwest of Portugal, Pimenta *et al*. (2013), found that 83% of Minhota breed population was infected with gastrointestinal parasites, a higher prevalence than occurred in our study (63,2%). The parasites found in cattle were Strongyle type eggs, *Strongyloides papillosus*, *Dyctiocaulus viviparus*, *Moniezia benedeni* and *Fasciola hepatica*. Crespo *et al*. (2013), also detected the same parasites, some of them with similar prevalence. The reason why animals showed high prevalence could be linked to the traditional husbandry in which animals graze freely contacting with contaminated pasture and contributing successively to re-contamination. Additionally, the reason why small ruminants presented a higher prevalence of parasites than cattle may be related to larger flocks: more density can influence the level of pasture contamination (Armour, 1980). Gastrointestinal nematodes were the most prevalent type of helminth in all hosts (84,5% in small ruminants; 55,3% in cattle; and 72,9% of the total prevalence) likewise it is corroborated by Crespo & Jorge (1999) that found a prevalence of 81,5% in sheep, and Pimenta *et al*. (2013) that detected a prevalence of 75% in cattle. This could be explained since nematodes are the most numerous among helminth parasites of domestic animals (Hendrix & Robinson, 2006) and its route of transmission are direct, i.e., do not need an intermediate host, thus the infection is more prone to occur. Strongyle type eggs were the most prevalent GIN with statistically significant differences between hosts, being more prevalent in small ruminants. They were equally common in other studies, both in small ruminants (see Crespo & Jorge, 1999; Lagares, 2008; Guerreiro, 2009; Duro, 2010; Anastácio, 2011; and Kantzoura *et al*., 2012) and in cattle (see Duro, 2010; Theodoropoulus *et al*., 2010; Crespo *et al*., 2013; and Pimenta *et al*., 2013). Small ruminants showed a higher ratio of infection and this may be related to i) the rearing relying on communal grazing pastures, which increase the risk of GI parasites infection (Papadopoulus *et al*., 2007); ii) the anthelminthic resistance being especially (but not exclusively) seen in parasites of small ruminants (Morgan *et al*., 2013), or iii) due to the host species differ in their susceptibility and tolerance to parasites (Hudson *et al*., 2006). These eggs occurred along the year and assumed a higher prevalence in spring and summer months associated with an increase in mean temperature, in all hosts. Since, in Portugal, moisture is the only limiting factor to larval development in grazing and consequent parasitic infection however, temperatures are favourable to it, throughout the year (Fazendeiro, 1989 cited in Crespo & Jorge 1999). In general, the total parasites found occurred all over the year and there was not significant differences in the overall prevalence of helminths per season. Except for *M. benedeni* and *S. papillosus* that seem to present a seasonal pattern since these parasites appeared mainly in summer months. This seasonal occurrence of *M. benedeni* may be associated with the active periods of oribatid mites, the intermediate host that carry the cysticercoid larvae, which correspond to summer in temperate regions (Radostits *et al*., 2007). On the other hand, in cattle there were significant differences in helminth prevalence along the year suggesting a seasonal influence. No parasitic form was observed in November and March. In fact, within cold months only in January parasites were observed. This may be due to a hypothetical deworming combined with developmental arrestment at the larval stage inside the host, the hypobiosis[4] (that is particularly common in gastrointestinal nematodes of ruminants in response to seasonal climate conditions) and host resistance, which in temperate areas of the northern hemisphere is associated with decreasing temperatures and photoperiod (Fernandez *et al.*, 1999; Langrová & Jankovská, 2004). The increase in prevalence over the following warmer months, could be interpreted by calves entering their first grazing season as have not acquired natural immunity, which become more susceptible to gastrointestinal nematodes infections (Shaw *et al.*, 1998); also, could be combined with the ability to limit parasite burden (resistance): once infected, hosts can protect themselves from subsequent harm by directly attack parasites and thereby reduce parasite loads (Råberg *et al*., 2009). Besides all this, we must not forget that coprological tests have limitations: a negative result lacks of predictive value since, in reality, the animal could be actually parasitized (Lagares, 2008). Strongyle was the only helminth type of eggs that occurred all the year; *S. papillosus* was only observed in January, a winter month that presented the lowest mean temperature and was the lowest rainfall period – there must be another cause to the lack of occurrence during the year of *S. papillosus* eggs, since in small ruminants this parasite occurred even in the warmer months. *M. benedeni* and *F. hepatica* only occurred in July. For *M. benedeni* it is applied, logically, the same told before for small ruminants.

[4] Hypobiosis: cessation of development at an early phase of parasitic nematodes in the host.

The seasonality of *F. hepatica* is also predictable for having a complex life cycle, intra and extra-host, which is completed in 3 or 4 months, under favourable conditions (Bowman, 2014). Furthermore, Costa (2010) in a bovine fasciolosis surveillance in southern Portugal (Alentejo) did not detect eggs in the coprological tests, however, serological tests went positive. As occurred in Cardoso (2010), where the serological showed positive animals whilst the coprological tests went negative. Gomes (2012), screening fasciolosis in fattening cattle did not obtain any positive results in simple sedimentation technique, in turn, with McMaster Modified technique (according to the protocol proposed by Conceição *et al*. (2002)) had positive results, as it happened with Malcata (2014). This can mean that the low prevalence of *F. hepatica* found in our study could have another expression given these examples. Or mainly in the case of small ruminants, could be false negatives.

Regarding to the occurrence of **mixed infections**, 78% of **small ruminants** and 55% of **cattle** samples were infected with one or more parasites. That was visible a build-up through the year while the co-infection ratio was higher in warmer months. Similar values were found by Ramos (2013) and Pimenta *et al*. (2013), where 53% and 45%, respectively, of two different native cattle breeds were infected with more than one type of helminth parasite. Mixed infections involving multiple genera and species are common and seem to be the rule rather than the exception in natural systems (Graham *et al*., 2007; Morgan *et al*., 2013; Roeber *et al.,* 2013), and usually have a greater impact than monospecific infections: in host fitness, in the severity of symptoms, in the release of infective stages into the environment and, ultimately, in the epidemiology of each parasite species within the host population (Graham *et al*., 2007; Pederson & Fenton, 2007; Roeber *et al*., 2013).

The monthly **faecal egg counts** were statistically significant in all hosts, which may suggests a seasonal influence on egg shedding coincident with temperature increase. Papadopoulus *et al*., (2003) also found that faecal egg counts for both sheep and goats were significantly affected by the month of the year, in Greece, a country also under Mediterranean climate conditions. In **cattle**, 39% of the faecal samples were positive to GIN. In contrast Malcata (2014) only found eggs in 6,3% of dairy cattle faeces, in southern Portugal. This can be understood since the majority of bovine milk production in continental Portugal are under intensive rearing systems, in contrast to other production systems, as is the case of the cattle population under study, where animals have free access to pasture and are more prone to parasitic infections occurring by faecal-oral transmission during grazing. July was the most prevalent month in which the infection level was moderate (max.: 550 EPG; EPG $\bar{x}$ = 157), however, according to Taylor (2010), the level of infection was generally 'low'. This predominance of low infection levels have also been reported by Crespo *et al*. (2013) and Cardoso (2010), both in beef cattle. The egg output showed two peaks: the first in spring (April) which coincided with a particular rainy month, and the second was in summer (July), matching an increase in mean temperature and relative humidity. In **small ruminants**, eggs were detected in 45% of collected fresh faecal samples. Crespo & Jorge (1999), in southern Portugal, found that 63,1% of sheep samples were positive to strongyle type egg output. May was the most prevalent month presenting a moderate level of infection (max.: 7600 EPG; EPG $\bar{x}$ = 1650) and according to the FEC interpretation of Tarazona (1986) the level of infection did not exceed the 'moderate' threshold (at individual level were only registered two cases of 'high' infection, one in spring and another in summer). The moderate infection level trend also occurred in two studies, carried out in the central (Lagares, 2008) and southern Portugal (Crespo & Jorge, 1999). The output was relatively low with two peaks: the first also in spring (May), after a rainy month, and the second in summer (August) coinciding with an increase in mean temperature and relative humidity, too. Crespo & Jorge (1999) also found the highest faecal egg count in spring (from March to May). Faecal egg counts in this study were generally low, which is rather typical in subclinical infections (Theodoropoulus et al., 1998) however showed a two-peak pattern, as already said. There is probably a relationship between climate conditions and the pattern of faecal egg output since the prevalence of infection showed a significant seasonal difference, which means that repeated cross-sectional studies are needed. The first EPG peak observed may be related, as already explained above, with activation of inhibited infective larvae, by the presence of young animals in pasture or decreased in immunity. For example, like Morgan & Djik (2012) stated, L3 of most species survive well over winter on pasture and infect susceptible hosts, especially newborns in the spring. Thus, Papadopulos et al. (2013) showed a similar pattern in Greece and interpreted that the subsequent decrease of FEC may be affected by the development of host immunity, as a result of better nutrition during spring and the beginning of summer. Thus, Morgan & Djik (2012), still in line with our findings, stating that rising temperatures, through spring, accelerate the development of L3 levels on pasture through summer, and the acquisition of immunity promotes a decrease in EPG levels in autumn and winter. However the epidemiological significance of the faecal egg output is often difficult to interpret due to its poor relation to the level of parasitic burden, thus further investigation is required into the variation of timing of the peaks of egg output and their magnitude from year to year (Martínez-González et al., 1998).

In future research is advisable, for instance, to standardize the methods and to do faecal cultures in order to identify the strongyle type eggs to species level given its importance in helminthfauna of livestock in the study area. It would be of great value to determine the pasture larval pattern and its relationship with egg deposition and worm burden (Martínez-González et al., 1998). And perhaps establishing partnerships with the agricultural cooperatives and livestock farmers in order to expand the size of the study to allow us to make a parasitological scan over time but also to understand what kind of health management is practiced by livestock farmers. Since it could cause economic losses in different ways, efforts should be made to control helminths infection, therefore this requires baseline knowledge of the parasitic fauna that occurs locally and in this point of view the present study may be a starting point and a good basis for a better understanding and effective control of these parasites.

3.6. References

ADRIMAG – Associação de Desenvolvimento Rural Integrado das Serras de Montemuro, Arada e Gralheira (s/d). Montanhas Mágicas – Viva a nossa Natureza!. [Brochure – Electonic version]. Arouca, 48 pp.

ALHO, A. M., NABAIS, J, CARVALHO, L. M. (2014). A importância da Técnica de Baermann na clínica de pequenos animais. *Revista Clínica Animal*. Publicações Ciência e Vida, pp. 28-31.

ANASTÁCIO, P. F. B. (2011). Parasitoses Gastrointestinais em Ovinos em Regime Extensivo e Intensivo em Explorações do Ribatejo. Tese de Mestrado, Universidade Técnica De Lisboa, Faculdade de Medicina Veterinária, Lisboa, 89 pp.

ARMOUR, J. (1980). The epidemiology of helminth disease in farm animals. *Veterinary Parasitology*, *6* (1), pp. 7-46.

BERENGUER, J.G. (2007). Manual de Parasitología: morfología y biología de los parásitos de interés sanitario. Barcelona: Publicaciones I Ediciones de la Universitat de Barcelona 35 pp.

BOWMAN, D. D. (2014). Georgis' Parasitology for Veterinarians. Elsevier, 10th Edition, 496 pp.

CARDOSO, J. M. G. (2010). Contribuição para o estudo do parasitismo gastrointestinal e hepático em bovinos de carne em regime extensivo no Concelho de Odemira. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 112 pp.

CHARTIER, C., RECHE, B. (1992). Gastrointestinal Helminths and Lungworms of French Dairy Goats: Prevalence and Geographical Distribution in Poitou-Charentes. *Veterinary Research Communications*, 16, pp. 327-335.

CHOUBISA, S. L., & JAROLI, V. J. (2013). Gastrointestinal parasitic infection in diverse species of domestic ruminants inhabiting tribal rural areas of southern Rajasthan, India. *Journal of Parasitic Diseases*, *37* (2), pp. 271-275.

CONCEIÇÃO, M. A. P., DURÃO, R. M., COSTA, I. H., DA COSTA, J. M. C. (2002). Evaluation of a simple sedimentation method (modified McMaster) for diagnosis of bovine fascioliosis. *Veterinary parasitology*, *105* (4), pp. 337-343.

COSTA, A. M. C. B. (2010). Fasciolose bovina: aspectos clínicos e epidemiológicos no Alentejo. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 91 pp.

CRESPO, M. V., & JORGE, A. T. (1999). Helmintes dos ovinos das regiões do Ribatejo e Alentejo. pp. 194-200.

CRESPO, M. V., MARIANO, P., & ROSA, F. (2013). Parasitismo em bovinos de raças de carne e brava no concelho de Coruche. pp. 277-284.

CRINGOLI, G., RINALDI, L., VENEZIANO, V., CAPELLI, G., MALONE, J. B (2002). A cross-sectional coprological survey of liver flukes in cattle and sheep from an area of the southern Italian Apennines. *Veterinary Parasitology*, 108 (2), pp. 137-143.

DASZAK, P., CUNNINGHAM, A. A., & HYATT, A. D. (2000). Emerging infectious diseases of wildlife--threats to biodiversity and human health. *science*, *287* (5452), pp. 443-449.

DIAKOU A., PAPADOPOULOS E. (2002). Prevalence of gastrointestinal parasites of cattle in Greece. *Journal of the Hellenic Veterinary Medical Society*, 53, pp. 304-309.

DOBSON, A., KUTZ, S., PASCUAL, M. AND WINFREE, R. (2003). Pathogens and Parasites in a Changing Climate. *Advances in Applied Biodiversity Science*, 4, pp. 33-38.

DURO, L. S. L. S. (2010). Parasitismo gastrintestinal em animais da Quinta Pedagógica dos Olivais. Especial referência aos mamíferos ungulados. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 135 pp.

FERNÁNDEZ, A. S., FIEL, C. A., & STEFFAN, P. E. (1999). Study on the inductive factors of hypobiosis of Ostertagia ostertagi in cattle. *Veterinary parasitology*,*81* (4), pp. 295-307.

FITZPATRICK, J. L. (2013). Global food security: The impact of veterinary parasites and parasitologists. *Veterinary Parasitology*, 195, pp. 233-248.

GOMES, C. A. V. C., (2012). Fasciolose em Bovinos de Engorda. Dissertação de Mestrado Integrado em Medicina Veterinária, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, 84 pp.

GPP – Gabinete de Planeamento e Políticas (2012). Programa de Desenvolvimento Rural 2014-2020 - Documento De Orientação. Ministério da Agricultura, do Mar, do Ambiente e do Ordenamento do Território. 68 pp.

GRAHAM, A. L., CATTADORI, I. M., LLOYD-SMITH, J. O., FERRARI, M. J., BJØRNSTAD, O. N. (2007). Transmission consequences of coinfection: cytokines writ large?. *Trends in parasitology*, *23*(6), pp. 284-291.

GUERREIRO, C. M. C. (2009). Influência do maneio na prevalência de parasitoses gastrointestinais em pequenos ruminantes: estudo comparativo entre a região do Alentejo e a região de Andaluzia. Dissertação de Mestrado, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa.

HENDRIX, C. M., & ROBINSON, E. (2006). Diagnostic parasitology for veterinary technicians. 3rd Edition. Mosby Elsevier. Missouri, USA, pp. 11-111.

Herbert, R. (2013). Confidence Interval Calculator. Available on <http://www.pedro.org.au/english/downloads/confidence-interval-calculator/>. Accessed on September 2015.

HOSTE, H., FRILEUX, Y., POMMARET, A., GRUNER, L., VAN QUACKEBEKE, E., KOCK, C. (1999). Importance du parasitisme par des strongles gastro-intestinaux chez les chèvres laitières dans le Sud-Est de la France. *INRA Productions Animales*, 12 (5), pp. 377-389.

HUDSON, P. J., CATTADORI, I. M., BOAG, B., DOBSON, A. P. (2006). Climate disruption and parasite–host dynamics: patterns and processes associated with warming and the frequency of extreme climatic events. *Journal of Helminthology*, *80* (2), pp. 175-182.

IACA – Associação Portuguesa dos Industriais de Alimentos Compostos para Animais (2014). Anuário IACA para 2014.

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014b). Serra de Montemuro. Plano Sectorial da Rede Natura 2000, pp. 1-11. Consultado em Dezembro. [electronic document]

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014a). Serras da Freita e Arada. Plano Sectorial da Rede Natura 2000, pp. 1-12. Consultado em Dezembro. [electronic document]

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014a). Serras da Freita e Arada. Plano Sectorial da Rede Natura 2000, 12 pp. Consultado em Dezembro, 2014. [electronic document]

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014b). Serra de Montemuro. Plano Sectorial da Rede Natura 2000, 11 pp. Consultado em Dezembro, 2014. [electronic document]

INE – Instituto Nacional de Estatística (2009). Recenseamento Agrícola 2009.

IPMA – Instituto Português do Mar e da Atmosfera (2014). Boletim meteorológico para a agricultura. Instituto Português do Mar e da Atmosfera, I.P., Vol. 47

IPMA – Instituto Português do Mar e da Atmosfera (2015). Boletim meteorológico para a agricultura. Instituto Português do Mar e da Atmosfera, I.P., Vol. 49-56.

KANTZOURA, V., DIAKOU, A., KOUAM, M. K., FEIDAS, H., THEODOROPOULOU, H., THEODOROPOULOS, G. (2012). Prevalence and Risk Factors of Gastrointestinal Parasitic Infections in Small Ruminants in the Greek Temperate Mediterranean Environment. *Parasitology International*, 62 (6), pp. 554-560.

LAGARES, A. F. B. F. (2008). Parasitoses de pequenos ruminantes na região da Cova da Beira. Dissertação de Mestrado Integrado em Medicina Veterinária, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 115 pp.

LANGROVÁ, I., & JANKOVSKÁ, I. (2004). Arrested development of Trichostrongylus colubriformis in experimentally infected rabbits. Effect of decreasing photoperiod, low temperature and desiccation. *Helminthologia*, *41*(2), pp. 85-90.

MALCATA, F. C. B. (2014). Prevalência e controlo dos parasitas gastrointestinais em explorações bovinas leiteiras em Portugal continental. Dissertação de Mestrado. Universidade de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 114 pp.

MARTÍNEZ-GONZÁLEZ, B., DÍEZ-BAÑOS, N., ROJO-VÁZQUEZ, F. A. (1998). An epidemiological study of gastrointestinal parasitism in dairy sheep flocks in León (NW Spain). *Small Ruminant Research*, 27, pp. 25-30.

MARTÍNEZ-VALLADARES, M., ROBLES-PÉREZ, D., MARTÍNEZ-PÉREZ, J. M., CORDERO-PÉREZ, C., FAMULARO, M., FERNÁNDEZ-PATO, N., GONZÁLEZ-LANZA, C., CASTAÑÓN-ORDÓÑEZ, L., ROJO-VÁZQUEZ, F. (2013). Prevalence of gastrointestinal nematodes and Fasciola hepatica in sheep in the northwest of Spain: relation to climatic conditions and/or man-made environmental modifications. *Parasites & Vectors*, Vol. 6, pp. 1-282.

MORGAN, E. R., CHARLIER, J., HENDRICKX, G., BIGGERI, A., CATALAN, D., VON SAMSON-HIMMELSTJERNA, G., DEMELER, J., MÜLLER, E., VAN DJIK, J., KENYON, F., SKUCE, P., HÖGLUND, J., O'KIELY, P., VAN RANST, B., DE WAAL, T., RINALDI, L., CRINGOLI, G., HERTZBERG, H., TOGERSON, P., WOLSTENHOLME, A. & VERCRUYSSE, J. (2013). Global change and helminth infections in grazing ruminants in Europe: impacts, trends and sustainable solutions. *Agriculture*, *3* (3), pp. 484-502.

MORGAN, E. R., VAN DIJK, J. (2012). Climate and the epidemiology of gastrointestinal nematode infections of sheep in Europe. *Veterinary Parasitology*, 189, pp. 8-14.

PAPADOPOULOS, E., ARSENOS, G., COLES, G. C., HIMONAS, C. (2007). Gastrointestinal nematode infection pattern of Greek dairy goats reared under extensive husbandry conditions and treated with anthelmintics at different times during the year. *Small Ruminant Research*, 69, pp. 68-73.

PAPADOPOULOS, E., ARSENOS, G., SOTIRAKI, S., DELIGIANNIS, C., LAINAS, T., ZYGOYIANNIS, D. (2003). The epizootiology of gastrointestinal nematode parasites in Greek dairy breeds of sheep and goats. *Small Ruminant Research*, 47 (3), pp. 193-202.

PEDERSEN, A. B., & FENTON, A. (2007). Emphasizing the ecology in parasite community ecology. *Trends in ecology & evolution*, *22* (3), pp. 133-139.

PEDREIRA, J., PAZ-SILVA, A., SÁNCHEZ-ANDRADE, R., SUÁREZ, J. L., ARIAS, M., LOMBA, C., DÍAZ, P., LÓPEZ, C., DÍEZ-BAÑOS, P., MORRONDO, P. (2006). Prevalences of gastrointestinal parasites in sheep and parasite-control practices in NW Spain. *Preventive Veterinary Medicine*, 75, pp. 56-62.

PERRY, B. D., & RANDOLPH, T. F. (1999). Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Veterinary parasitology*, *84* (3), pp. 145-168.

PIMENTA, S., MALHEIRO, A., DORES, J., DANTAS, M., MATEUS, T. (2014). Diversidade de parasitas gastrointestinais em bovinos da raça Minhota: estudo preliminar. *Impactum.* Coimbra, pp. 16-20.

PORDATA (2015). Estimativas Anuais da População Residente.

PORDATA (2015). Números dos Municípios e Regiões de Portugal.

PORDATA (2015). Ofertas de emprego (média anual) disponíveis nos centros de emprego e formação profissional: total e por sector de actividade económica.

PORDATA (2015). Recenseamentos Gerais da População.

RÅBERG, L., GRAHAM, A. L., READ, A. F. (2009). Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364* (1513), pp. 37-49.

RADOSTITS, O. M., GAY, C. C., KENNETH, W. H. & PETER, D. C. (2007). Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats. 10th Edition. Saunders Elsevier. 1582p.

RAMOS, J. C. (2013). Avaliação das Parasitoses Gastrointestinais em Bovinos de Raça Brava durante a Primavera e Verão. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa.

ROCHA, D., SÁ, A. A., BRILHA, J. (2012). Considerações Preliminares Sobre o Património Natural e Cultural do Território "Montemuro e Gralheira" (Portugal). In: Henriques, M. H., Andrade, A. I., Quinta-Ferreira, M., Lopes, F. C., Barata, M. T., Pena dos Reis, R- & Machado, A (Coord.), *Para Aprender Com A Terra: memórias e notícias de geociências no espaço lusófono*. Imprensa da Universidade de Coimbra. Coimbra, pp. 241-249.

ROEBER, F., JEX, A. R., & GASSER, R. B. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance-an Australian perspective. *Parasites and Vectors*, *6*, 153 pp.

SEVİMLİ, F. (2013). Checklist of small ruminant gastrointestinal nematodes and their geographical distribution in Turkey. Turkish Journal of Veterinary and Animal Sciences, Vol. 37, pp. 369-379.

SHAW, D. J., VERCRUYSSE, J., CLAEREBOUT, E. DORNY, P. (1998). Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. *Veterinary parasitology*, *75* (2), pp. 115-131.

TARAZONA, J. M. (1986). A method for the interpretation of parasite egg counts in faeces of sheep. *Veterinary parasitology*, *22* (1), pp. 113-119.

TAYCE, J., ACEVEDO, P., VICENTE, J., GORTAZAR, C. (2008). Minimum sampling effort for reliable non-invasive estimations of excretion abundance of Elaphostrongylus cervi L1 in red deer (Cervus elaphus) populations. Journal of Helminthology, Vol. 82, no. 3, pp. 255-261.

TAYLOR, M. A. (2010). Sustainable Parasite Control in Cattle : An Overview and the New Guidelines. *Cattle Practice*, 18, pp. 142-145.

THEODOROPOULOS, G., KOUTSOTOLIS, K., NIKOLAOU, E., KALOGIANNIS, D., PETRAKOS, G. (1998). Research note Seasonal variation of gastrointestinal nematodes of sheep in the region of Joannina, Greece. *International Journal for Parasitology*, 28 (8), pp. 1287-1292.

THEODOROPOULOS, G., PERISTEROPOULOU, P., KOUAM, M. K, KANTZOURA, V., THEODOROPOULOU, H. (2010). Survey of gastrointestinal parasitic infections of beef cattle in regions under Mediterranean weather in Greece. *Parasitology International*, 59 (4), pp. 556-559.

THIENPONT, D., ROCHETTE, F., & VANPARIJS, O. F. J. (1986). *Diagnóstico de las helmintiasis por medio del examen coprológico*. Segunda Edición. Janssen Research Foundation. Beerse, Bélgica, 36 pp.

THORNTON, P. K. (2010). Livestock production: recent trends, future prospects.*Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365* (1554), pp. 2853-2867.

TINAR, R., AKYOL, Ç. V., ÇIRAK, V. Y., ŞENLIK, B., BAUER, C. (2005). Investigations on the seasonal patterns of strongyle infections in grazing lambs, and the occurrence of anthelmintic resistance on sheep and goat farms in western Anatolia, Turkey. *Parasitology Research*, 96 (1), pp. 18-23.

UENO, H., GONÇALVES, P. C. (1998). Manual para diagnóstico das helmintoses de ruminantes. Japan International Cooperation Agency, 4ª Edição, 143 pp.

VALCÁRCEL, F., ROMERO C. G. (1999). Prevalence and Seasonal Pattern of Caprine Trichostrongyles in a Dry Area of Central Spain. *Journal of Veterinary Medicine*, 46, pp. 673-681.

YUKARI, B. A. (2005). Seasonal activity of gastro-intestinal nematodes in goats in Burdur region, Turkey. *Turk J Vet Anim Sci*, *29*, pp. 441-448.

ZAJAC, A. M., & CONBOY, G. A. (2012). *Veterinary clinical parasitology*. John Wiley & Sons.

ZANZANI, S. A., GAZZONIS, A. L., DI CERBO, A., VARADY, M., & MANFREDI, M. T. (2014). Gastrointestinal nematodes of dairy goats, anthelmintic resistance and practices of parasite control in Northern Italy. *BMC veterinary research*, *10* (1), 114 pp.

CHAPTER IV

Perspectives of parasite transmission among domestic and wild ruminants in central Portugal

4. PERSPECTIVES OF PARASITE TRANSMISSION AMONG DOMESTIC AND WILD RUMINANTS IN CENTRAL PORTUGAL

4.1. Abstract

Parasitic infections can interfere with individual's well-being and its impacts can lead to a change in population dynamics, affecting the entire structure of a community or ecosystem. Parasites can be classified in three major groups of organisms: protozoa, helminths and arthropods. This work is a literature review and focus on interspecific parasitic infections caused by helminths (helminthosis) which can be shared between domestic and wild ungulates. In this review, we will explore the interspecific infections, its associated risk factors and some control measures. As this theme can be easily extended to the human-domestic-wildlife interface, it needs to be discussed by a multidisciplinary point of view.

Key words: Helminths, Cross-transmission, Domestic-wild ungulates

4.2. Introduction

The domestic/wildlife interface is becoming a global issue of growing interest, following this line this review addresses the role of helminths in this multiple-host context. Multiple diseases affecting livestock have already been identified in wildlife, especially in wild ungulates considering their close ecological and phylogenetic relationship with domestic ones (Martin *et al*., 2011). In addition, host species in the same guild and with similar resource utilization patterns will provide opportunities for host-switching, which could lead to convergence of parasite faunas (Hoberg & Brooks, 2008).

Scott (1988) states that parasitic infections have individual level impact since parasites may indirectly affect host survival and reproduction. And since the susceptibility of the infected host to predation increases, the competitive fitness consequently decreases and the animal's ability to hold a territory alters, also the age at sexual maturity delay leading to a decrease in fertility. Hereupon, infection may cause impact on population density, which consequently influences the population genetic heterogeneity. This clearly proposes that parasites have an impact at community structure and ecosystems. As Scott (1988) stated "*that's why it is important to understand the population biology of a given parasite species within its range of potential hosts. There may be differences not only in the pathogenicity of the infection among different species of host, but also in the susceptibilities of hosts to infection either because of innate differences among hosts or because of differing exposure*".

It is irrefutable that more investigation in this field is needed: understanding the dynamics of multiple host parasites is important due to domestic animal production and welfare, wildlife conservation (especially for endangered species) and also because zoonosis can compromise human health (Power & Mitchell, 2004; Gortázar, 2007), as humans can also share some of their parasite fauna with animals. Besides the obvious importance of scientific research in this topic, it is also of utter relevance the establishment of disease control programs coordinated between agriculture, conservation, environment and public health sectors (Artois *et al*., 2001).

The aim of this review is to highlight the cross-transmission of parasitic infections between domestic (livestock) and wild ungulates, by collecting previous reported studies in this field, and to enumerate the inherent factor risks and present some control measures.

4.3. Methods

We undertook a literature review using search engines such as SCOPUS, Google Scholar, JSTOR and ISI Web of Science, with the following keywords: "impact of diseases", "livestock", "wildlife", or "helminth", "cross-transmission", "wild ungulates" and "livestock". We did not restrict our research to time or country. Within parasitic diseases, we selected those that mentioned helminths, and a special attention was given to studies that included roe deer (*Capreolus capreolus*) within the wild ungulates. Unpublished reports, academic theses or dissertations, conference proceedings and other non-refereed publications, were also taken into consideration. All this information resulted in a review that emphasizes interspecific parasitic infections, risk factors and control strategies.

4.4. Results

Many wildlife ethiological agents also infect sympatric domestic animals. Then, the role of different host populations in the maintenance and spread of infection becomes important to conservation (Morgan *et al*., 2006). Walker & Morgan (2014) concluded that goats, sheep, donkeys and camelids are the most liable to nematodes carried by wildlife and, in general, the number of parasites shared between wild and domestic species is high: 18–76% of parasitic nematode species found in wild hosts are also found to infect domestic hosts, and 42–77% of parasitic nematode species found in domestic hosts are also found to infect wild hosts. The same authors showed an overlap of 412 nematode species between 76 wild and 8 domestic ungulate host species. Within *Nematoda*, the generality of trichostrongylids species are equally refered as domestic (sheep, goats and cattle) as wild parasites (roe deer *Capreolus capreolus*, chamois *Rupicapra rupicapra* and alpine ibex *Capra ibex*) (Pérez, 2001). Indeed the majority of the literature research comprised studies about nematodes and this can be explained because they are ubiquitous and generalist parasites (Walker & Morgan, 2014).

i) Interspecific parasitic infections

Studying and controlling an infectious disease implies knowing all actors involved in its transmission (Martin *et al*., 2011).

In northern Portugal (Peneda-Gerês National Park), Figueiredo (2011) studied three populations of goats: domestic, feral and wild. The author observed a very similar parasite fauna among the three populations, probably due to the cosmopolitan nature of parasites and possible occupation of the same areas. In Galicia, Spain, Iglesias (2012) concluded that roe deer is not involved in trematodes and lung nematodes cross-transmission to domestic ruminants (namely cattle and sheep), however they share ten genus of gastrointestinal nematodes (GIN). Pato *et al.* (2009) obtained the same results and suggested that roe deer could be a reservoir host for GIN, in Galicia. Still in Europe, a research driven by Hrabok *et al*. (2006) in Scandinavia demonstrated unequivocally that reindeers are highly suitable hosts for both bovine and ovine infective nematode larvae, particularly for the latter.

Additionally, other examples arrive from elsewhere: in Kazakhstan, there are reports of sharing helminthfauna between ungulates. Particularly, the case of saigas (*Saiga tatarica*) that share home range and several species of parasites with domestic sheep, goats, cattle and camels - some are transmitted predominantly from saigas to livestock, and others from livestock to saigas (Morgan *et al*., 2004). Also in Zambia, other antelope - lechwe - (*Kobus leche kafuensis*) had several species of nematode which also infect domestic hosts, the lechwe were in contact with domestic animals in a wetland area and the authors concluded that this is likely to lead to bidirectional transmission of parasites between them (Phiri *et al*., 2011).

Table 6 is a schematization of research studies of helminths shared between wild and domestic ungulates in Europe.

Table 6 – Helminths shared between wild and domestic ungulates in Europe (Adapted from Navarrete *et al*., 1990; Simpson, 2002; Böhm *et al*., 2007; Vázquez *et al*., 2010; Martin *et al*., 2011).

Parasite	Ungulate species[5]	Livestock species
B. trigonocephalum	Elk	Sheep and goats
Chabertia ovina	Roe deer	Sheep and goats
Cooperia spp.	Red deer	Cattle, sheep and goats
Cysticercus tenuicollis (Taenia hydatigena)	Red deer, roe deer and elk	Cattle, sheep and goats
Dicrocoelium dendriticum	Red and roe deer	Cattle, sheep, goats
Dictyocaulus spp.	Red deer and roe deer	Cattle
Elaphostrongylus spp.	Red deer and roe deer	Sheep and goats
Equinococcus granulosus	Elk and red deer	Sheep and goats
Fasciola hepatica	Red deer, roe deer, Iberian ibex, elk	Cattle, sheep, goats
H. contortus[6]	Roe deer	Sheep
Moniezia benedeni	Elk	Cattle
Moniezia spp.	Roe deer	Cattle, sheep, goats
Muellerius capillaris	Roe deer	Sheep and goats
Nematodirus spp.	Roe deer	Cattle, sheep and goats
O. bifurcum	Red deer, roe deer, elk	Cattle, sheep and goats
Oesophagostomum spp.	Red deer and roe deer	Cattle, sheep and goats
Ostertagia sp.	Red deer and roe deer	Cattle and sheep
Skrjabinagia kolchida	Red deer and roe deer	Cattle, sheep and goats
Teladorsia sp.	Red deer	Sheep
Trichostrongylus spp.	Red deer	Cattle, sheep and goats
Trichuris ovis	Elk, red deer and roe deer	Domestic Ruminants

[5] Elk: *Alces alces*; Iberian ibex: *Capra pyrenaica*; Red deer: *Cervus elaphus*; Roe deer: *Capreolus capreolus*

[6] Cerutti *et al*. (2010) genetically confirmed that *Haemonchus contortus* species from wild and domestic animals is the same in alpine ruminants host species.

ii) Risk factors

A parasitic infection causes impacts at individual, population and community level, as already mentioned. But which are the risk factors that leave the animals susceptible to infection?

Firstly, the human pressure, i.e., the general pattern seen worldwide today is the expansion of urban areas into natural areas which, in addiction to extensive agriculture practices, lead to an increase of probability of contact between domestic animals and wildlife populations. The increasing trend of wild ungulates distribution range and densities also contributed to this. As the host density increases, the higher the net transmission and the higher the average parasite burden per host (Scott, 1988). As a consequence, the domestic and feral animals will be increasingly present in areas occupied by wild animals like is the example of the Iberian ibex (*Capra pyrenaica*) in Portugal. According to Figueiredo (2011), this is one of the main threats to the conservation of the Iberian ibex due to the increased possibility of disease transmission between domestic and feral goat populations and competition for natural resources, which may induce ibex to select less suitable habitats, characterized by areas of scarce and poor vegetation, providing nutrition in less quantity and quality, aggravating health status and body condition scoring. Like Morgan *et al*. (2004) said "*ignoring the role of sympatric hosts in parasite dynamics compromises efforts to control parasites in multi-species systems*".

Besides human land-use and future changes in host distributions, mosaic faunas due to climate change will lead to additional opportunities for previously undescribed host–parasite interactions as well as the general risk of emerging infectious diseases (Walker & Morgan, 2014).

In the last years, animal's translocation projects have increased and are now considered a conservation tool. However, translocation can also be considered a potential risk for the spread of parasites, previously not present in the new habitat. Attempting to conserve one species by introducing it into an apparently suitable habitat, one can be introducing new species of parasites into native animals or may be exposing the introduced species to new infections present in the new habitat (Scott, 1988).

Sharing the same (probably highly contaminated) pastures is a risk factor for intra and interspecific transmission. It is called the faecal-oral transmission route (Böhm *et al*., 2007). In many parts of the world grazing land is shared between wild and domestic species, as well as water sources, leading to the potential for parasitic transmission, mainly nematodes, due to its complex – but direct – life cycle that involves partial development outside of the host (Gortázar, 2007; Walker & Morgan, 2014). Actually, transmission and consequent pathogenicity is dependent on a large number of factors, intrinsic and extrinsic to the host population (Scott, 1988).

iii) Disease control strategies

The importance of survey resident parasite species and know -their distribution will, as far as possible, avoid its introduction into new areas (through translocations), preventing the impact on host physical condition, or even their role as vectors of zoonosis or diseases that may affect humans and domestic livestock (Ruiz-Fons *et al*., 2006). The best way to ensure updated information is to establish a detailed surveillance program. This should be undertaken with the aim of identifying endemic parasites and absence or presence of infection. If it is impossible to obtain samples (faeces or urine samples for parasite eggs, blood smears for larvae, or skin for ectoparasites), it may be possible to use tracer or sentinel animals as indicators of endemic transmission (Scott, 1988).

However, longitudinal monitoring is also required. This can not only be used to detect changes in parasite populations worthy of further investigation but also to identify key diseases to include in future screening work due to their impacts on longevity or reproduction (Matthews *et al*., 2006).

A special focus should also be in translocation projects, a conservation tool widely used. However there is a notorious shortage of protocols or measures to appropriate health screening at the time of its implementation, which could compromise the individual survival and consequently jeopardize species recovery (Matthews *et al*., 2006). To conserve a host population or species, it is necessary to conserve its environment, too. The potential influence of infection and disease must be included in the list of consideration factors in a conservation program, especially those relating to susceptibility to disease (Scott, 1988).

Nevertheless, cautious must always be taken when analyzing the results. A positive result leaves little doubt of the presence of an existing infection, however, negative results may indicate a recent infection in which parasites have not produced eggs or larvae (or are not sufficiently sensitive to detect small numbers of eggs). Detection of infection in a host population level may be somewhat more complex. A particularly important characteristic of most macroparasites (include helminths) is their tendency to be overdispersed within the host population such that many individuals are uninfected or lightly infected whereas a few individuals are very heavily infected. One consequence of this dispersion pattern is that examination of a small sample of the host population may suggest the absence of infection even though some animals may be heavily infected (Scott, 1988).

Disease control in wildlife is not easy. Several factors need to be considered such as wild animals are by definition free-living, therefore sometimes access to a large sample of the population is difficult, thus detecting signs of infection and disease may be complex; also population size or dynamics cannot always be estimated with confidence (Artois *et al*., 2001). But when a disease occurs some control strategies must be taken. Artois *et al* (2001) proposes:

1. Manipulating host population size - reduces the density of infected animals and those who are more susceptible, by culling (or different methods like poisoning, gassing, shooting or trapping) or the most recently alternative which is contraception.
2. Immunization: *per os* vaccination.
3. Attacking directly the etiological agents: deworming.

4.5. Discussion

Diseases caused by parasites despite subtlety, do represent a serious problem to biodiversity conservation, human health and livestock welfare and productivity.

The results obtained after the literature review suggest that transmission of parasites in domestic/wildlife interface is a reality that must not be ignored by biologists, veterinarians, doctors and decision makers. The need to obtain a radical knowledge regarding the evolution of host-parasite relationship and their epidemiological outline should be a priority in scientific research particularly in human and veterinary parasitology. Be aware of parasite fauna allows us to establish management strategies for both wild and domestic ungulates.

However, it is very important to bet on genetic methods in order to provide efficient tools to understand the overlap between populations of generalist parasites in different host species and understand the infectious strains circulating among them.

4.6. Conclusions

It was clear that in this particular area we need the know-how of biologists, ecologists, veterinarians, epidemiologists and medical doctors. Thus, an interdisciplinary collaboration is an unquestionable requisite to the success of management programs, which aim to decrease cross-transmission events or to control its consequences for human and veterinary public health.

4.7. References

ARTOIS, M., DEPNER, K. R., GUBERTI, V., HARS, J., ROSSI, S., RUTILI, D. (2002). Classical swine fever (hog cholera) in wild boar in Europe. *Revue scientifique et technique (International Office of Epizootics)*, 21 (2), pp. 287–303.

BÖHM, M., WHITE, P. C. L., CHAMBERS, J., SMITH, L., HUTCHINGS, M. R. (2007). Wild deer as a source of infection for livestock and humans in the UK. *Veterinary Journal*, 174 (2), pp. 260–276.

CERUTTI, M. C., CITTERIO, C. V., BAZZOCCHI, C., EPIS, S., D'AMELIO, S., FERRARI, N., LANFRANCHI, P. (2010). Genetic variability of *Haemonchus contortus* (Nematoda: Trichostrongyloidea) in alpine ruminant host species. *Journal of helminthology*, 84 (3), pp. 276–283.

FIGUEIREDO, P. J. (2011). Rastreio parasitológico em populações de caprinos silvestres, assilvestrados e domésticos no PNPG. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária, Lisboa, 101 pp.

GORTÁZAR, C. (2007). Enfermedades compartidas entre el ganado ovino y caprino y la fauna silvestre. *Veterinary Research*, 12 (3), pp. 10–12.

HOBERG, E. P., & BROOKS, D. R. (2008). A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography*, *35* (9), pp. 1533-1550.

HRABOK, J. T., OKSANEN, A., NIEMINEN, M., RYDZIK, A., UGGLA, A., & WALLER, P. J. (2006). Reindeer as hosts for nematode parasites of sheep and cattle. *Veterinary parasitology*, *136* (3), pp. 297-306.

IGLESIAS, M.A. (2012). Prevalencia y factores de riesgo asociados a la infección por endoparásitos en rumiantes domésticos y silvestres de la provincia de Lugo. Dissertação de Doutoramento. Universidad de Santiago de Compostela, Facultad de Veterinaria, Lugo.

MARTIN, C., PASTORET, P., BROCHIER, B., HUMBLET, M., SAEGERMAN, C. (2011). A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. *Veterinary Research*, 42 (1), 70 pp.

MATHEWS, F., MORO, D., STRACHAN, R., GELLING, M. and BULLER, N. (2006). Health surveillance in wildlife reintroductions. *Biological Conservation*, 131 (2), pp. 338–347.

MORGAN, E. R., LUNDERVOLDB, M., MEDLEYB G.F., SHAIKENOVC, B.S., TORGERSOND, P.R. (2006). Assessing risks of disease transmission between wildlife and livestock: the Saiga antelope as a case study. Biological Conservation, 131 (2), pp. 244-254.

MORGAN, E., MILNER-GULLAND, E. J., TORGERSON, P. R., MEDLEY, G. F. (2004). Ruminating on complexity: Macroparasites of wildlife and livestock. *Trends in Ecology and Evolution*, 19 (4), pp. 181–188.

NAVARRETE, I.; REINA, D.; HABELA, M.; NIETO, C.G.; SERRANO, F.; PÉREZ, E. (1990). Parasites of roe deer (Capreolus capreolus) in Cáceres province, Spain. 32 Internationalen Symposiums über die Erkrankungen der Zoo- und Wildtiere. Eskilsuna (Sweden).

PATO, F. J., VÁZQUEZ, L., DÍEZ-BAÑOS, N., LÓPEZ, C., SÁNCHEZ-ANDRADE, R., FERNÁNDEZ, G., DÍEZ-BAÑOS, P., PANADERO, R., DÍAZ, P., MORRONDO, P. (2013). Gastrointestinal nematode infections in roe deer (*Capreolus capreolus*) from the NW of the Iberian Peninsula: Assessment of some risk factors. *Veterinary Parasitology*, 196 (1-2), pp. 136–142.

PÉREZ, J. M. (2001). Distribución, genética y estatus sanitario de las poblaciones andaluzas de la cabra montés. Universidade de Jaén, Sevilha: Consejería de Medio Ambiente.

PHIRI, A. M., CHOTA, A., MUMA, J. B., MUNYEME, M., SIKASUNGE, C. S. (2011). Helminth parasites of the Kafue lechwe antelope (*Kobus leche kafuensis*): a potential source of infection to domestic animals in the Kafue wetlands of Zambia. *Journal of helminthology*, *85* (01), pp. 20-27

POWER, A. G. & MITCHELL, C. E. (2004). Pathogen spillover in disease epidemics. *The American naturalist*, 164 (5), pp. S79–S89.

RUIZ-FONS, F., FERNÁNDEZ-DE-MERA, I. G., ACEVEDO, P., Höfle, U., Vicente, J., de la Fuente, J., & Gortazár, C. (2006). Ixodid ticks parasitizing Iberian red deer (*Cervus elaphus hispanicus*) and European wild boar (*Sus scrofa*) from Spain: geographical and temporal distribution. *Veterinary Parasitology*, 140 (1), pp. 133-142.

SCOTT, M. E. (2010). The Impact of Infection and Disease on Animal Populations : Implications for Conservation Biology. *Society for Conservation Biology*, 2, (1), pp. 40–56.

SIMPSON, V. R. (2002). Wild animals as reservoirs of infectious diseases in the UK. *Veterinary Journal*, 163 (2), pp. 128–146.

VÁZQUEZ L.,PAINCEIRA A., DACAL V., PATO F.J., PANADERO R., LÓPEZ C., DÍAZ P., ARIAS M.S., FRANCISCO I., DÍEZ-BAÑOS P. & MORRONDO P. (2010). Long-term study of internal parasitic infections in free-ranging roe deer (*Capreolus capreolus*) from NW Spain. *Rev. Ibero-Latinoam. Parasitol*. 69 (2), pp. 172-177.

WALKER, J. G. & MORGAN, E. R. (2014). Generalists at the interface: Nematode transmission between wild and domestic ungulates. *International Journal for Parasitology: Parasites and Wildlife*, 3 (3), 9 pp.

CHAPTER V

Conclusions and Future Perspectives

5. CONCLUSIONS AND FUTURE PERSPECTIVES

This chapter contains a critical review of what was done in practical work either in theoretical work and also includes brief recommendations for future researches, both in methodological issues as paths to follow. Furthermore, the general conclusions of the thesis are also highlighted.

This thesis showed that the most common livestock species in central Portugal (western part) were infected with helminths and also suggested a possible seasonal pattern in the rate of infection (Chapter III).

By developing this work I learned that parasitic infections can lead to changes in population dynamics by affecting the entire structure of a community or ecosystem, because a harmful effect in individual survival or reproduction determines the population density and subsequently the genetic heterogeneity, which will eventually shape the community. Hereupon, a parasitological survey is important as a health management routine, but not only for the livestock species *per se* but also to other species who share the same habitat as cross transmission between wild and domestic ungulates can occur (Chapter IV). In the specific case of the study area, is of utter relevance to establish an adequate health management and contribute for a decrease in livestock production losses, as the primary sector has a crucial role in the sustainable development of this area. I also highlight the role of infectious diseases, especially the cross-transmission topic, since a roe deer re-introduction programme is going on under the study area, as a conversation tool for the endangered Iberian wolf (Chapter IV). This animal translocation could be a good way of parasitic spread: i) from the translocated animals into the place of intervention, or ii) resident parasites that can infect non-immune translocated animals. The cross-transmission is an issue of interest in this case since the introduced ungulate and the domestic resident ones have a close ecological and phylogenetic relationship, which makes them prone to become sympatric animals and share some parasites, mainly due to the resources sharing between them (water and pastures, for instance). This represents a risk factor in the epidemiological context. At least, it is also important not to forget that infections caused by helminth parasites are directly influence by local climate since their life cycles include free-living stages.

Hereupon, it is justified to do surveillance programmes on a regional scale, taking in account the environmental factors and in a multiple-host context, since disease caused by parasites, even though subtlety, is deleterious to biodiversity conservation (especially endangered species), human health (due to zoonosis) and the welfare and productivity of livestock. So, investigate this issue is utter relevant in order to establish adequate and practicable prevention and control programmes where the focus are the agent, the host and the environment in a multidisciplinary point of view. These programmes can only be effectively designed and implemented if all stakeholders of interest are involved, i.e., biologists, veterinarians, medical doctors and particularly livestock farmers.

Next I will present the main conclusions of the manuscripts, the methodological limitations and make some suggestions to overcome them in the future, and present some guidelines to pursue in further investigation.

Chapter III:

- Most of the livestock were infected with several helminth parasites.
- Small ruminants (sheep and goats) were the hosts with higher prevalence of parasites.
- The gastrointestinal nematodes were the most common, namely the strongyle type eggs.
- *Moniezia benedeni* and *Fasciola hepatica* showed a seasonal trend, appearing only in July.
- Mixed infection were more prevalent than single infection.
- The nematodes faecal egg counts followed a two peaks pattern as in small ruminants as in cattle, in the same seasons.

Chapter IV:

- Wild and domestic ungulates are sympatric hosts.
- Helminths can be transmitted between domestic and wild ungulates, especially nematodes that are the most generalist.
- Some parasites found in Chapter III were also found in roe deer: *Fasciola hepatica*, *Trichuris ovis*, *Moniezia* spp., *Nematodirus* spp., *Muellerius capillaris*, *Dictyocaulus* spp. and several strongylids.
- Management programmes must include the wildlife-domestic-human context in a multidisciplinary point of view.

Methods limitations and ways to overcome it:

- We were unable to collect faeces directly from the rectal bulb which can biased our results. Nevertheless, we tried to overcome this problem, by waiting to the animal to defecate and collect the samples, or by only collecting (very) fresh samples. In the future, it is advisable to establish partnerships with veterinarians of agricultural cooperatives in order to make a more correct sampling.
- Another limitation was the optimization of the laboratory procedures in the middle of the investigation. In further works it is desirable to adopt a more suitable methodology from the very beginning. We suggest the methods described in Chapter II.
- We faced some methodological limitations such as the failure to identify strongyle type eggs to genus/species level. The way to overcome this involves to do faecal cultures or if possible molecular tests, that despite its celerity and sensitivity it is much more expensive.

Where to go from here?

- From now on it is from utter relevance to do repeated cross-sectional studies in order to continue the surveillance. Only in this way it is possible to confirm seasonal trends or screening other risk factors, optimize the sanitary management, pay attention to possible anthelminthic resistance and control outbreak diseases.

- Another recommendation is to elaborate a parasitic surveillance programmes to wild species. Not only focused in ungulates but also in carnivorous, because there are parasites that complete their life cycle when the carnivorous (definitive host) eats the herbivorous (intermediate host). This is of major importance since the top predator, Iberian wolf, are facing a very high risk of extinction. Additionally and importantly, roe deer samples must also be conducted in order to make a real parasitic scan.

- To conclude, would be interesting if future studies could address other etiologic agents (protozoa, viruses, bacteria) and applied another type of examination of samples like immunological or molecular tests, as to proceed to the sanitary inspection of carcasses. This would allow a complete health screening of the wild and domestic population under this area.

CHAPTER VI

References

6. REFERENCES

ADRIMAG – Associação de Desenvolvimento Rural Integrado das Serras de Montemuro, Arada e Gralheira (s/d). Montanhas Mágicas – Viva a nossa Natureza!. [Brochure – Electonic version]. Arouca, 48 pp.

ALHO, A. M., NABAIS, J., CARVALHO, L. M. (2014). A importância da Técnica de Baermann na clínica de pequenos animais. *Revista Clínica Animal*. Publicações Ciência e Vida, pp. 28-31.

ALMEIDA, J. D. (2009). Flora e Vegetação das Serras Beira-Durienses. Tese de Doutoramento em Biologia, Universidade de Coimbra, Faculdade de Ciências e Tecnologia. Coimbra, 963 p.

ANASTÁCIO, P. F. B. (2011). Parasitoses Gastrointestinais em Ovinos em Regime Extensivo e Intensivo em Explorações do Ribatejo. Tese de Mestrado, Universidade Técnica De Lisboa, Faculdade de Medicina Veterinária. Lisboa, 89 p.

ANDERSON, R. M., & MAY, R. M. (1978). Regulation and Stability of Host-Parasite Population Interactions: I. Regulatory Processes. *Journal of Animal Ecology*, *47*(1), pp. 219–247.

BERENGUER, J.G. (2007). Manual de Parasitología: morfología y biología de los parásitos de interés sanitario. Barcelona: Publicaciones I Ediciones de la Universitat de Barcelona 35 pp.

BÖHM, MONIKA, WHITE, PIRAN C L, CHAMBERS, JULIA, SMITH, LESLEY AND HUTCHINGS, M. R. (2007). Wild deer as a source of infection for livestock and humans in the UK. *Veterinary Journal*. 2007. Vol. 174, no. 2, pp. 260–276.

BOWMAN, D. D. (2014). Georgis' Parasitology for Veterinarians. *Elsevier*, 10th Edition, 496 p.

BRUCKNER, G., DONALDSON, A. I., JAMES, A., MCDERMOTT, J., LEYLAND, T., MORRIS, R. S., PERMIN, A., RWEYEMAMU, M. M., WARD, D. & WEBB, R. (2002). Improved animal health for poverty reduction and sustainable livelihoods. *Series title: FAO Animal Production and Health Papers*, *153*, 52. Rome, Italy. pp. 47.

BUSH, A.O., LAFFERTY, K. D., LOTZ, J. M. AND SHOSTAK, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology*, 83 (4), pp. 575-583.

CARDOSO, J. M. G. (2010). Contribuição para o estudo do parasitismo gastrointestinal e hepático em bovinos de carne em regime extensivo no Concelho de Odemira. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 112 p.

CERUTTI, M. C., CITTERIO, C. V., BAZZOCCHI, C., EPIS, S., D'AMELIO, S., FERRARI, N., LANFRANCHI, P. (2010). Genetic variability of Haemonchus contortus (Nematoda: Trichostrongyloidea) in alpine ruminant host species. *Journal of helminthology*, 84 (3), pp. 276–283.

CHINTOAN-UTA C, MORGAN ER, SKUCE PJ, COLES GC. (2014). Wild deer as potential vectors of anthelmintic-resistant abomasal nematodes between cattle and sheep farms. *Proceedings of the Royal Society B: Biological Sciences*, 281, Issue: 1780.

COSTA, J. C., AGUIAR, C., CAPELO, J. H., LOUSÃ, M., & NETO, C. (1998). Biogeografia de Portugal Continental. pp. 5-56.

DOBSON, A. P., HUDSON, P. J. (1986). Parasites, disease and the structure of ecological communities. *Trends in Ecology & Evolution*, vol. 1, no. 1, pp. 11-15.

DOBSON, A., KUTZ, S., PASCUAL, M. AND WINFREE, R. (2003). Pathogens and Parasites in a Changing Climate. *Advances in Applied Biodiversity Science*, No 4, pp. 33-38.

European Comission (2015). Natura 2000 Network. Disponível em <http://ec.europa.eu/environment/nature/natura2000/index_en.htm>, Acedido em Dezembro de 2014

FIGUEIREDO, P. J. (2011). Rastreio parasitológico em populações de caprinos silvestres, assilvestrados e domésticos no PNPG. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, pp. 101.

FOREYT, W. J. (2001). Veterinary parasitology: Reference manual. 5th edition. Blackwell Publishing. Iowa, USA. pp. 246.

GOMES, C. A. V. C. (2012). Fasciolose em Bovinos de Engorda. Dissertação de Mestrado Integrado em Medicina Veterinária, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 84 p.

GREER, A. W., KENYON, F., BARTLEY, D. J., JACKSON, E. B., GORDON, Y., DONNAN, A. A., MCBEAN, D. W., JACKSON, F. (2009). Development and field evaluation of a decision support model for anthelmintic treatments as part of a targeted selective treatment (TST) regime in lambs. *Veterinary Parasitology*, 164 (1), pp. 12-20.

GUERRA, D., ARMUA-FERNANDEZ, M. T., SILVA, M., BRAVO, I., SANTOS, N., DEPLAZES, P., & DE CARVALHO, L. M. M. (2013). Taeniid species of the Iberian wolf (*Canis lupus signatus*) in Portugal with special focus on *Echinococcus* spp. *International Journal for Parasitology: Parasites and Wildlife*, *2*, pp. 50-53.

GUERREIRO, C. M. C. (2009). Influência do maneio na prevalência de parasitoses gastrointestinais em pequenos ruminantes: estudo comparativo entre a região do Alentejo e a região de Andaluzia. Dissertação de Mestrado, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, pp. 135.

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014a). "Serras da Freita e Arada". Plano Sectorial da Rede Natura 2000, pp. 1-12. Consultado em Dezembro, 2014. [electronic document]

ICNB – Instituto para a Conservação da Natureza e Biodiversidade (2014b). "Serra de Montemuro". Plano Sectorial da Rede Natura 2000, pp. 1-11. Consultado em Dezembro, 2014. [electronic document]

IFAH (2015). Parasitas. Disponível em: <http://www.ifaheurope.org/food-producing-animals/success-stories/parasites.html>, Acedido em: Outubro de 2015

INE – Instituto Nacional de Estatística (2009). Recenseamento Agrícola 2009.

INE – Instituto Nacional de Estatística (2011). Contas Nacionais Anuais - Base 2011.

INE – Instituto Nacional de Estatística (2011). Contas Regionais - Base 2011.

KESSING, F. (2008). Part I. Effect of Ecosystem on Disease – Introduction. pp. 9-11. In Ostfeld, R. S., Keesing, F., & Eviner, V. T. (Eds), Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems. United Kingdom: Princeton University Press.

KUMAR, N., RAO, T. K. S., VARGHESE, A., RATHOR, V. S. (2013). Internal parasite management in grazing livestock. *Journal of Parasitic Diseases*, *37* (2), pp. 151-157.

Kutz, S. J., Hoberg, E. P., Nagy, J., Polley, L., & Elkin, B (2004). 'Emerging' Parasitic Infections in Arctic Ungulates. *Integrative and Comparative Biology*. 44 (2), 109–118.

LAGARES, A. F. B. F. (2008). Parasitoses de pequenos ruminantes na região da Cova da Beira. Dissertação de Mestrado Integrado em Medicina Veterinária, Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 115 pp.

MADEIRA DE CARVALHO, L. M., BERNARDO, F.A., PAZ-SILVA, A. (2012). 14. The Role of Fungi in the Control of Animal Parasites – Classification, Mode of Action and Practical Applications. pp. 271-308. In A. Paz-Silva & M.S. Arias Vázquez (Eds.), Fungi: Types, Environmental Impact and Role in Disease. Nova Science Publishers. Hauppauge, New York, 531 pp.

MADEIRA DE CARVALHO, L.M., GILLESPIE, A.T., SERRA, P.M., BERNARDO, F.A., FARRIM, A.P., FAZENDEIRO, I. (2007). Eficácia do fungo nematófago *Duddingtonia flagrans* no controlo biológico da estrongilidose equina no Ribatejo. *Revista Portuguesa de Ciências Veterinárias*, 102 (563-564), pp. 233-247.

MARCOGLIESE, D. J. (2005). Parasites of the superorganism: are they indicators of ecosystem health?. *International journal for parasitology*, *35* (7), pp. 705-716.

MARTIN, C., PASTORET, P., BROCHIER, B., HUMBLET, M. AND SAEGERMAN, C. (2011). A survey of the transmission of infectious diseases/infections between wild and domestic ungulates in Europe. *Veterinary Research*, pp. 42-70.

MAS-COMA, S., VALERO, M. A., & BARGUES, M. D. (2008). Effects of climate change on animal and zoonotic helminthiases. *Rev Sci Tech*, *27*(2), pp. 443-57.

MAS-COMA, S., VALERO, M. A., BARGUES, M. D. (2009). Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology*, Vol. 163, no. 4, pp. 264-280.

MATOS, C. A. P. (2000). Recursos Genéticos Animais e Sistemas de Exploração Tradicionais em Portugal. *Archivos de Zootecnia*, 49, pp. 363-383.

MITCHELL, S. E., ROGERS, E. S., LITTLE, T. J., & READ, A. F. (2005). Host-parasite and genotype-by-environment interactions: temperature modifies potential for selection by a sterilizing pathogen. *Evolution*, *59* (1), pp. 70-80.

MORGAN, E. R., WALL, R. (2009). Climate change and parasitic disease: farmer mitigation?. *Trends in Parasitology*, 25 (7), pp. 308-313.

MORGAN, E., CHARLIER, J., HENDRICKX, G., BIGGERI, A., CATALAN, D., VON SAMSON-HIMMELSTJERNA, G., DEMELER, J., MÜLLER, E., VAN DIJK, J., KENYON, F., SKUCE, P., HÖGLUND, J., O'KIELY, P., VAN RANST, B., DE WAAL, T., RINALDI, L., CRINGOLI, G., HERTZBERG, H., TORGERSON, P., WOLSTENHOLME, A., VERCRUYSSE, J. (2013). Global Change and Helminth Infections in Grazing Ruminants in Europe: Impacts, Trends and Sustainable Solutions. *Agriculture*, 3 (3), pp. 484-502.

MORGAN, E., MILNER-GULLAND, E. J., TORGERSON, R., MEDLEY, G. F. (2004). "Ruminating on complexity: macroparasites of wildlife and livestock". TRENDS in Ecology and Evolution, Vol.19, No.4, pp. 181-188.

PATO, F. J., VÁZQUEZ, L, DÍEZ-BAÑOS, N., LÓPEZ, C., SÁNCHEZ-ANDRADE, R., FERNÁNDEZ, G., DÍEZ-BAÑOS, P., PANADERO, R., DÍAZ, P., MORRONDO, P. (2013). "Gastrointestinal nematode infections in roe deer (*Capreolus capreolus*) from the NW of the Iberian Peninsula: Assessment of some risk factors". *Veterinary Parasitology* 196, pp. 136–142.

PORDATA (2015). Estimativas Anuais da População Residente.

PORDATA (2015). Números dos Municípios e Regiões de Portugal.

PORDATA (2015). Ofertas de emprego (média anual) disponíveis nos centros de emprego e formação profissional: total e por sector de actividade económica.

PORDATA (2015). Recenseamentos Gerais da População.

RAMOS, J. C. (2013). Avaliação das Parasitoses Gastrointestinais em Bovinos de Raça Brava durante a Primavera e Verão. Dissertação de Mestrado. Universidade Técnica de Lisboa, Faculdade de Medicina Veterinária. Lisboa, 87 pp.

RIÁDIGOS, S. M., VÁZQUEZ, M. S. A., CANO, E.V., FRAGA, P.P., UZAL, D.G., MARCOS, M. F., QUINTERO, R. B., SÁNCHEZ-ANDRADE, R., FERNÁNDEZ, MADEIRA DE CARVALHO, L.M. (2014). Control biológico de nematodos parásitos en antílopes (Cervicapra cervicapra) en cautividad. Revista Pastagens e Forragens, 34, pp. 47-56.

RIBEIRO, A. (2006). A evolução geodinâmica de Portugal. In: Dias, R., Araújo, A., Terrinha, P & Kullberg, C., (Eds.), *Geologia de Portugal no contexto da Ibéria*. Universidade de Évora. Évora, 27 pp.

ROCHA, D., SÁ, A. A., BRILHA, J. (2012). Considerações Preliminares Sobre o Património Natural e Cultural do Território "Montemuro e Gralheira" (Portugal). In: Henriques, M. H., Andrade, A. I., Quinta-Ferreira, M., Lopes, F. C., Barata, M. T., Pena dos Reis, R- & Machado, A (Coord.), *Para Aprender Com A Terra: memórias e notícias de geociências no espaço lusófono*. Imprensa da Universidade de Coimbra. Coimbra, pp. 241-249.

ROEBER, F., JEX, A. R., & GASSER, R. B. (2013). Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance-an Australian perspective. *Parasit Vectors*, 6, 153 pp.

ROSE, H., HOAR, B., KUTZ, S. J., & MORGAN, E. R. (2014). Exploiting parallels between livestock and wildlife: Predicting the impact of climate change on gastrointestinal nematodes in ruminants. *International Journal for Parasitology: Parasites and Wildlife*, 3, pp. 209-219.

SCHWEIZER, G., BRAUN, U., DEPLAZES, P., TORGERSON, P. R. (2005). Estimating the financial losses due to bovine fasciolosis in Switzerland. *The Veterinary Record*, *157*, 188-193.

SCOTT, M. E. (1988). The impact of infection and disease on animal populations: implications for conservation biology. *Conservation biology*, pp. 40-56.

SHAPIRO, L. S. (2010). Pathology & Parasitology for Veterinary Technicians. Delmar – Cengage Learning, 2nd edition, 320 pp.

SIMPSON, V. R. (2002). Wild Animals as Reservoirs of Infectious Diseases in the UK. *The Veterinary Journal*, 163, pp. 128-146.

STROMBERG, B. E. (1997). Environmental factors influencing transmission. *Veterinary Parasitology*, *72* (3), pp. 247-264.

TAVARES, R. G., STAGGEMEIER, R., BORGES, A. L. P., RODRIGUES, M. T., CASTELAN, L. A., VASCONCELOS, J, ANSCHAU, M. E. & SPALDING, S. M. (2011). Molecular techniques for the study and diagnosis of parasite infection. *Journal of Venomous Animals and Toxins including Tropical Diseases*, *17* (3), pp. 239-248.

TAYCE, J., ACEVEDO, P., VICENTE, J., GORTAZAR, C. (2008). Minimum sampling effort for reliable non-invasive estimations of excretion abundance of *Elaphostrongylus cervi* L1 in red deer (*Cervus elaphus*) populations. *Journal of Helminthology*, 82 (3), pp. 255-261.

THIENPONT, D., ROCHETTE, F., & VANPARIJS, O. F. J. (1986). *Diagnóstico de las helmintiasis por medio del examen coprológico*. Segunda Edición. Janssen Research Foundation. Beerse, Bélgica, 36 pp.

TORRES, R. T., SILVA, N., BROTAS, G., & FONSECA, C. (2015). To Eat or Not To Eat? The Diet of the Endangered Iberian Wolf (*Canis lupus signatus*) in a Human-Dominated Landscape in central Portugal. *PloS one*, *10* (6).

UENO, H., GONÇALVES, P. C. (1998). Manual para diagnóstico das helmintoses de ruminantes. Japan International Cooperation Agency, 4ª Edição, 143 pp.

VIEIRA, A. (2005). Avaliação das potencialidades naturais e paisagísticas da Serra de Montemuro. Actas do X Colóquio Ibérico de Geografia, APG/AEG, Universidade de Évora, Évora, 13 pp.

WALKER, J. G. AND MORGAN, E.R. (2014). Generalists at the interface: Nematode transmission between wild and domestic ungulates. *International Journal for Parasitology: Parasites and Wildlife*, 3, pp. 242–250.

ZAJAC, A. M., & CONBOY, G. A. (2012). Veterinary Clinical Parasitology. 8th Edition. Wiley-Blackwell. 368 pp.

CHAPTER VII

Annexes

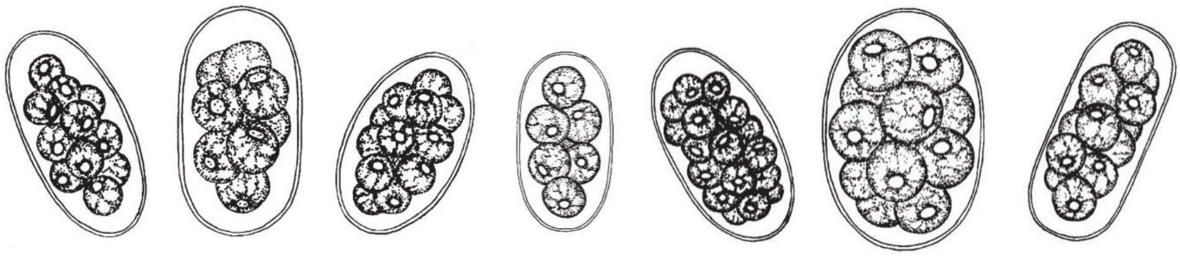
7. ANNEXES

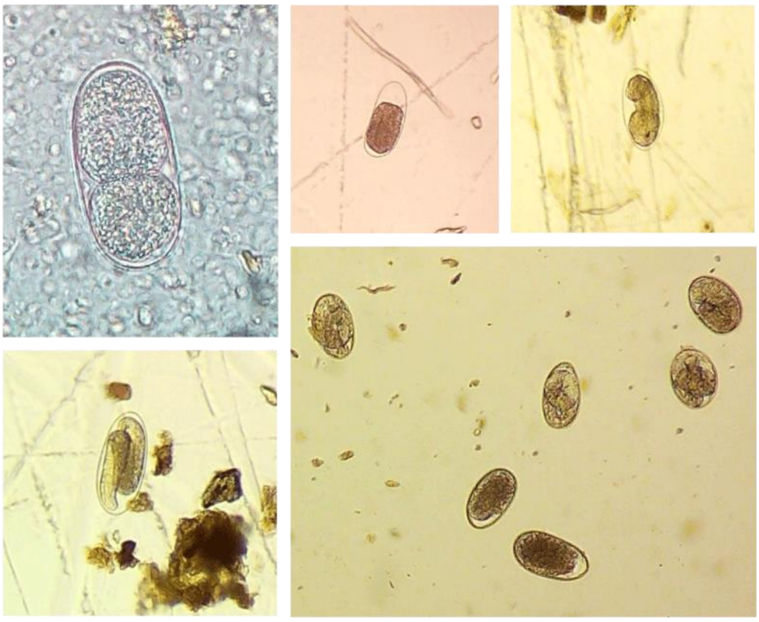
7.1. Annex I

Photographic record of parasites observed microscopically throughout the investigation in the different coprological techniques. The images used below are originals of the author and the illustrations are from Gary A. Averbeck authorship.

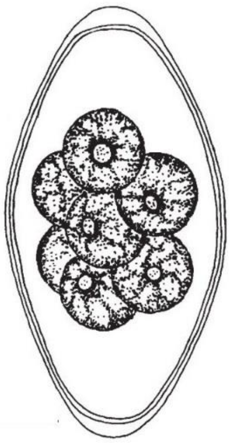
Gastrointestinal Nematodes

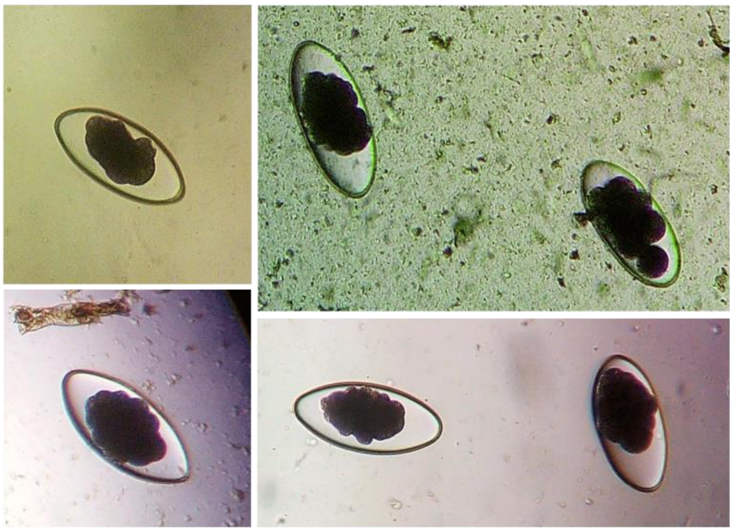
- Strongyle type eggs



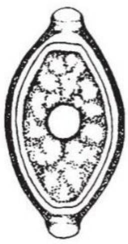


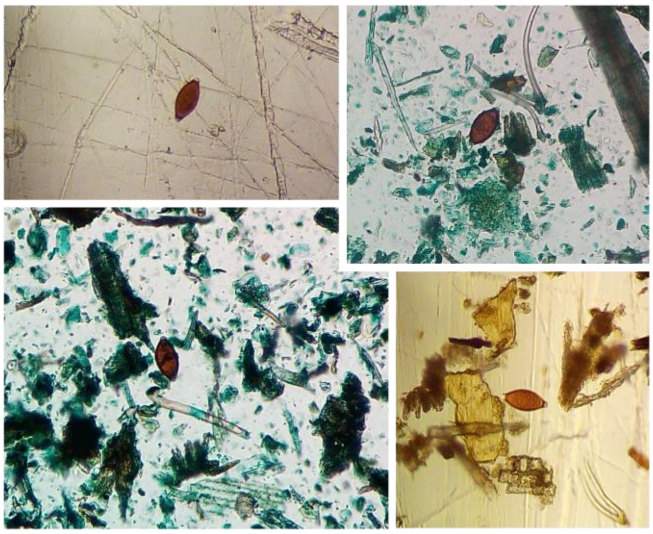
- *Nematodirus* spp.



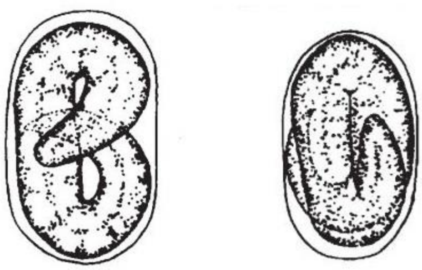


- *Trichuris ovis*

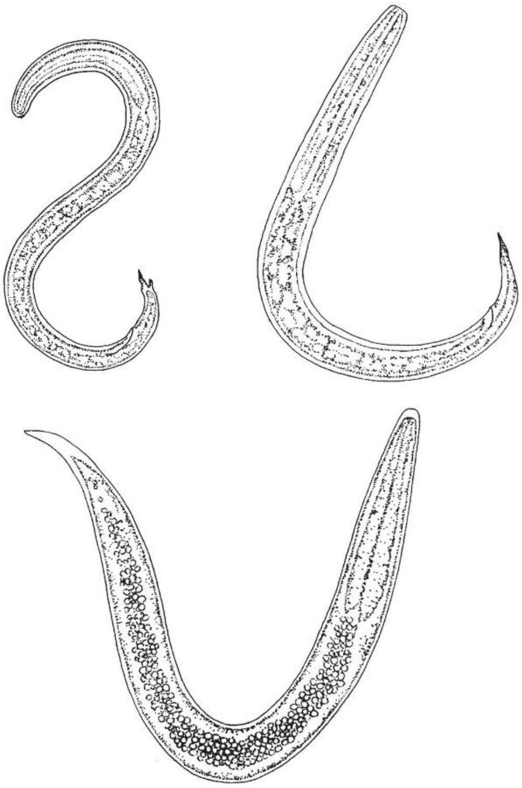




- *Strongyloides papillosus*

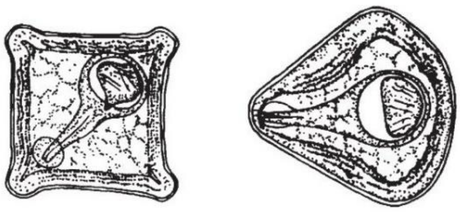


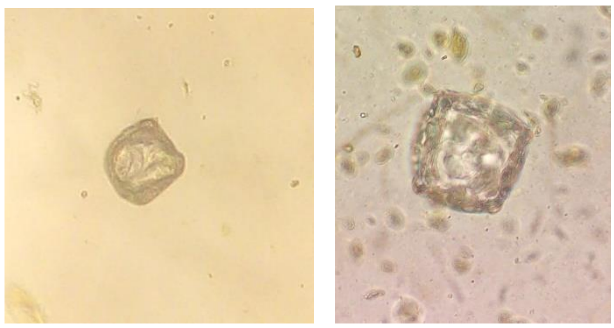
Pulmonary Nematodes



Cestodes

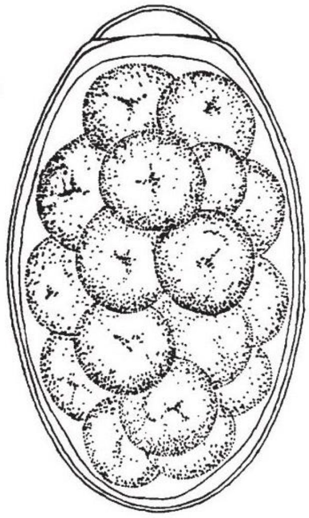
- *Moniezia* spp.





Trematodes

- *Fasciola hepatica*



Protozoa

- *Eimeria* spp.