UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

PREVALENCE OF GASTROINTESTINAL PARASITES AND ANTHELMINTIC EFFICACY IN SHEEP AND GOATS UNDER DIFFERENT MANAGEMENT AND DEWORMING SYSTEMS IN THE REGION OF LISBON AND TAGUS VALLEY, PORTUGAL

MARIA INÊS CAETANO ANTUNES

ORIENTADOR:

Doutor Miguel Luís Mendes Saraiva Lima

COORIENTADOR:

Doutor Luís Manuel Madeira de Carvalho

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MARIA INÊS CAETANO ANTUNES

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Nome: Maria Inês Caetano Antunes

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Faculdade de Medicina Veterinária da Universidade de Lisboa, 17 de Março de 2021

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“Eles não sabem, nem sonham,

que o **sonho comanda a vida**,

que sempre que um homem sonha

o mundo pula e avança

como bola colorida

entre as mãos de uma criança.”

António Gedeão

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Porque "*aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós*". Antoine de Saint-Exupéry

Um enorme obrigado a todos, sem exceção.

ABSTRACT

Infections caused by gastrointestinal parasites have been described as one of the most important issues regarding small ruminant production. They induce major losses, causing a reduction in weight gain, poor feed utilization and consequently a decreased productivity. They can also be fatal, so their control measures mean a lot of investment. The main objectives of this study were to characterize the presence and level of parasitism of small ruminants in nine farms located in the region of Lisbon and Tagus Valley, as well as, the presence and level of anthelmintic resistance cases in five of the nine farms. The farms had different types of production and deworming systems, giving a generalized assessment of the current parasitological situation regarding small ruminants in that region. The anthelmintic efficacy study was performed in a dairy goats' farm using eprinomectin (Eprinex® Pour-on), in two mixed (both sheep and goats) farms using fenbendazole (Panacur® 2,5%) and in two sheep flocks using an association of closantel and mebendazole (Seponver® Plus).

The overall presence of gastrointestinal parasites in the nine farms was 89%. All farms were found to have positive animals to at least one type of gastrointestinal parasite, which pronounces a widespread infection with gastrointestinal parasites in the region. The most frequent encountered eggs were from strongyle type, followed by oocysts of *Eimeria* spp., eggs from *Strongyloides papillosus* and *Moniezia expansa*. Regarding the ranking of the parasitism level based on the eggs per gram (EPG) counts, five farms had more than 50% of the animals ranked in the low level of infection category (with less than 500 EPG), three farms had all three types of classifications with similar proportions (about 35%) and one farm had 75% of the animals ranked in the high level of infection category, with more than 1500 eggs per gram. Regarding the anthelmintic efficacy study, four out of five farms where the study was conducted presented anthelmintic resistance: two farms against fenbendazole and two farms concerning the association of closantel and mebendazole.

The present study shows that even though there was a generalized infection by gastrointestinal parasites in the region, this infection appeared not to have fatal repercussions when at low levels. However, when anthelmintic efficacy was required it was not at the levels as it was supposed to be, according to the literature the first one to be reported in in the region of Lisbon and Tagus Valley, which announces an increase of anthelmintic resistant nematode strains in small ruminant production in our country.

Keywords: Gastrointestinal parasites, Infection levels, Anthelmintic efficacy, Small Ruminants, Lisbon and Tagus Valley

RESUMO

As infeções provocadas por parasitas gastrointestinais têm sido descritas como um dos fatores mais importantes relacionados com a produção de pequenos ruminantes. Estas provocam graves prejuízos, reduzindo o ganho médio diário e a utilização dos alimentos, levando uma menor produtividade. Estas infeções podem também tornar-se fatais, pelo que as suas medidas de controlo exigem grandes investimentos. Uma das condutas mais comuns para ultrapassar esta situação prende-se com o uso frequente e desnecessário dos anti-helmínticos, sem avaliar a real necessidade da sua aplicação. Todavia, uma vez que esta atividade leva ao aparecimento e aumento das resistências por parte dos parasitas, esta prática requer uma avaliação e consequente alteração. Por forma a adequar a abordagem ao seu controlo, torna-se imperativo o conhecimento dos parasitas gastrointestinais mais comuns na produção dos pequenos ruminantes. Estes consistem em protozoários do Filo Apicomplexa, helmintes da Classe Trematoda, da Classe Cestoda e do Filo Nematoda. No Filo Apicomplexa temos *Eimeria* spp., um género de coccídia transmitido através da contaminação fecal de comida e água. Trata-se de um parasita intracelular, que destrói as células do seu hospedeiro e que provoca doença sobretudo em animais jovens ou debilitados. *Cryptosporidium* sp., também pertencente ao Filo Apicomplexa, trata-se de um parasita que infeta as células epiteliais do trato gastrointestinal de mamíferos, aves, répteis e peixes. Algumas espécies podem ser zoonóticas, o que aumenta a sua importância quando se lida com animais potencialmente infetados ou com águas potencialmente contaminadas. A Classe Trematoda compreende duas subclasses principais: Monogena e Digenea. Na subclasse Digenea, encontramos parasitas com um estilo de vida heteroxeno, ou seja, que requerem um hospedeiro intermediário (moluscos) e que apenas parasitam vertebrados. É o caso da *Fasciola hepatica*, de distribuição cosmopolita e que pode ser encontrado no fígado e ductos biliares de mamíferos herbívoros e de humanos. Os seus ovos são eliminados com a bílis para o lúmen intestinal e para o exterior através das fezes. *Dicrocoelium dendriticum* também se trata de um trematode encontrado nos ductos biliares de ruminantes, camelídeos, coelhos e outros mamíferos. Na Classe Cestoda encontramos parasitas achatados e segmentados, cujas formas adultas são hermafroditas. Nesta Classe inclui-se o género *Moniezia*, de distribuição cosmopolita e cujo ciclo de vida se inicia com a ingestão pelo hospedeiro intermediário de fezes contaminadas com ovos de *Moniezia* spp. Por fim, no Filo Nematoda, encontramos predominantemente parasitas de corpo cilíndrico e com um ciclo de vida direto. Neste Filo inserem-se os géneros *Haemonchus, Teladorsagia, Trichostrongylus, Cooperia, Nematodirus, Chabertia, Oesophagostomum, Bunostomum, Trichuris* e *Strongyloides*.

Atualmente existem três principais grupos de anti-helmínticos utilizados no tratamento das helmintoses nos pequenos ruminantes: os benzimidazóis, como o febendazol, albendazol e mebendazol; as lactonas macrocíclicas como a ivermectina, eprinomectina e moxidectina e os Imidazotiazóis como o levamisol. Existe ainda o grupo das Salicinalinidas e fenóis substitutos onde se insere o closantel.

Não obstante, o seu uso indiscriminado tem levado ao desenvolvimento de resistências aos anti-helmínticos, que tem vindo a ser reportado a nível mundial, sobretudo no grupo dos benzimidazóis e das lactonas macrocíclicas; estas estirpes resistentes de nematodes gastrointestinais têm sido encontradas nos Estados Unidos da América, no Brasil, em África, na Austrália, Nova Zelândia e Europa.

Para o aparecimento das resistências contribui o facto de que, embora sejam vistos e tratados como semelhantes, os ovinos e os caprinos diferem entre si de diversas formas, sendo que os caprinos possuem uma taxa metabólica superior e requerem, portanto, doses superiores no que diz respeito à administração de fármacos. A maioria dos anti-helmínticos não se encontram licenciados para esta espécie e as doses apropriadas para a mesma são raramente conhecidas. Os caprinos geralmente requerem doses 1.5 a 2 vezes superior à dos ovinos, contudo, uma vez que são tratados conjuntamente e de acordo com a dose recomendada para estes últimos, acabam por receber uma dose inferior à necessária, promovendo o aparecimento da resistência anti-helmíntica.

O aparecimento de estirpes de nematodes resistentes aos anti-helmínticos tem sido frequentemente reportado em pequenos ruminantes, o que levou à necessidade de criar novas abordagens no controlo e tratamento das parasitoses. Um ponto fundamental para o combate à resistência anti-helmíntica trata-se da manutenção da população em refúgio, constituída pelos parasitas presentes em animais não tratados, pelas suas fases de vida livre (por exemplo, na pastagem) e pelos seus estádios não afetados pelo tratamento. Sugere-se então que um produtor, aquando da passagem dos animais para o pasto, deverá deixar os animais mais saudáveis por tratar, para que os parasitas suscetíveis possam sobreviver e reproduzir-se com parasitas resistentes, propagando assim os genes suscetíveis e atrasando o desenvolvimento da resistência aos anti-helmínticos. Além da manutenção da população em refúgio, outras medidas deverão ser implementadas, tais como: a aplicação do método FAMACHA©, suplementação com proteína por forma a aumentar a resistência e resiliência ao parasitismo, a introdução de fungos nematófagos na alimentação, formulação de vacinas, o uso de plantas com propriedades anti-helmínticas e a seleção de animais resistentes ao parasitismo.

A situação de resistência aos anti-helmínticos na produção de pequenos ruminantes em Portugal é desconhecida, pelo que os principais objetivos deste estudo foram caracterizar a presença e o nível de parasitismo de pequenos ruminantes em nove

explorações localizadas na região de Lisboa e Vale do Tejo, assim como avaliar a presença e o nível de resistência anti-helmíntica em cinco das nove explorações.

As explorações selecionadas possuíam diferentes sistemas de produção e desparasitação, por forma a demonstrar de uma forma generalizada o estatuto parasitário dos pequenos ruminantes na região. Uma exploração encontrava-se localizada no distrito de Lisboa, uma no distrito de Setúbal e sete no distrito de Santarém. Quatro das explorações encontravam-se em regime intensivo e cinco em regime extensivo, sendo que das primeiras, duas eram constituídas apenas por caprinos, uma por ovinos e uma por ambas as espécies, ou seja, mista. As explorações extensivas eram constituídas por três rebanhos de ovinos e dois rebanhos mistos. As idades dos animais estavam compreendidas entre os seis meses e os nove anos e as colheitas foram efetuadas entre Setembro de 2018 e Janeiro de 2020. As fezes foram colhidas diretamente da ampola retal dos animais e identificadas e analisadas individualmente, por forma a averiguar o nível de parasitismo através da contagem de ovos por grama pela técnica de McMaster. Foram também realizadas coproculturas para averiguar os géneros de estrôngilos gastrointestinais predominantes. O estudo da eficácia anti-helmíntica foi realizado numa exploração de cabras leiteiras recorrendo à eprinomectina (Eprinex® Pour-on), em duas explorações mistas recorrendo ao uso de febendazol (Panacur® 2,5%) e em duas explorações de ovinos usando uma associação de closantel e mebendazol (Seponver® Plus).

A presença geral de parasitas gastrointestinais nas nove explorações foi de 89.27%, com uma diferença significativa entre ovinos e caprinos (76.85% e 92.78%, respetivamente). Todas as explorações demonstraram ter animais positivos a pelo menos um tipo de parasita gastrointestinal, o que revela uma infeção generalizada por parasitas gastrointestinais na região. Os ovos detetados com maior frequência foram os do tipo estrongilídeo (88.88%), seguido de oocistos de *Eimeria* spp. (66.66%), ovos de *Strongyloides papillosus* (55.55%) e de *Moniezia expansa* (11.11%). A média da contagem de ovos por grama (OPG) nos ovinos foi de 2029, com contagens com valores entre 0 e 21300. Nos caprinos, a média de OPG foi de 606 com contagens com valores entre 0 e 5850. Em relação à contagem dos oocistos por grama (OOPG), foram encontradas médias de 47 OOPG, com valores entre 0 e 2500, e 109 OOPG, com valores entre 0 e 850, respetivamente. Na classificação do nível de parasitismo baseado na contagem de OPG, cinco explorações tiveram mais de 50% dos seus animais classificados no nível baixo de infeção (menos de 500 OPG), três explorações tiveram os três tipos de classificação em proporções idênticas e uma exploração teve 75% dos seus animais classificados no nível alto de infeção, com mais de 1500 OPG. Por fim, no estudo da eficácia dos anti-helmínticos, quatro das cinco explorações apresentaram resistência: duas ao febendazol e duas à associação de closantel e mebendazol.

O presente estudo demonstrou que embora se tenha observado uma infeção generalizada por parasitas gastrointestinais na região, esta infeção não revelou ter repercussões fatais quando se encontrava em níveis baixos. Contudo, quando foi necessária a eficácia dos anti-helmínticos, esta não se encontrou aos níveis esperados, e segundo a literatura, a primeira a ser reportada na região de Lisboa e Vale do Tejo, o que sugere um aumento de estirpes de nematodes resistentes aos desparasitantes no nosso País.

Palavras-chave: Parasitas gastrointestinais, Níveis de infeção, Eficácia anti-helmíntica, Pequenos ruminantes, Lisboa e Vale do Tejo

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LIST OF ABBREVIATIONS, INITIALS AND ACRONYMS

% – Percentage

AHR – Anthelmintic Resistance

ATP – Adenosine Triphosphate

AVM – Avermectin

BZD – Benzimidazole

CI – Confidence Interval

EPG – Eggs Per Gram

EPN – Eprinomectin

FECRT – Faecal Egg Count Reduction Test

GABA – Gamma Amino Butyric Acid

GI – Gastrointestinal

GIN – Gastrointestinal Nematode

IVM – Ivermectin

L1 – First stage larva

L2 – Second stage larva

L3 – Third stage larva

L4 – Fourth stage larva

L5 – Fifth stage larva

LEV – Levamisole

MBZ – Mebendazole

MDR – Multi Drug Resistant

ML – Macrocyclic Lactone

mL – milliliter

MOX – Moxidectin

OPG – Oocysts Per Gram

SC – Subcutaneous

WAAVP – World Association for the Advancement of Veterinary Parasitology

PART I – DESCRIPTION OF THE TRAINING PERIOD

The training period occurred between September 2018 and January 2019 and it was performed under the supervision of Professor Miguel Saraiva Lima at *Faculdade de Medicina Veterinária, Universidade de Lisboa.* The author occasionally accompanied the classes of Professor Ricardo Bexiga and Professor Patrícia Simões. This training period consisted in accompanying the Farm Animals Clinics ambulatory work with the students, which gave the author the opportunity to visit different types of animal production systems, such as feedlots, dairy farms and beef farms. These visits allowed the analysis of the most common characteristics and problems of each type of animal production system. It was also an excellent opportunity to learn since the visits took place in a pedagogic environment. In the dairy goats' farms, the caseload consisted mostly in pregnancy toxaemias, pneumonias and hoof-related problems and surgeries such as cesareans or procedures like draining abscesses, hooves trimming and necropsies. The author also had the opportunity to collect faecal samples for the present study. In cattle farms, both dairy and beef, with a wide range of clinical cases, it was possible to perform several physical exams with posterior discussion of most likely differential diagnosis and treatment. In Figure 1, some pictures from this training period are presented. It was also possible to observe mostly cases of pneumonias in calves, ketosis and mastitis in lactating cows and the surgical treatment for left displaced abomasum, as well as a leg amputation in a calf. The author has participated in several vaccinations and disbudding procedures in calves. Regarding the beef farms, there was a visit to a farm with a case of oak poisoning in a cow, caused by the excessive ingestion of acorns. In a lambs' feedlot, most of the observed cases consisted in urolithiases in males. During this time, the author also worked at the Parasitology and Parasitological Diseases Laboratory,

Figure 1 - Some moments from the author's first training period. From left to right: Collecting faeces directly from the rectum of a goat; Surgical treatment for left abomasum displacement; Acorns found inside the rumen of a cow at the moment of necropsy; Handling goats. (Originals)

performing faecal egg counts, sedimentation and flotation techniques and coprocultures in order to collect data.

As an extra training period, for two weeks in February 2019 and for four weeks in May 2019 the author accompanied the work of the veterinarians Dr. Luís Fragoso and Drª Maria Inês Romeiras from *Vetequilíbrio* and *Agrupamento de Defesa Sanitária do Baixo Tejo*, which goes from the region of Ribatejo to Alentejo. The workload consisted mostly in sanitary procedures in ruminants such as bovine tuberculosis testing, vaccinating, deworming and ear tagging. There was also the opportunity to follow the clinics work with some cases like diarrhoeas in calves, placenta's retention in cows, downer cows, haemonchosis in sheep and surgeries such as castrations and wound repairs. It was also possible to follow the reproductive management of some farms, performing rectal palpations, echography, inseminations and andrological examinations. As it was a mixed practice, there was also the opportunity to see some small animals and equine medicine, such as vaccinating and deworming, reproductive echography and wound treatments. With this traineeship it was possible to establish contacts with some small ruminants' farmers in order to use their animals for the present study. In the Figure 2, some pictures from the second training period are presented.

Figure 2 - Some moments from the second training period. From left to right: Sanitary procedures in sheep; Sanitary procedures in *Raça Brava* cattle; Sanitary procedures in *Raça Mertolenga* cattle; Wound repair in a horse with left hindlimb caught in barbed wire; Vaccinating and deworming dogs. (Originals)

Between February 2019 and April 2019, the author went to England with the Erasmus Program to do a traineeship at Westpoint Farm Vets in Sevenoaks, under the supervision of Dr. Rui D'Orey Branco. It consisted in accompanying the work of the ambulatory vets through the south of England and London surrounding areas, following a vast diversity of clinical cases, surgeries and sanitary procedures. In the dairy farms' context, the author participated in several vaccinations, calf scoring (measuring temperature, nasal discharge, ocular discharge, coughing) and disbudding procedures in calves, as well as blood sampling from jugular in calves and coccygeal vein in cows. It was also possible to participate in reproductive routines, performing rectal and vaginal palpations and seeing surgeries such as caesareans, teat removal, left abomasum displacement surgical treatment, removal of a palpebral mass and an umbilical hernia resolution in a calf. Regarding the small holders, it was possible to attend the new clients' visits, learning more about the communication with the owners and their concerns about their pets (mostly small ruminants, camelids and pigs). The livestock work consisted mostly in the bovine tuberculosis testing, but also some cow's dehorning, resolution of uterine torsion and prolapse, placenta's retention, caesareans and pregnancy diagnosis. The author also saw some small ruminants' cases such as pregnancy's toxaemia, caesareans and vaginal prolapses in sheep, lameness and respiratory problems. The author has also participated in a Dairy Cows Hoof-Trimming Workshop and a Lambing Workshop.

During the period in England, the author got to spend two weeks at Milbourn Equine Vets in Hawkhurst and two weeks as an extern at the Bell Equine Veterinary Clinic in Mereworth. At Milbourn Equine Vets the traineeship consisted in following the work of the ambulatory vets, such as vaccinations, dentistry, lameness tests, pre-purchase exams, eye ulcer treatment and nasolacrimal canal flushing, laminitis diagnosis, sedations, euthanasia and imaging exams such as radiography, echography, gastroscopy and respiratory endoscopy with tracheal collection. At the Bell Equine hospital, the externship gave the opportunity to observe several internal medicine cases, lameness tests, imaging exams such as radiography, echography and endoscopy and to participate in both orthopaedic and soft tissues surgeries. The author was also involved in the hospital routines, being present in the rounds and helping with the hospitalized animals, performing physical exams and drugs administration. During these two weeks, one day per week the author would go out with one of the ambulatory vets, visiting the yards and helping with the measurements of body weight and condition and collecting blood samples from the jugular vein of several ponies. In the Figure 3, some pictures from this training period are presented.

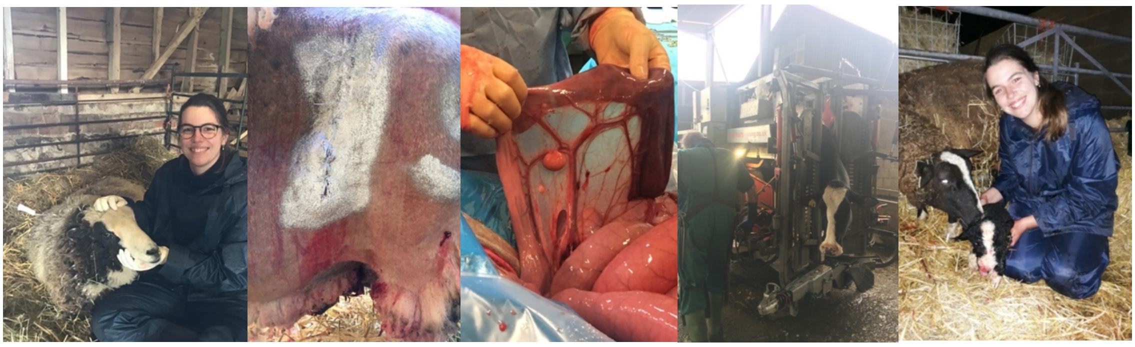

Figure 3 - Some moments the Erasmus traineeship. From left to right: Treating a sheep with pregnancy toxaemia; Suture from a caesarean in a sheep; Colic surgery in a horse; Dairy Cattle Hoof-Trimming Workshop; Newborn lamb. (Originals)

By the end of April 2019, the author applied to a volunteer program with the *Garrano* horses of *Serra d'Arga*, a project between Kyoto, Coimbra and Paris Universities. As the applicant was accepted, the month of June 2019 was spent in Viana do Castelo, living with the researchers and the other volunteers. The work consisted mostly in the collection of data such as recording videos with cameras and drones, photographing, taking notes on the horses' behaviour and collecting faecal samples. In Figure 4, some pictures from this volunteer program are presented.

Figure 4 - Some moments from the volunteer at the *Garrano* horses of *Serra D'Arga* Project. From left to right: The author with the researchers; a group of the resident *Garrano* horses, the author with the researchers and other volunteers. (Originals)

PART II – INTRODUCTION

Gastrointestinal parasitic infections have been described as one of the most important issues regarding small ruminant production. They induce major losses, causing a reduction in weight gain, poor feed utilization and consequently a decreased productivity. They can also be fatal, so their control measures mean a lot of investment (Sharma, Vatsya, & Kumar, 2016). A common way to overcome this is by using anthelmintic drugs repeatedly and indiscriminately, without regarding the real need of its use. However, as this may cause an increase of anthelmintic resistance, this option or strategy needs to be changed. Nowadays, there are three major groups of anthelmintic drugs for the treatment of small ruminants: benzimidazoles, such as fenbendazole, albendazole and mebendazole; macrocyclic lactones such as ivermectin, eprinomectin and moxidectin and imidazothiazoles such as levamisole. There are also the salicylanilides and substituted phenols group, in which closantel is included (Kaplan 2004, Bowman 2014). All over the world, the development of anthelmintic resistance against anthelmintic classes such as benzimidazoles and macrocyclic lactones have been reported. In the United States of America, Brazil, Africa, Australia, New Zealand and Europe, anthelmintic efficacy studies have been performed and gastrointestinal nematodes (GIN) resistant strains have been found (Kaplan 2004, 2012). In Europe, multiple resistance to the three major anthelmintic classes were described in sheep flocks in Scotland (Sargison et al. 2007, 2010). Bosco (2020), performed a study in 10 sheep farms in Italy, observing high anthelmintic efficacy for albendazole and ivermectin in eight farms, “normal” efficacy for macrocyclic lactones in two farms, “reduced” efficacy for albendazole in one farm and “suspected” efficacy in another farm. Furgasa (2017) describes the presence of resistant strains of GIN against albendazole and ivermectin, in a study performed in sheep in Haramaya University farms (Ethiopia). In India, Sudan (2013) reported resistance in GIN in goats in the fenbendazole treated group, as well as suspected resistance to ivermectin.

Since the situation of anthelmintic resistance in Portugal regarding small ruminants is barely known, the present study aims the evaluation of the level of parasitism in sheep and goats under different management and deworming systems.

PART III – LITERATURE REVIEW

1. SMALL RUMINANT PRODUCTION IN PORTUGAL

The small ruminant production holds an important role in the economy of Portugal, especially in the rural regions, where it helps fighting socio-economic desertification and promotes agricultural activities to the local populations. Sheep and goats have a higher capacity of obtaining nutrients in rugged lands or poor soils, compared to larger species, and the extensive management system allows not only a diversified nutrition but also helps preventing fires and soil erosion.

In Portugal, sheep are reared for meat, milk and wool purposes. Lambs originated from dairy and meat farms are sold for human consumption and the milk from dairy farms is mainly used for cheese production (for example, *Queijo Serra da Estrela, Queijo de Azeitão, Queijo de Nisa, Queijo de Serpa*). Goats are also reared for meat and milk purposes. Kids are reared for human consumption and milk used in cheese production (for example, *Queijo de Cabra Transmontano*).

In the last years, the small ruminant production has been declining, mostly due to the low profitability, mandatory sanitary rules and diminished number of young farmers interested in this field (Cabo et al. 2017).

In 2018, the total number of sheep in Portugal was 2 208 000 animals and their meat production has decreased 0.4% compared with 2017. For this situation contributed the fact that less animals were sent to slaughter, which may be justified by the increasing exportation of lambs to another countries (for example, Israel). The milk production (69.9 million liters) registered a lower volume (1.6% less than 2017). The most representative region for sheep production is Alentejo, with a total of 1 000 361 animals, followed by the Center and North regions (with 471 000 and 283 000 animals, respectively).

As for goats, the total number of animals in Portugal in 2018 was 333 000. The number of slaughtered animals presented an increase of 0.9% compared to 2017. Milk production (27.1 million litters) has improved by 8.8%, justified by the increased productivity of the dairy goats. Cheese production increased 0.8%, with a total of 84 000 tones. This evolution resulted from a higher production of goat cheese (in a total of 3 800 tones), which has raised 29.7% compared to 2017. The most representative region for goat farming is the Center, with a total of 110 000 animals, followed by Alentejo and the North regions (with 102 000 and 82 000, respectively) (Instituto Nacional de Estatística 2019).

2. COMMON GASTROINTESTINAL PARASITES IN SMALL RUMINANTS PRODUCTION

2.1. PHYLUM APICOMPLEXA

The phylum Apicomplexa belongs to the Kingdom Protozoa and its organisms are unicellular and eukaryotic, meaning they consist in a single cell with nucleus with the genetic information stored in chromosomes (Taylor et al. 2016). Most are free-living organisms, and some may move by means of a single flagellum or several flagella (that in some species can form an undulating membrane), by cilia, which consist in fine short hairs, pseudopodia, which are prolongations of the cytoplasm or simply by gliding movements (like the extracellular stages of *Eimeria*). Protozoa usually feed through pinocytosis or phagocytosis (Sancho 2009; Bowman 2014).

2.1.1. *Eimeria* spp.

Eimeria is a genus of coccidia that is transmitted mainly by faecal contamination of food and water. These organisms are intracellular parasites and destroy their host cells, causing disease mostly in young or debilitated animals (Bowman 2014).

2.1.1.1. Life cycle

The life cycle of *Eimeria* is considered direct and consists in both asexual (merogony or schizogony) and sexual (gametogony) multiplication (Taylor et al. 2016). By sexual multiplication, all the *Eimeria* spp. produce unsporulated oocysts that are expelled in the faeces. These oocysts sporulate within 1-4 days (according to temperature and humidity) and gather in their interior four sporocysts containing two sporozoites (a total of eight sporozoites per oocyst). The sporulated oocyst is the infective form of *Eimeria* and when ingested by the host the sporozoites enter the epithelial and lamina propria cells, where they continue their development. They first become a trophozoite which grows larger and become a schizont. The schizont will produce merozoites to invade new fresh cells and become second-generation schizonts. The limit of these schizogony generations for most *Eimeria* species is three. In the new cell, the merozoites develop to become a microgamont (male) or macrogamont (female). The female grows, stores nutrients and induces the hypertrophy of nucleus and cytoplasm of the host cell, becoming mature (macrogamete). The male becomes multinucleated through various nuclear divisions and each nucleus end up becoming a microgamete, a unicellular flagellated organism (Bowman 2014). This is the only phase in which the coccidias can move by themselves (Urquhart et al. 1996).

2.1.1.2. Eimeria-induced coccidiosis

Coccidiosis is the name of the disease caused by organisms known as coccidias, which includes the genus *Eimeria*. The infection occurs by the ingestion of sporulated oocysts that further develop and reproduce in epithelial cells of the intestine. The oocysts can be found in faeces of perfectly healthy animals and usually the disease only manifests in young or debilitated individuals. For this reason, the diagnosis requires the identification of the oocysts in the faeces (through sugar or salt flotation concentrates of faeces), collection of the animal/herd history, the clinical signs and, if that is the case, the necropsy (Bowman 2014). Though the similarities, sheep and goats do not share the same species of *Eimeria*. According to Sancho (2009), sheep can harbour the following species: *Eimeria ahsata, E. intricata, E. bakuensis, E. crandallis, E. weybridgensis, E. granulosa, E. faurei, E. parva, E. ovinoidalis and E. marsica.* Taylor et. al (2016) also refers the species *E. gilruthi, E. pallida* and *E. punctata* as sheep coccidias. The following table contains the *Eimeria* species, host and site, based on Taylor et. al (2016).

Table 1 - Some Eimeria species, host and site, based on Taylor et. al (2016).

Species	Sheep	Goats	Site
Eimeria ahsata	+		Small intestine
Eimeria alijevi		+	Small and large intestine
Eimeria arloingi		+	Small intestine
Eimeria aspheronica		+	Unknown
Eimeria bakuensis	+		Small intestine
Eimeria capralis		+	Unknown
Eimeria caprina		+	Small and large intestine
Eimeria caprovina		+	Unknown
Eimeria charlestoni		+	Unknown
Eimeria christenseni		+	Small intestine
Eimeria crandallis	+		Small and large intestine
Eimeria faurei	+		Small and large intestine
Eimeria gilruthi	+	+	Abomasum
Eimeria granulosa	+		Unknown
Eimeria hirci		+	Unknown
Eimeria intricata	+		Small and large intestine
Eimeria jolchijevi		+	Unknown
Eimeria marsica	+		Unknown
Eimeria masseyensis		+	Unknown
Eimeria ninakohlyakimovae		+	Small and large intestine
Eimeria ovinoidalis	+		Small and large intestine
Eimeria pallida	+	+	Unknown
Eimeria parva	+		Small and large intestine
Eimeria punctata	+	Occasionally	Unknown
Eimeria weybridgensis	+		Small intestine

2.1.1.3. Treatment and control

Eradicating coccidiosis from a herd can be a hard task due to the parasite's capacity of reproducing and the endurance of the oocysts in the environment. The best way to control this problem is through a good management and prophylactic administration of anticoccidials (Urquhart et al. 1996). The approved drugs for controlling coccidiosis in sheep are decoquinate, lasalocid and sulfaquinoxaline (Bowman 2014). According to Taylor (2003), toltrazuril and diclazuril can also be used. Usually, the most susceptible animals are the ones exposed to stress situations, especially lambs at weaning or when placed in feedlots. As for goats, the recommended drugs are decoquinate and monensin. Amprolium, if overdosed, may lead to polio encephalomalacia from thiamine deficiency. If kids are not dehydrated, sulfa drugs can also be used in order to control coccidiosis (Bowman 2014).

2.1.2. *Cryptosporidium* sp.

Cryptosporidium sp. is a small parasite that infects the epithelial cells from the gastrointestinal tract of mammals, birds, reptiles and fish. Some species can be zoonotic, which requires caution when handling potentially infected animals or contaminated waters (Bowman 2014; Taylor et al. 2016).

2.1.2.1. Life cycle

Cryptosporidium sp. has a monoxenous life cycle, similar to *Eimeria* spp. However, the sporulation occurs on the host's interior which means oocysts are immediately infective (Urquhart et al. 1996; Taylor et al. 2016). Oocysts containing four sporozoites are discharged in the faeces and are very resistant in the environment. When ingested by the susceptible host, the sporozoites are released and invade the microvillous border of gastric glands or the lower half of small intestine. It is in the microvillous border that schizogony, gametogony, fertilization and sporogony occur (Bowman 2014). Two types of oocysts can be produced: the majority has a thick wall and are passed in the faeces; the second type are thin-walled and suffer excystation internally, causing autoinfection. That is the reason for the manifestation of chronic infection in healthy hosts and lethal hyperinfection in immune-deficient hosts (Taylor et al. 2016).

2.1.2.2. Cryptosporidiosis

Cryptosporidiosis is a noteworthy disease due to the capacity of *Cryptosporidium* sp. causing debilitating diarrhoea in young and immune-deficient hosts, though inapparent infection is relatively common in most healthy hosts (Bowman 2014). Transmission is faecal-oral and may be through direct contact with infected hosts or with contaminated food, water or fomites (Chalmers and Giles 2010). According to Ulutaş and Voyvoda (2004), cryptosporidial infection rates were higher in diarrhoeic lambs than in the non-diarrhoeic ones, which shows that *Cryptosporidium* spp. are important pathogens that participate and exacerbate lamb's neonatal diarrhoea. The differences in the presence of oocysts in the environment or the *Cryptosporidium* spp. populations' infectivity may be linked with the geographical region where the animals are raised, explaining the differences in this parasites' prevalence. The table 2 comprehends the *Cryptosporidium* species, host (whenever includes sheep and/or goats) and site, based on Taylor et. al (2016).

Table 2 - Cryptosporidium species, host and site, based on Taylor et. al (2016).

Species	Host	Site
Cryptosporidium bovis	Cattle, sheep	Small intestine
Cryptosporidium hominis	Human, sheep, dugongs	Small intestine
Cryptosporidium parvum	Cattle, sheep, goat, horse, pig, deer, human	Small intestine
Cryptosporidium ubiquitum	Deer, ruminants, rodents, carnivores	Small intestine
Cryptosporidium xiaoi	Sheep, goat	Small intestine

2.1.2.3. Treatment and control

Cryptosporidium sp. has no specific treatment due to the lack of antiparasitic treatment options, vaccines and the resistance to bleach-based disinfectants (Chalmers and Giles 2010). This means that the quality of the sanitary conditions of animal husbandry are crucial, as well as appropriate grazing practices (Ulutaş and Voyvoda 2004). Halofuginone lactate can be used as prophylactic treatment to help reduce egg shedding and washing materials with hot water and detergent is also recommended (Chalmers and Giles 2010).

2.2. CLASS TREMATODA

The class Trematoda belongs to the phylum Platyhelminthes and has two main subclasses: Monogenea and Digenea. Since Monogenea comprehends mainly parasites of fish and amphibious animals, for purposes of this work only the Digenea will be contemplated. The organisms from this subclass require an intermediate host (molluscs, where asexual generations usually occur) and only parasite vertebrates (where sexual generations can be found). The adult trematodes are usually called flukes and can appear in the intestine, bile ducts, lungs, blood vessels, or other organs of the final host (Bowman 2014; Taylor et al. 2016).

2.2.1. Genus *Fasciola*

Fasciola hepatica has a worldwide distribution and can be found in the liver and bile ducts of herbivorous mammals and humans. The eggs are conducted with the bile to the intestinal lumen and to the exterior with the faeces. As illustrated on Figure 5, if in contact with water, a ciliated larva (miracidium) will develop inside of the egg. It finally hatches in two to four weeks at summer temperatures and swims to find the intermediate host which is the lymnaeid snail (*Galba truncatula*). When inside the snail, loses the cilia and forms a sporocyst. Through growth and multiple divisions, the germinal cell becomes a germinal ball and progresses into a redia. This redia has germinal balls on the inside and the ones from the second-generation rediae end up developing into cercarias. It evolves in a month or two of summer temperatures and leaves the redia through a birth pore. Passes the snail's tissues and goes into the water, where it swims until it finds a plant, encysts and forms a metacercaria (the infective stage). When ingested by a susceptible host (mammals), the cyst wall is digested, and the fluke enters the wall of the intestine and through the peritoneal space migrates to the liver. After a few weeks, young flukes enter the bile ducts and mature, becoming sexually active adult flukes. After about one month of the infection, fluke start laying eggs. The life cycle of *F. hepatica* is completed in two to three months and in sheep and cattle is responsible for mortality and high morbidity, characterized by weight loss, anaemia and hypoproteinaemia. The infection by *F. hepatica* can have different clinical signs, depending on the parasite's stage and association with *Clostridium novyi*. An acute form can occur during the liver's invasion by young flukes. The high trauma and inflammatory reaction cause severe abdominal pain and reluctance to move. If there is enough trauma, *C. novyi* multiplies and produces toxins, leading to death. The chronic form (most common) is associated with the

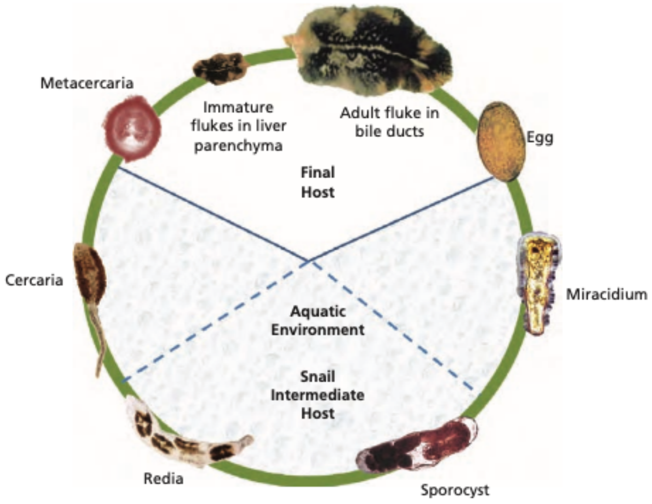

Figure 5 - Life cycle of *Fasciola hepatica.* Adapted from Taylor et al. 2016.

presence of adult flukes in the bile ducts and as mentioned before, is characterized by loss of body condition, anaemia and hypoproteinaemia. The treatment of *F. hepatica* infection can be performed through the use of clorsulon (associated or not with ivermectin), closantel, triclabendazole (for immature and mature forms), rafoxanide, oxyclozanide, nitroxynil and albendazole (Urquhart et al. 1996; Bowman 2014).

2.2.2. Genus *Dicrocoelium*

Dicrocoelium dendriticum is a parasite that can be found in the smaller bile ducts of ruminants, camelids, rabbits and other mammals (Taylor et al. 2016). In order to complete its life cycle, *D. dendriticum* needs two intermediate hosts. The first one is a snail (e.g., *Cionella lubrica*) and the second one an ant (e.g., *Formica fusca*). Even though trematodes life cycles are usually associated with water, this specie's adapted to terrestrial hosts. The embryonated eggs eliminated in faeces are ingested by the snail, in which cercariae develop into sporocysts. The cercariae leave the sporocysts through slime balls (mucus secreted by the snail around the cercariae) and will later be ingested by the ant, which feeds on this slime balls. Inside the ant, the cercariae encyst as metacercariae. The definitive host gets infected by ingesting the ant while grazing. Metacercariae are digested in the intestine and migrate to the bile duct. The infection by *D. dendriticum* may not cause evident illness in the short term, but due to the extended life of this parasite, progressive hepatic cirrhosis occurs in older sheep, leading to weight losses, poor wool production and reduced milk yielding. The recommended treatment for *D. dendriticum* is albendazole and netobimin (Urquhart et al. 1996; Bowman 2014).

2.3. CLASS CESTODA

The organisms from Cestoda, which also belongs to the Phylum Platyhelminthes, are known as tapeworms, since they have an elongated flat body, with segments (proglotids) that are wider than long, and that can extend from millimetres to several meters. The body is composed by a chain of segments (strobila) containing progressively maturing reproductive units and in the end a scolex (with the purpose to attach to the host's intestine). In each segment, the reproductive organs (both feminine and masculine) can be immature (without developed sexual organs), mature (with differentiated feminine and masculine organs) or ovigerous (only the uterus full of eggs). The cestodes' life cycle is considered indirect. Adult forms are hermaphrodites and can be found in the definitive host, while immature forms (metacestodes) can be found in the intermediate hosts (Sancho 2009; Bowman 2014).

2.3.1. Genus *Moniezia*

The genus *Moniezia* belongs to the Family Anoplocephalidae, has a worldwide distribution and comprehends the species *Moniezia benedeni* (cattle) and *M. expansa* (sheep and goats). Bowman (2014) also refers *M. caprae* as a tapeworm of goats. The life cycle begins with the ingestion by the intermediate host (free-living oribatid mite) of faeces containing *Moniezia* spp. eggs. After ingestion, the oncosphere (first generation larva) is released from the egg, perforates the intestine and develops to a cysticercoid larva (second-generation larva). The definitive host gets infected by accidentally ingesting the mite while grazing. Inside the ruminant, the arthropod is digested, and the cysticercoid larva attaches to the small intestine's wall by the scolex. It matures and, by auto fecundation, starts producing eggs (containing the onchosphere) which are later excreted individually with the faeces, in group or inside the proglotid. Adult *Moniezia* spp. are usually non-pathogenic, but mass infections can cause intestinal obstruction. The infection is more common in young animals and ovigerous proglotids can be found in faeces. The recommended treatment includes niclosamide, bunamidine or benzimidazoles such as albendazole and fenbendazole and praziquantel, being this one the most effective drug against cestodes. Pasture renewal is also recommended in order to control the presence of the intermediate host (Sancho 2009; Bowman 2014; Taylor et al. 2016).

2.4. PHYLUM NEMATODA

The parasites from the Phylum Nematoda are known as roundworms and have a large body cavity (pseudocoelom), containing fluid under pressure. The digestive tract is considered complete and sexual dimorphism is present (females are usually bigger, and males possess a differentiated posterior end, in some groups showing a copulatory bursa). The life cycles from the superfamilies Trichostrongyloidea, Strongyloidea and Ancylostomatoidea are usually direct, with the first stage larva (L1) and second stage larva (L2) as free-living organisms and the third stage larva (L3) as the infective agent. The life cycle begins with the eggs being expelled with faeces to the environment. If the environment conditions are suitable, the embryo develops to a L1, which gets out of the egg and feeds on the organic material. The L1 develops to a L2 that later, through the same nutrition, becomes a L3 (more resistant than the two first stages). The L3 migrates from faeces to the surrounding grass and is ingested by the susceptible host, where in the abomasum or intestine penetrates the gastric glands and becomes a fourth stage larva (L4). After a new development the L4 becomes a fifth stage larva (L5), originating adults. When sexually mature, female and male copulate and start producing eggs, restarting the cycle (Sancho 2009; Bowman 2014).

2.4.1. FAMILY TRICHOSTRONGYLIDAE

2.4.1.1. Genus *Haemonchus*

The species that belong to the genus *Haemonchus* can be found in the abomasum of ruminants. The buccal cavity has a lancet-like tooth which allows the obtention of blood from the mucosal vessels. These parasites are also called as "barber pole", due to the fact that females have a uterus filled with eggs in spirals around a blood-filled gut (Taylor et al. 2016). The species found in sheep and goats is *Haemochus contortus* and its life cycle, illustrated in the Figure 6, is direct, with the eggs hatching in the pasture and the L1 developing to L3 (the infective agent) in a period of five days. The infection with this parasite is characterized by anaemia, due to its hematophagous habits, that can remove from one fifth to one tenth of the circulating erythrocyte volume per day, depending on the severity of the infection. Young animals seem to be more affected, though adults submitted to stress can also suffer from fatal anaemia, when the body cannot compensate the blood loss. The most evident sign of this infection is the pale colour of the skin and mucous membranes, as well as the submandibular oedema (bottle jaw) (Urquhart et. al 1996; Bowman 2014).

Figure 6 - Life cycle of Haemonchus contortus. Adapted from Bowman 2014.

2.4.1.2. Genus *Teladorsagia*

Teladorsagia circumcincta can be found in the abomasum of sheep and goats (Bowman 2014). The life cycle is direct and usually takes about three weeks to be completed. Under certain circumstances, the ingested L3 can enter in a stage of hypobiosis before the fully development to L4 (Urquhart et. al 1996). The infection by *Teladorsagia* sp. can cause chronic abomasitis, diarrhoea, anaemia and submandibular oedema (due to the hypoproteinaemia), although animals keep a normal appetite (Bowman 2014).

2.4.1.3. Genus *Trichostrongylus*

The parasites from the genus *Trichostrongylus* can be found in the small intestine and abomasum of ruminants. The life cycle is direct, with the particularity of the L3 only losing its sheath in the abomasum (Urquhart et al. 1996). The L3, which is the infective larva, is very resistant and can endure the winter in the pasture. When spring comes, ruminants get exposed to the parasite and become infected by ingesting the larva. Most *Trichostrongylus* infections are asymptomatic, however, these parasites can cause watery diarrhoea and weight loss, especially if stressed or malnourished animals get infected with a high number of parasites (Bowman 2014).

2.4.1.4. Treatment and control of haemonchosis, teladorsagiosis and trichostrongylosis

The recommended drugs for infections by trichostrongylids are the macrocyclic lactones such as ivermectin, doramectin, eprinomectin and moxidectin; benzimidazoles such as albendazole, mebendazole and fenbendazole and imidazothiazoles (levamisole). Animals should be treated before moving to a different pasture in order to reduce its contamination and, if possible, switching the pasture every year between small ruminants and cattle, to decrease the specific parasites presence, is also a recommendable practice (Urquhart et. al 1996; Bowman 2014).

2.4.2. FAMILY COOPERIDAE

2.4.2.1. Genus *Cooperia*

The worms from genus *Cooperia* are parasites of the small intestine of ruminants and the most important species of sheep and goats are *Cooperia oncophora* and *C. curticei*. The life cycle is direct, and the infection begins with the ingestion of the L3, which exsheath and migrates to the intestine where it develops and becomes an adult (Taylor et al. 2016). It is not usual for these parasites to cause disease, nevertheless, they can cause loss of appetite and subsequently a decrease in the animal's growth (Urquhart et. al 1996). The recommended treatment is the same as for the rest of the trichostrongylids mentioned above.

2.4.3. FAMILY MOLINEIDAE

2.4.3.1. Genus *Nematodirus*

The genus *Nematodirus* can be found in the small intestine of ruminants, camelids and rabbits and the species of more importance in sheep and goats are *Nematodirus battus, N. filicollis* and *N. spathiger* (Taylor et al. 2016). The life cycle is direct, as the other trichostrongyloids, but *Nematodirus* is considered unique within these, since the development from L1 to L3 happens inside the eggshell and different species demand specific hatching requirements, usually when weather is warmer. This particularity means that usually there is a wave of disease caused by *Nematodirus* sp., in the late spring. The most affected animals are usually lambs, to whom it may cause severe diarrhea (Bowman 2014). The prophylactic treatment is recommended between May and June, when the presence of L3 in the pasture is higher. Animals should be treated, with three weeks of interval, with benzimidazoles, avermectins or moxidectin (Jackson and Coop 2007).

2.4.4. FAMILY STRONGYLIDAE

2.4.4.1. Genus *Chabertia*

It is not rare to find sheep and goats infected by a small number of *Chabertia ovina* worms*,* which is considered the largest nematode found in the colon of ruminants. The life cycle is direct: the infective larva (L3) is ingested and penetrates the mucosa of the small intestine (and sometimes that of the caecum and colon) and develops to a L4. The L4 emerges and travels to the caecum, where it becomes a L5. After this development, the young adults travel to the colon and start laying eggs, that will posteriorly be eliminated with the faeces and hatch on the ground, releasing the L1 that develops till the infective stage (Taylor et. al 2014). The L3 can survive the winter and L4 can enter in a stage of hypobiosis, where it encapsulates in the large intestine walls and emerges when temperatures are higher. The mature adults feed on the intestinal mucosa and may cause intestinal bleeding and diarrhoea, leading to anaemia and weight loss (Urquhart et. al 1996). The recommended treatment is the same as for the rest of the trichostrongylids mentioned above.

2.4.4.2. Genus *Oesophagostomum*

The genus *Oesophagostomum* has a worldwide distribution and is of high concern in the tropical and subtropical regions (Urquhart et. al 1996). The worms from this genus can be found in the caecum and colon and the most important species in sheep and goats are *Oesophagostomum columbianum, O. venulosum, O. asperum* and *O. multifoliatum* (Taylor et al. 2016). The life cycle begins with the ingestion of the L3 that later penetrates the mucosa of the intestine (where it can form a nodule) and develop to a L4. The L4 emerges and migrates to caecum and colon and becomes a L5 (adult form). In some temperate regions, L4 can enter in a stage of hypobiosis to survive the winter, even though L3 can survive the winter in the pasture. Acute inflammation may occur due to reactions to the L3 nodules, that later can caseate and calcify and interfere with intestinal motility. They can cause severe and fetid diarrhoea, leading to weakness, anaemia and weight loss (Urquhart et. al 1996; Bowman 2014). The recommended treatment is the same as for the rest of the trichostrongylids mentioned above.

2.4.5. FAMILY ANCYLOSTOMATIDAE

2.4.5.1. Genus *Bunostomum*

Bunostomum sp. is one of the largest nematodes of the small intestine of ruminants and the most important species in sheep and goats is *Bunostomum trigonochepalum*. The life cycle usually begins with the ingestion of the infective larva (L3) which travels to the small intestine, where it develops and becomes an adult hookworm. The infection can also be percutaneous, where the larva penetrates the skin and migrates through the tissues to the lung, becomes a L4 and re-enters the gastrointestinal tract. The recommended treatment in ruminants is avermectins, moxidectin, levamisole and benzimidazoles (Bowman 2014; Taylor et al. 2016).

2.4.6. FAMILY TRICHURIDAE

2.4.6.1. Genus *Trichuris*

Adults of the genus *Trichuris* are found in the caecum and colon of mammals. The life cycle starts with eggs (lemon-shaped with a plug in each pole) being expelled with faeces (Sancho 2009). Inside the egg, the infective first-stage larva develops and hatches after being ingested by a susceptible host, where it continues the development in the epithelium of the intestine. Ruminants are frequently infected, but asymptomatic. Only young animals usually show disease if massively infected. The recommended treatment for sheep is an ivermectin drench (Bowman 2014; Taylor et. al 2016).

2.4.7. FAMILY STRONGYLOIDIDAE

2.4.7.1. Genus *Strongyloides*

The parasites from the genus *Strongyloides* belong to the order Rhabditida and are characterized by an oesophagus with a rhabditiform shape. It is only the female that is parasitic and can be found in the small intestine of young animals (Taylor et al. 2016). The infection by *Strongyloides papillosus* is usually moderate and asymptomatic, but disease can occur in neonates or immunodeficient animals, or if a significant number of parasites is present (Bowman 2014). According to Pienaar et al. (1999), severe disease can also occur in goats with a light infection by *Strongyloides* sp. The transmission can be transmammary, cutaneous or by the ingestion of the L3. Treatment for strongyloidiasis is rarely necessary, but the recommended drugs are the benzimidazoles, ivermectin or moxidectin (Bowman 2014, Urquhart et. al 1996).

3. COMMON ANTHELMINTICS IN SMALL RUMINANT PRODUCTION

3.1. BENZIMIDAZOLES

This drug class is used against fungi, protozoa, and helminths, being widely used in human and veterinary medicine. The prototype of the first generation of benzimidazoles (BZDs) was thiabendazole, which has provided a major breakthrough in the treatment of parasitic diseases, leading to the development of this anthelminthic class (Jaeger and Carvalho-Costa 2017). Since benzimidazoles have a higher affinity for nematode tubulin than mammalian tubulin, they offer selective activity against parasites, resulting in a low mammalian toxicity (Bowman 2014). Benzimidazoles also have a broad spectrum of activity with high efficacy, are easy to administrate and have a low cost of production which resulted in an unwisely usage in livestock parasitic infections in the years following its development. Due to this fact, some natural selection of parasite genotype has followed, conferring resistance to this type of drugs, which is aggravated by a cross-resistance phenomenon in BZDs (Jaeger and Carvalho-Costa 2017).

3.1.1. FENBENDAZOLE

3.1.1.1. Indications

Fenbendazole is an old benzimidazole that works effectively as a broad spectrum anthelmintic. It is often used as a treatment against numerous intestinal helminthiasis of animals. This drug's effectiveness is shown at the doses of 7.5-10 mg/kg against nematodes, 15 mg/kg against Protostrongylidae lungworms, and 100 mg/kg against infections of *Fasciola* spp. and *Dicrocoelium dendriticum* infections in sheep, administered orally (Arkhipov et al. 2019).

3.1.1.2. Pharmacokinetics

According to Plumb (2018), fenbendazole is only absorbed after oral administration and is metabolized to the active compound, oxfendazole (sulfoxide) and the sulfone. In sheep, almost half of a dose of this drug is excreted unchanged in the faeces and less than 1% in the urine, while the rest is metabolized.

3.1.1.3. Contraindications

Although this anthelmintic is considered safe and non-toxic, some cases of diarrhoea and vomiting have been registered (Arkhipov et al. 2019). This drug is not approved for use in horses destined for food purposes (Plumb 2018).

3.1.2. ALBENDAZOLE

3.1.2.1. Indications

Albendazole, which is structurally related to mebendazole, is an anthelminthic benzimidazole indicated against nematodes, cestodes and protozoa infections. Its dosage is 7.5 mg/kg orally against susceptible parasites in sheep (Plumb 2018).

3.1.2.2. Pharmacokinetics

Albendazole is reckoned to be the best orally absorbed benzimidazole, since approximately 47% of an oral dose was recovered in the urine as metabolites after a 9-day treatment. The active metabolites reach its peak plasma concentration 20 hours after administration (Plumb 2018). Pinkrah (2017) described that albendazole is metabolized by the liver, and by binding to tubulins, this anthelmintic reduces the energy reserves of the parasites, which end up dying and getting expelled in the faeces. It is recognized that it can disrupt cell division in the parasite, leading to a reduction in egg production.

3.1.2.3. Contraindications

According to Plumb (2018), this drug has been associated with embryotoxic effects in sheep when given early in pregnancy. It may also cause gastrointestinal and hepatic dysfunction.

3.1.3. MEBENDAZOLE

3.1.3.1. Indications

Mebendazole (MBZ) is a broad-spectrum anthelmintic used in human and veterinary medicine for removal and control of liver flukes, tapeworms, stomach worms, intestinal worms and lungworms. It is absorbed from the gastrointestinal tract (Galtier et al. 1994). The recommended dosage for the control of nematodes in sheep is 12.5mg/kg, orally (Kelly et al. 1975).

3.1.3.2. Pharmacokinetics

Mebendazole causes degenerative changes in the tegument and intestinal cells of the parasite, by binding to the colchicine-sensitive site of tubulin and consequently inhibiting its polymerization or assembly into microtubules. The loss of the cytoplasmic microtubules leads to a lower uptake of glucose by the larval and adult stages of the susceptible parasites and depletes their glycogen stores. Degenerative changes in the endoplasmic reticulum, in the mitochondria of the germinal layer, and the subsequent release of lysosomes result in decreased production of Adenosine Triphosphate (ATP). Due to diminished energy production, the parasite is weakened and eventually dies (Wishart et al. 2018).

3.1.3.3. Contraindications

Mebendazole is similar to albendazole and its usage should be avoided during pregnancy, since these agents are embryotoxic. It may also cause gastrointestinal upset (Plumb 2018).

3.2. SALICYLANILIDES AND SUBSTITUTED PHENOLS

According to Taylor et al. (2016), the salicylanilides and substituted phenols are used efficiently as flukicides for cattle and sheep being highly effective against adult and immature flukes from *Fasciola hepatica.* Some of these drugs are very effective against blood-sucking nematodes.

3.2.1. CLOSANTEL

3.2.1.1. Indications

This salicylanilide is a broad-spectrum anthelminthic used in cattle and small ruminants, being efficient against several bloodsucking nematodes, arthropods and trematodes. It can be administered orally and via intramuscular route. The recommended dosage is 10 mg/kg, orally (Ecco et al. 2006).

3.2.1.2. Pharmacokinetics

According to Ecco et al. (2006), high concentrations of this drug can be found in the plasma one day after administration although tissue levels are usually lower. Closantel is a poorly metabolized compound since 80% of the dose is excreted by faeces and less than 0.5% by urine. The primary route of metabolism leads to monoiodoclosantel metabolites. Similarly, to other salicylanilides, 99% of closantel is extensively bound to plasma proteins (mainly albumin), which lengthens drug levels in plasma and limits its distribution to tissue (Swan 1999). After the administration of closantel to lactating dairy cows, a parallel decline of closantel concentrations in plasma and milk have been shown with a plasma/milk concentrations ratio in the order of 50/1. From available data in dairy cows, it can be concluded that residues of closantel persist in milk and that the parent compound is the main residue in this food commodity (Iezzi et al. 2014).

3.2.1.3. Contraindications

Accidental overdose, which can result in poisoning, has been described in goats, cattle, sheep and dogs. Clinical signs include nervous disturbances one to two days after administration of the drug. Ecco et al. in 2006 also described the spontaneous (iatrogenic) poisoning of kids with closantel, and the clinical (e.g. blindness) and pathological findings associated with the toxicosis.

3.3. MACROCYCLIC LACTONES

Macrocyclic lactones (MLs), or macrolides, are potent lipophilic parasiticides widely used for control of internal and external parasites in domestic animals and livestock (Bassissi et al. 2004). MLs consist of avermectins and milbemycins and are considered to be the most effective parasiticides, considering they have a very low toxicity. Due to this fact, MLs are widely used and the parasites' resistance to these drugs has increased (Bowman 2014). We will further describe Ivermectin, as it is the most popular ML and Eprinomectin, as this was the only ML used in our research.

3.3.1. IVERMECTIN

3.3.1.1. Indications

Ivermectin (IVM) is semisynthetic derivative of Avermectin (AVM) and has activity against a wide range of endoparasites (namely nematodes) and ectoparasites in livestock (cattle, sheep, goats, horses, and pigs), pets, wild animals and fish, being considered a broad-spectrum endectocide (Bowman 2014). Due to its low toxicity, high efficiency and safety, this compound is also used as an antiparasitic agent in humans. Its recommended usage is 0.2 mg/kg, administered subcutaneously (González et al. 2006). There are several IVM sheep drenches available in the market and these are also used as extra label in goats, occasionally dosed 1.5x to 2x the label dose in sheep. In these cases, withdrawal period must also be superior (Bowman 2014).

3.3.1.2. Pharmacokinetics

According to Plumb (2018), IVM enhances the release of gamma amino butyric acid (GABA) at presynaptic neurons. GABA acts as an inhibitory neurotransmitter and blocks the post-synaptic stimulation of the adjacent neuron in nematodes or the muscle fibre in arthropods causing paralysis of the parasite and eventually death. As liver flukes and tapeworms do not use GABA as a peripheral nerve transmitter, IVM is ineffective against these parasites. The IVM is excreted in faeces as active drug and is toxic for aquatic animals and dung-feeding insects (Bowman 2014).

3.3.1.3. Contraindications

Ivermectin can induce serious adverse effects by killing the larvae when they are in vital areas and may also cause discomfort or transient swelling at the injection site. Administering a maximum of 10 ml at the injection site can help minimize these effects. The injectable products for use in cattle should only be given subcutaneously (SC). In cattle, toxic effects generally do not appear until dosages of 30x the recommended one are injected. At dosages of 8 mg/kg, symptoms of ataxia, listless and occasionally death were observed in cattle (Plumb 2018).

3.3.2. EPRINOMECTIN

3.3.2.1. Indications

According to Arsenopoulos et al. (2019), Eprinomectin (EPN) is a modern ML with a high efficacy against gastrointestinal roundworms, lungworms and some ectoparasites in cattle. In order to overcome the shortfall of anthelmintic drugs with zero withdrawal period in milk, the off-label use of EPN was adopted by some dairy sheep farmers and nowadays its use has been officially registered in dairy sheep, being a promising anthelmintic drug with easy and welfare friendly administration.

3.3.2.2. Pharmacokinetics

Eprinomectin binds selectively to glutamate-gated chloride ion channels which occur in invertebrate nerve and muscle cells. This leads to an increase in the permeability of cell membrane to chloride ions, leading to paralysis and death of the susceptible parasite. Like ivermectin, eprinomectin also enhances the release of GABA at presynaptic neurons. These compounds are generally not toxic to mammals as they do not have glutamate-gated chloride channels and these complexes do not readily cross the blood-brain barrier (Plumb 2018).

3.3.2.3. Contraindications

Eprinomectin has a broad safety margin and zero milk withdrawal period when compared to other anthelmintic drugs. When given up to 5x the recommended dosage, calves have not shown any signs of adverse effects (Arsenopoulos et al. 2019).

4. RESISTANCE TO ANTHELMINTICS IN SMALL RUMINANTS

Helminthic infections are an important cause for reduction in both productive and reproductive performance in small ruminants all over the world (Sharma et al. 2016). In order to control this issue, farmers have been administering anthelmintic (AH) drugs to their animals at frequent intervals, most of the times without concerning the correct principles to apply, such as the timing and frequency of deworming or which molecules to use. This fact has led to an increased development of multi drug resistant (MDR) populations of gastrointestinal nematodes (Crook et. al 2016). The first reports of anthelmintic resistance (AHR) date to the late 1950s and early 1960s, with some *Haemonchus contortus* in sheep resisting the treatment with phenothiazine (Drudge et al. 1957 cited by Kaplan 2004). Though the prevalence of resistant nematodes is lower in Europe, resistance to all three major AH classes has been described in Scotland and Switzerland (Kaplan 2012). In table 3, some AH drugs and the first published report of its resistance in sheep is presented.

One of the main problems resides in the fact that sheep and goats differ in many ways, namely because goats have a higher metabolic rate and usually require higher dose rates for drugs. Most of the anthelmintics used in goats have not been licensed for this species and correct dosage rates are barely known (Várady et. al 2011). Goats usually require 1.5-2 times the recommended dosage for sheep, which means that since they are frequently treated together with sheep or according to dosage for that specie, goats are constantly being underdosed, promoting the selection of resistant strains (Papadopoulos 2008).

Table 3 - Year of approval of anthelmintic drugs and first published report of its resistance in sheep. Adapted from Kaplan 2004.

Drug	Host	Year of initial drug approval	First published report of resistance
Benzimidazoles			
Thiabendazole	Sheep	1961	1964
Imidothiazoles			
Levamisole	Sheep	1970	1979
Macrocyclic Lactones			
Ivermectin	Sheep	1981	1988
Moxidectin	Sheep	1991	1995

Since Multi Drug Resistant worms have been appearing more frequently in small ruminants, new approaches to deworming and controlling these parasites have been developing. According to Van Wyk (2001), refugia, which includes the worm population in non-dewormed animals, the free-living stages on the environment (for example, in the pasture) and the parasitic stages that are not affected by the treatment, should be considered the most important factor in the parasite management of a flock. As illustrated in the Figure 7, this means that when the farmer decides to treat the flock (usually prior to moving to a new pasture), they should keep some animals untreated, so susceptible parasites can survive and reproduce with resistant worms, propagating susceptible genes and delaying the onset of AHR. Papadopoulos (2008) reports that besides refugia, additional measures to control AHR must be taken: apply the FAMACHA© system, supplementation with protein in order to increase immunity and resilience to parasitism,

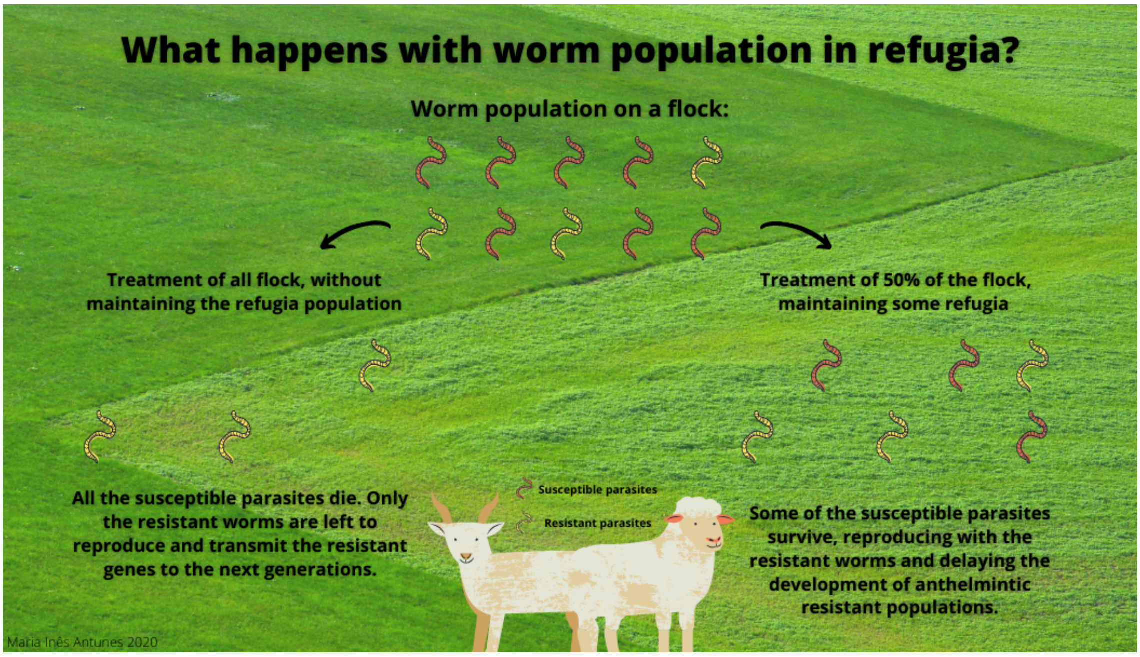

Figure 7 - Scheme explaining how to keep refugia population. (Original)

the introduction of nematophagous fungal spores, formulation of vaccines, use of plants with anthelmintic properties and selective breeding of animals that tolerate nematodes.

Though anthelmintic resistance in cattle and horses is not at the same level as the small ruminants, there is some growing evidence of the increased development of MDR nematodes in these species (Kaplan 2004). There are some studies regarding the emergent appearance of anthelmintic resistance in Portugal. One of the studies (Madeira de Carvalho et al. 2003) addressed this subject by calculating anthelmintic efficacy in horses using Faecal Egg Count Reduction Test (FECRT). A population of parasites was considered resistant when the percentage of reduction was ≤ 90% and doubtful between 90-95%. As result of this study, *in vivo* anthelmintic resistance for Pyrantel pamoate oral paste and *in vitro* anthelmintic resistance for Benzimidazole regarding horse strongyles was recorded for the first time in Portugal. The situation of anthelmintic resistance in Portugal regarding small ruminants is barely known, which accentuates the need to perform studies in order to acknowledge the current paradigm.

PART IV - EXPERIMENTAL STUDY: PREVALENCE OF GASTROINTESTINAL PARASITES AND ANTHELMINTIC EFFICACY IN SHEEP AND GOATS UNDER DIFFERENT MANAGEMENT AND DEWORMING SYSTEMS IN THE REGION OF LISBON AND TAGUS VALLEY, PORTUGAL

1. OBJECTIVES

The main objectives of this study were to:

a) Characterize the presence and level of parasitism in small ruminant farms located in the region of Lisbon and Tagus Valley in order to evaluate the current situation.
b) Assess the anthelmintic efficacy in the process of deworming with two individual molecules: eprinomectin and fenbendazole, and an association of two molecules: closantel and mebendazole. The farms used in this study were selected by the type of production (intensive (stabled) and extensive (pasture)), the animal species (sheep and/or goats) and according to the availability of the farmers.

2. MATERIAL AND METHODS

1.1. Geographic location of the farms and time of the visits

This study was performed between September 2018 and January 2020 and the small ruminants' farms were located in the district of Santarém (Farms B, BO, L, M, P Q and R), one farm in Lisbon (F) and one in Setúbal (C). The dairy goats' farms (B and C) and the lambs' feedlot (BO) were visited between September 2018 and January 2019, and the faecal samples were collected throughout this period. The samples from the sheep and goats from the farm F were collected in September 2018. The remaining farms (R, M, L, Q, P) were visited between May 2019 and January 2020. The experimental study for the anthelmintic efficacy was performed in January 2019 in the farms R and M in August 2019 and in the farms L and Q was performed in January 2020.

1.2. Anthelmintics used in the experimental study

The anthelmintic chosen for the dairy goats in farm (C) was eprinomectin (Eprinex® Pour-On) and was administered pour-on, at a dosage of 0,5 mg/kg bodyweight, due to the fact that no meat and milk withdrawal is required. In the sheep flocks from the farms R and M the anthelmintic used was mebendazole in combination with closantel (Seponver® Plus), administered orally, at a dosage of 15 mg/kg bodyweight, since it was the anthelmintic used by the farm veterinarians. In the mixed farms (both with sheep and goats) L and Q, the chosen molecule was fenbendazole

(Panacur® 2,5%), administered orally, at a dosage of 5 mg/kg bodyweight, in order to assess its anthelmintic efficacy.

1.3. Management and prior deworming system

The animals from the dairy goats' farms, which consist of an intensive type of production, were not regularly dewormed, which means that by the time of the study, they had not been dewormed in the previous year. The animals from the extensive production farms, were usually dewormed with Seponver® Plus, every six months, so the time of the study was selected in accordance with the subsequent required deworming. Before the usage of Seponver® Plus, these flocks were being dewormed with the same frequency (every six months) with ivermectin (Oramec®) and triclabendazole (Fasinex® 5%) for a period of three years; before that, the used anthelmintic was netobimin (Hapasil®).

1.4. Target animals

The target animals were sheep and goats, with different types of management and breeds. The dairy goats were mostly from the breeds Sannen and Alpine, and a crossbreed between both. The sheep and goats from the extensive production system were crossbreds, essentially for meat purposes. The animals selected for the study had ages ranging between a minimum of six months and a maximum of nine years. Each sample corresponded to an animal who was identified and analysed individually. Four farms belonged to small holders, having a total number of animals below 20. The dairy goats' farms (B and C) had around 200 and 100 animals respectively, and in farm C, 10 animals were selected to participate in the anthelmintic efficacy study. Table 4 shows the distribution of samples collected by farm in order to perform the anthelmintic efficacy study, in which R, M, L and Q are the small holders farms (extensive system) and C the dairy goats' farm (intensive system).

Table 4 - Number of animals submitted to the anthelmintic efficacy study and number of fecal samples collected.

Farm	Study Group	Control Group	Total of animals	Total of samples
R	11	9	20	40
M	8	6	14	28
L	13	9	22	44
Q	8	6	14	28
C	5	5	10	20
Total	45	35	80	160

1.5. Protocol

For the anthelmintic efficacy experimental study, on the day 0 (T0), faecal samples were collected from all of the animals from both study and control groups, and the individuals from the study group were dewormed with the anthelmintic previously chosen. Faecal sample collection from all the animals was repeated on day 15 (T15) and the control group was dewormed (Madeira de Carvalho et al. 2003, Várady et al. 2011). In order to assess the level of egg shedding from the remaining farms, some samples were collected from the farms F, BO, B, P and some more from farm C. The level of egg shedding from the farms where the experimental study was undertaken (R, M, L, Q and C) was determined according to the results from the first sample collection on day 0 (T0). Table 5 shows the number of faecal samples collected in these farms. For the present study, a total of 285 samples were collected, identified and posteriorly analysed in the laboratory.

Table 5 - Number of fecal samples collected in order to assess the level of egg shedding.

Farm	Total
F	10
BO	10
B	57
P	20
C	38
R	20
M	14
L	22
Q	14
Total	205

1.6. Sample collection and storage

The sample collection was performed directly from the animals' rectum, as represented in figure 8, using plastic bags, gloves and lubricant when needed. When the rectal palpation was not feasible, fresh samples were collected from the ground soon after defecation. The samples were identified, stored in a cooling container and transported to the laboratory's refrigerator, where they were maintained at a temperature of 4ºC and posteriorly analysed within the following 48 hours.

Figure 8 - Collecting faeces directly from a sheep's rectum. (Original)

1.7. Laboratory work

The faecal samples were analysed in the Parasitology and Parasitic Diseases laboratory of the Centre for Interdisciplinary Research in Animal Health (CIISA-FMV-UL). The egg shedding level was determined by counting the eggs per gram (EPG) through the McMaster slide chamber technique and faecal cultures were performed in order to assess the most prevalent/abundant genus of gastrointestinal strongyles (Madeira de Carvalho 2002, Cringoli et al. 2010, Van Wyk & Mayhew 2013). The laboratory work was performed during the field work period, meaning it was developed between September 2018 and January 2020, every time a sample analysis was required.

1.7.1. McMaster slide chamber technique

The McMaster slide chamber technique performed for this study consisted in the homogenisation of 2 grams of faeces in 28 mL of a saturated sugar solution. The solution obtained was filtered through a metallic filter, then homogenised again and collected with a Pasteur pipette to posteriorly fill the McMaster slide chambers. Five minutes post filling the chambers, the slides were ready to be observed at the microscope. The total egg/oocyst count was performed in the two chambers, by multiplying the number of eggs/oocysts found by 50 (Madeira de Carvalho, 2002).

1.7.2. Faecal cultures

Faecal cultures were performed by weighting 50-70 grams of homogenised and moistened faeces in a cup of plastic and covered with punctured aluminium foil. The cups were placed in an incubator for about 14 days at a temperature of 26-28ºC. After the incubation time, the cups were filled with water and inverted in a Petri dish, filling the surrounding with about 10 mL of water. After 24h, the remaining water was collected with a Pasteur pipette and transferred to 10 mL centrifuge tubes. The tubes were centrifuged at 1500 rpm for about 3 minutes and the isolated larvae were observed at the microscope. The identification of the L3 larvae was made according to Van Wyk & Mayhew (2013).

1.8. Data analysis

Data was stored, organized and statistically analysed in Microsoft® Office Excel for Mac version 16.33 and R® version 3.6.2. The effectiveness of the anthelmintics was evaluated in an Excel spreadsheet created by Angus Cameron (AusVet Animal Health Services for the University of Sidney). These calculations are based on those of the RESO FECRT analysis program Version 2, by Leo Wursthorn and Paul Martin of CSIRO, Animal Health Research Laboratory. The calculations are based on those published in 1989 by CSIRO 'Anthelmintic Resistance': Report of the Working Party for the Animal Health Committee of the SCA. The descriptive statistics of mean and range, calculated in Excel, were used for data pertaining to small ruminants' faecal samples. The overall occurrence of gastrointestinal parasites was calculated by dividing the number of positive faecal samples by the total number of samples examined. A generalized linear model was employed in order to assess if there was a significant difference in the presence of GI parasites between sheep and goats and the normal distribution of samples was verified by the *Shapiro-Wilk* test on R®. Results were considered as statistically significant when *p*-value was less than 0.05. Since there is limited data for the classification of the level of parasitism for goats, the evaluation for the two species was made according to the classification for the level of parasitism in sheep, proposed by Hansen & Perry (1990), cited by Mederos et al. (2010): in general, if the number of EPG is less than 500, the infection level is considered low; if it is between 500 and 1500 is considered moderate, and above 1500 EPG is considered a high level of infection. The anthelmintic efficacy was evaluated by the Faecal Egg Count Reduction Test (FECRT) according to Coles et al. (1992, 2006), where the percentage reduction in egg counting is calculated by the formula:

$$100\,(1 - X_{\mathrm{T}}/X_{\mathrm{C}})^{1}$$

Following this calculation, and according to World Association for the Advancement of Veterinary Parasitology (WAAVP), if the percentage reduction in egg count is less than 95% and the lower limits of 95% confidence level is less than 90%, it means that resistance is present.

3. RESULTS

3.1. Presence of gastrointestinal parasites

Starting with the presence of gastrointestinal (GI) parasites, in the 97 sheep and 108 goat faecal samples examined, 183 were positive for GI parasites on the McMaster technique, giving an overall presence of GI parasites in the 9 farms of 89.27% (95% confidence interval (CI) 79% to 89%). There was a significant difference in the occurrence of GI parasites between sheep (76.85%) and goats (92.78%) (*p*=0.001). Table 6 shows the occurrence of GI parasites in sheep and goats and the proportion and

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[1] X_{T} = Arithmetic mean of the study group egg count at T15; X_{C} = Arithmetic mean of the control group egg count at T15.

type of gastrointestinal parasite egg/oocyst according to farm is shown in Table 7. All farms were found to have positive animals to at least one type of GI parasite, which pronounces a widespread infection with GI parasites on the 9 farms analysed in the present study.

Table 6 - Occurrence and types of sheep and goat gastrointestinal parasite eggs/oocysts in faecal samples.

Specie	No. Examined	Positive	Types of parasite egg/oocyst observed			
			Strongyle type	*Strongyloides papillosus*	*Moniezia expansa*	*Eimeria* spp.
Sheep	97 (47.32%)	83 (76.85%)	82 (84.54%)	3 (3.09%)	2 (2.06%)	12 (12.37%)
Goat	108 (52.68%)	90 (92.78%)	19 (17.59%)	63 (58.33%)	-	55 (50.93%)
Total	205 (100%)	183 (89.27%)	101 (49.27%)	66 (32.20%)	2 (0.98%)	67 (32.68%)
P-value	-	0.001	0	0	0.988	0

Table 7 - Number of small ruminants sampled from each farm and proportion of faecal samples positive for gastrointestinal parasites.

Farm	No. Examined	Positive	Types of parasite egg/oocyst observed			
			Strongyle type	*Strongyloides papillosus*	*Moniezia expansa*	*Eimeria* spp.
B	57	40 (70.18%)	4 (7.02%)	31 (54.39)	-	33 (57.89%)
BO	10	8 (80%)	1 (10%)	2 (20%)	2 (20%)	6 (60%)
C	38	34 (89.47%)	6 (15.79%)	32 (84.21%)	-	22 (57.89%)
F	10	1 (10%)	-	1 (10%)	-	-
L	22	22 (100%)	21 (95.25%)	1 (4.55%)	-	-
M	14	14 (100%)	14 (100%)	-	-	1 (7.14%)
P	20	20 (100%)	20 (100%)	-	-	2 (10%)
Q	14	14 (100%)	14 (100%)	-	-	-
R	20	20 (100%)	20 (100%)	-	-	3 (15%)

The most frequent eggs encountered were from strongyle type (88.88%), followed by oocysts of *Eimeria* spp. (66.66%), eggs from *Strongyloides papillosus* (55.55%) and eggs from *Moniezia expansa* (11.11%). The Table 8 shows the proportion of infection by parasite on the 9 farms where faecal samples were collected.

Table 8 - Rate of infection by type of parasite on the farms.

Parasite	Faecal positive farms	Faecal negative farms	Occurrence (%)
Strongyle type	8	1	88.88
Strongyloides papillosus	5	4	55.55
Moniezia expansa	1	8	11.11
***Eimeria* spp.**	6	3	66.66

The Table 9 shows the mean counts and range of faecal egg counts in eggs per gram (EPG) for gastrointestinal helminths and oocyst counts in oocysts per gram (OPG) for coccidia in sheep and goats. The mean EPG count in sheep was 2029, with animals with counts between 0 and 21,300. Regarding the OPG counts, mean was 47 with animals with counts between 0 and 2500. As for goats, mean EPG count was 606, with animals with counts between 0 and 5850 and mean OPG was 109, with animals with counts between 0 and 850.

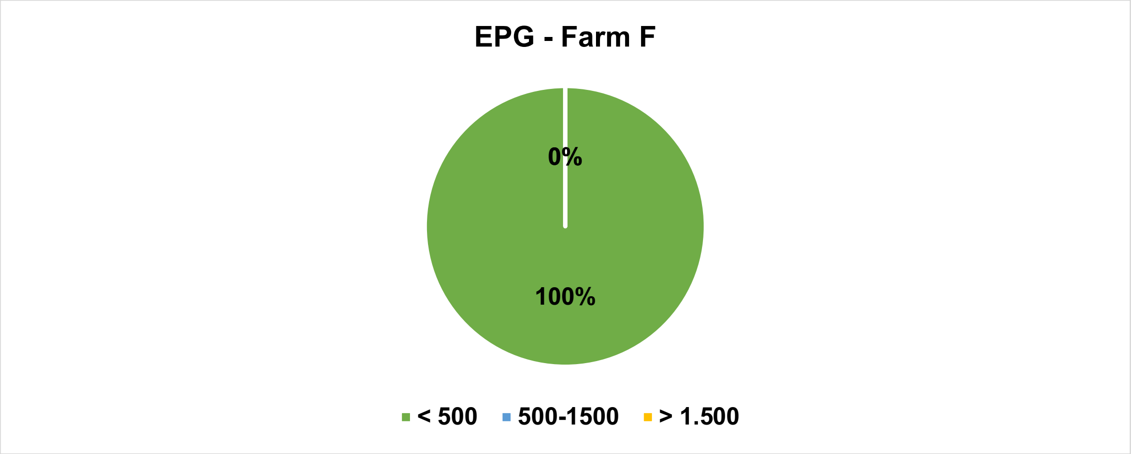
Table 9 - Faecal egg and oocyst count (EPG/OPG) from sheep and goats from the 9 farms.

Animal	EPG	OPG
Sheep		
Mean	2028.87	47.42
Range	0-21,300	0-2,500
Goats		
Mean	606.02	109.26
Range	0-5,850	0-850

3.2. Level of parasitism

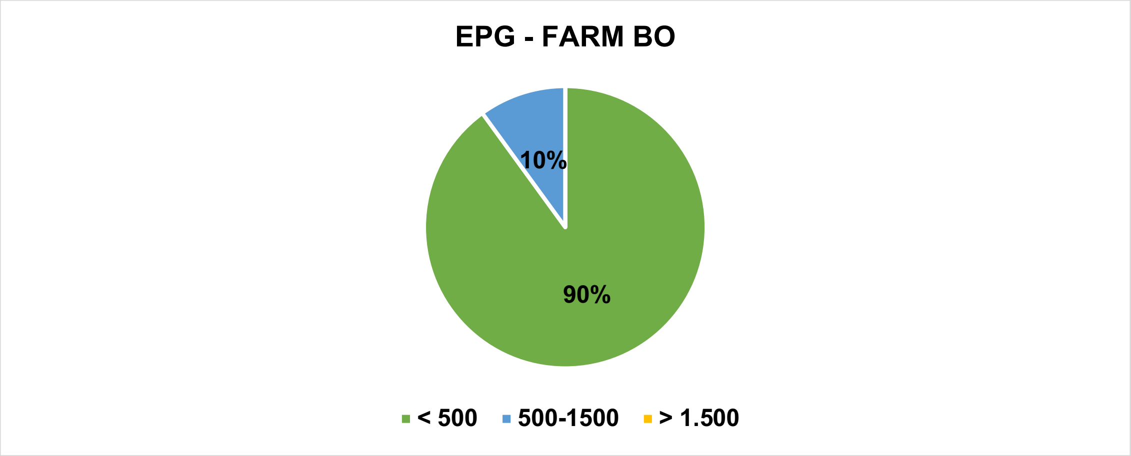
Regarding the classification of the level of parasitism based on the EPG counts, 5 farms (F, BO, C, B, L) had more than 50% of the animals ranked in the low level of infection category (EPG below 500). Farms M, P and Q had all 3 types of classifications with similar proportions. Farm R had 75% of the animals ranked in the high level of infection category, with more than 1500 EPG counts. In the Graphic 1, for Farm F (N=10), the level of parasitism was considered low, with 100% (10) of the animals with an EPG count below 500.

Graphic 1 - Level of Parasitism in the Farm F (n=10)



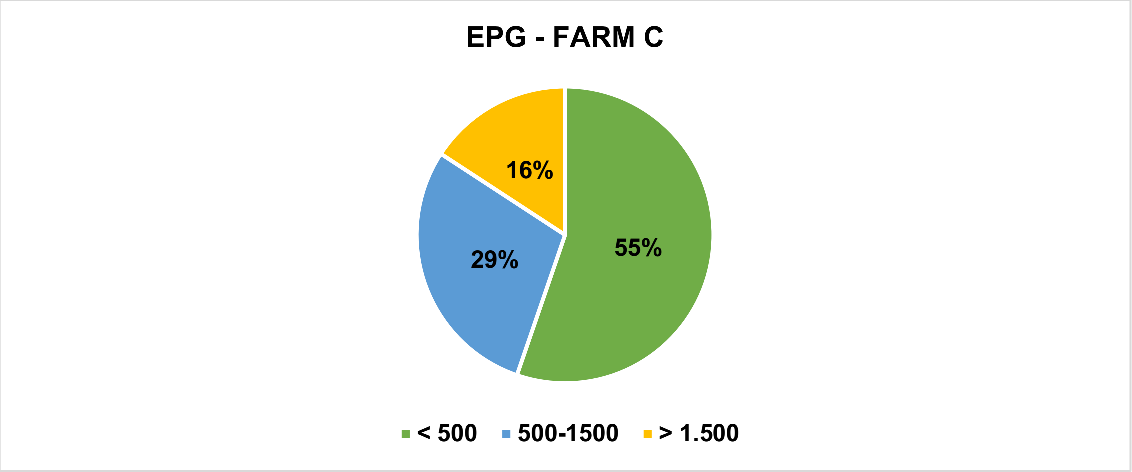
In Graphic 2, for Farm BO (n=10), the level of parasitism was considered low for 90% (9) of the animals and moderate for 10% (1).

Graphic 2 - Level of Parasitism in the Farm BO (n=10)



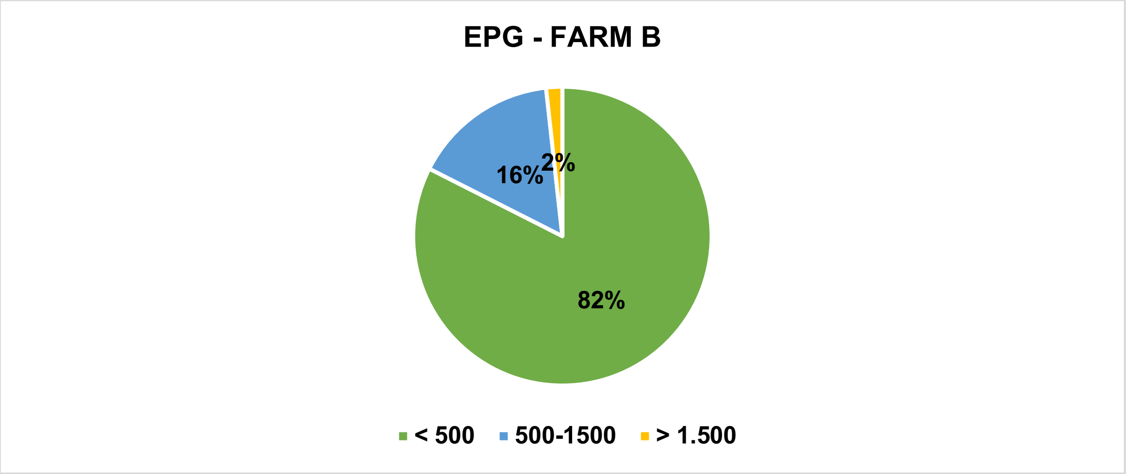
In Graphic 3, for Farm C (n=38), the level of parasitism was considered low for 55% (21) of the animals, moderate for 29% (11) and high for 16% (6) of the animals.

Graphic 3 - Level of Parasitism in the Farm C (n=38)



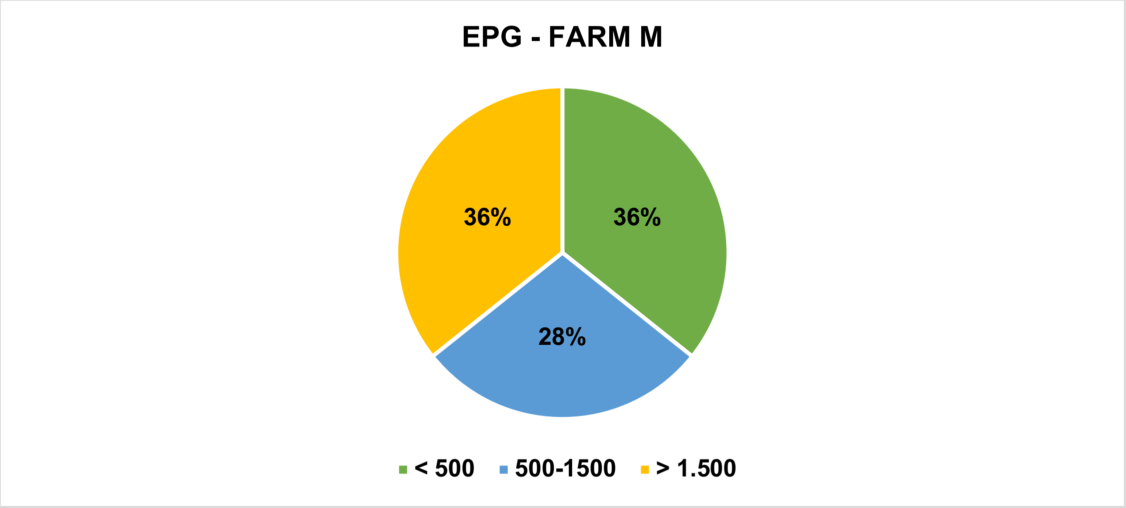
In Graphic 4, for Farm B (n=57), the level of parasitism was considered low for 82% (47) of the animals, moderate for 16% (9) and high for 2% (1) of the animals.

Graphic 4 - Level of Parasitism in the Farm B (n=57)



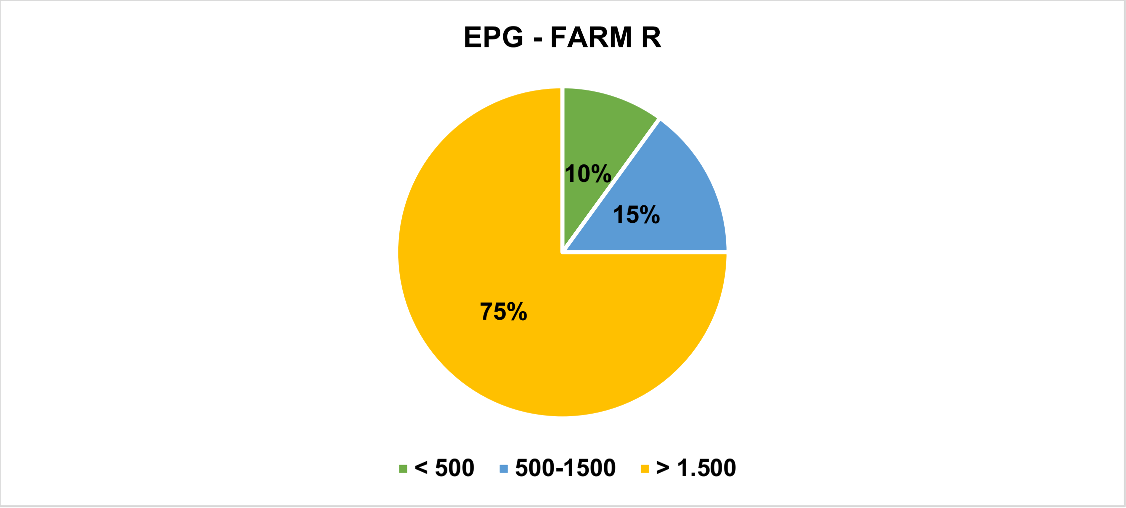
In Graphic 5, for Farm M (n=14), the level of parasitism was considered low for 36% (5) of the animals, moderate for 28% (4) and high for 36% (5) of the animals.

Graphic 5 - Level of Parasitism in the Farm M (n=14)



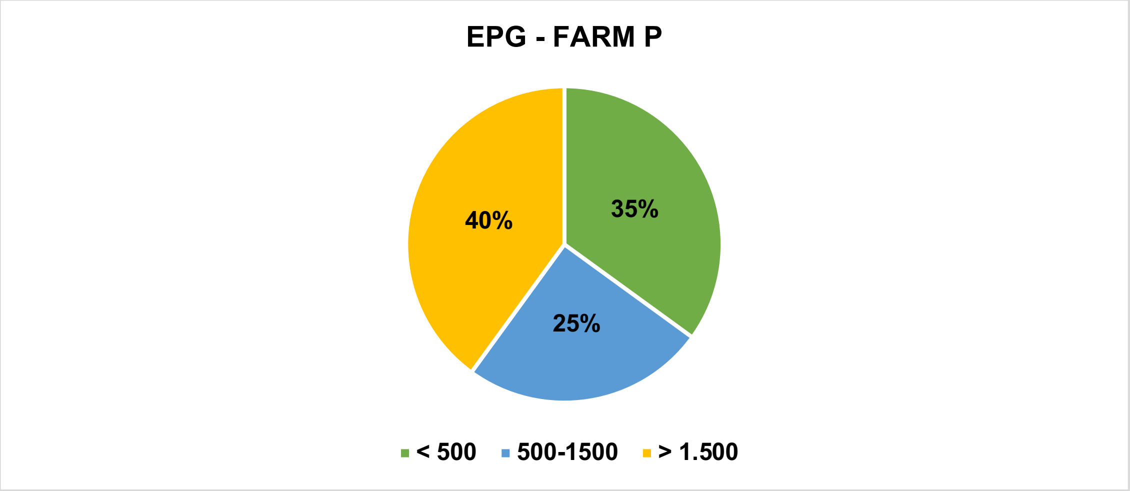
In Graphic 6, for Farm R (n=20), the level of parasitism was considered low for 10% (2) of the animals, moderate for 15% (3) and high for 75% (15) of the animals.

Graphic 6 - Level of Parasitism in the Farm R (n=20)



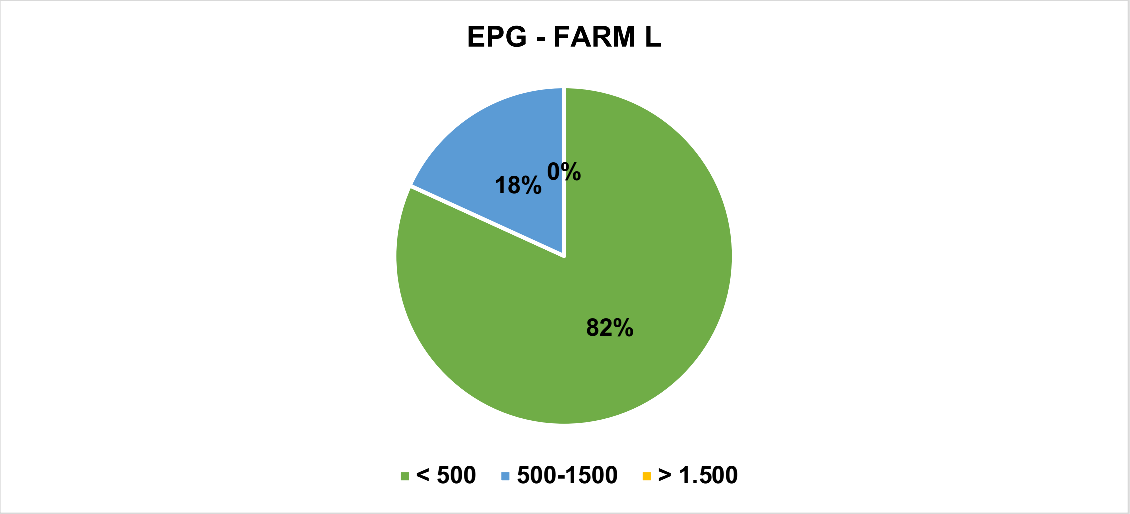
In Graphic 7, for Farm P (n=20), the level of parasitism was considered low for 35% (7) of the animals, moderate for 25% (5) and high for 40% (8) of the animals.

Graphic 7 - Level of Parasitism in the Farm P (n=20)



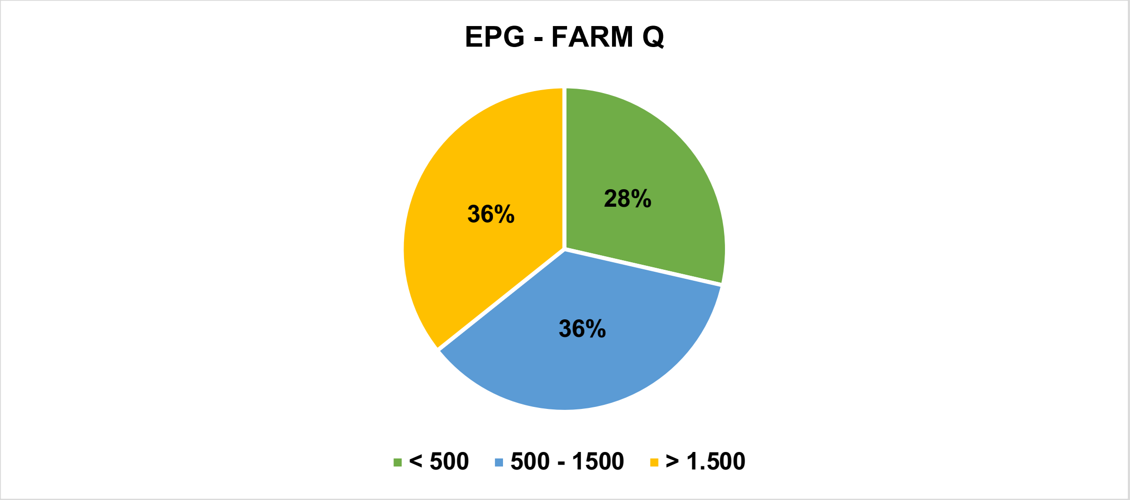
In Graphic 8, for Farm L (n=22), the level of parasitism was considered low for 82% (18) of the animals, and moderate for 18% (4).

Graphic 8 - Level of Parasitism in the Farm L (n=22)



In Graphic 9, for Farm Q (n=14), the level of parasitism was considered low for 28% (4) of the animals, moderate for 36% (5) and high for 36% (5) of the animals.

Graphic 9 - Level of Parasitism in the Farm Q (n=14)



3.3. Anthelmintic efficacy study

Regarding the anthelmintic efficacy study, 4 out of 5 farms where the study was conducted presented anthelmintic resistance (L, Q, R and M).

In Farm C (dairy goats), where the anthelmintic chosen was eprinomectin, the FECRT showed a percentage reduction of 100%. However, since there was a reduced number of animals on this trial and its faecal samples had low EPG counts, the results from this calculation ended up not being significant due to the low count. On Table 10, the calculation for drench effectiveness on Farm C is shown and on Table 11, the summary results for the most prevalent genera are shown. In this particular farm, only *Strongyloides papillosus* L3 were found after coprocultures.

Table 10 - Drench effectiveness in Farm C.

FECRT for Farm C (Dairy Goats)

Drench	Pre-Test	Control	**Eprinomectin**
Number	5	5	5
Arith. Mean	410	250	0
Var (FEC)	13000	10000	0
% Reduction			**100**
Var (Reduction)			
Upper 95% CL			100
Lower 95% CL			**82**
Drench effectiveness			**Low Count**[2]

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[2] Drench Effectiveness: When the result is shown as "Low Count" this indicates insufficient egg were seen for that species to accurately determine if the efficacy was greater than 90%. "Low Count" is flagged when the observed efficacy is 100% and the lower confidence limit for that result is less than 90% efficacy.

Table 11 - Summary results for Farm C.

Percent Fecal Egg Count Reduction (FECR)	
Drench	**Eprinomectin**
All species	100
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	
Sp. *Haemonchus*:	
Sp. Other:	100
Drench Effectiveness	
Drench	**Eprinomectin**
All species	**Low Count**
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	
Sp. *Haemonchus*:	
Sp. Other:	**Low Count**
Lower CL for Percent FECR	
Drench	**Eprinomectin**
All species	82
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	
Sp. *Haemonchus*:	
Sp. Other:	82

In Farm L (both sheep and goats), where the anthelmintic chosen was fenbendazole, the FECRT showed a percentage reduction of 48%, which means that anthelmintic resistance is present. On Table 12, the calculation for drench effectiveness on Farm L is shown and on Table 13, the summary results for the most prevalent genera are shown. In this farm, additionally to *Haemonchus contortus* and *Trichostrongylus* spp. there were also *Oesophagostomum* spp., *Chabertia ovina* and *Strongyloides papillosus* L3 found after coprocultures.

Table 12 - Drench effectiveness in Farm L.

FECRT for Farm L (Sheep and Goats)

Drench	Pre-Test	Control	**Fenbendazole**
Number	13	9	13
Arith. Mean	438	228	119
Var (FEC)	65897	40694	24808
% Reduction			**48**
Var (Reduction)			0,22
Upper 95% CL			81
Lower 95% CL			**-41**
Drench effectiveness			**Resistant**

Table 13 - Summary results for Farm L.

Percent Fecal Egg Count Reduction (FECR)	
Drench	**Fenbendazole**
All species	48
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	-78
Sp. *Haemonchus*:	79
Sp. Other:	95
Drench Effectiveness	
Drench	**Fenbendazole**
All species	**Resistant**
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	**Resistant**
Sp. *Haemonchus*:	**Resistant**
Sp. Other:	**Resistant**
Lower CL for Percent FECR	
Drench	**Fenbendazole**
All species	-41
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	-378
Sp. *Haemonchus*:	44
Sp. Other:	86

In Farm Q (both sheep and goats), where the anthelmintic chosen was also fenbendazole, the FECRT showed a percentage reduction of 84%, which means that anthelmintic resistance is present, even though this result is closer to 95% than the 48% found in Farm L. On Table 14, the calculation for drench effectiveness on Farm Q is shown and on Table 15, the summary results for the most prevalent genera are shown. In this particular farm, additionally to *Haemonchus contortus* and *Trichostrongylus* spp. there were also *Chabertia ovina* and *Strongyloides papillosus* L3 found after coprocultures.

Table 14 - Drench effectiveness in Farm Q.

Farm Q (Sheep and Goats)

Drench	Pre-Test	Control	**Fenbendazole**
Number	8	6	8
Arith. Mean	956	1675	269
Var (FEC)	837455	1835750	132813
% Reduction			**84**
Var (Reduction)			0,34
Upper 95% CL			95
Lower 95% CL			**46**
Drench effectiveness			**Resistant**

Table 15 - Summary results for Farm Q.

Percent Fecal Egg Count Reduction (FECR)	
Drench	**Fenbendazole**
All species	84
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	87
Sp. *Haemonchus*:	76
Sp. Other:	
Drench Effectiveness	
Drench	**Fenbendazole**
All species	**Resistant**
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	**Resistant**
Sp. *Haemonchus*:	**Resistant**
Sp. Other:	
Lower CL for Percent FECR	
Drench	**Fenbendazole**
All species	46
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	56
Sp. *Haemonchus*:	18
Sp. Other:	

In Farm R, where the anthelmintic chosen was closantel plus mebendazole, the FECRT showed a percentage reduction of 66%, which means that anthelmintic resistance is present. On Table 16, the calculation for drench effectiveness on Farm R is shown and on Table 17, the summary results for the most prevalent genera are shown. In this particular farm, additionally to *Haemonchus contortus* and *Trichostrongylus* spp. there were also *Chabertia ovina* and *Strongyloides papillosus* L3 found after coprocultures.

Table 16 - Drench effectiveness in Farm R.

FARM R (Sheep)

Drench	Pre-Test	Control	**Closantel plus Mebendazole**
Number	11	9	11
Arith. Mean	7264	2517	859
Var (FEC)	23780545	9973750	200909
% Reduction			**66**
Var (Reduction)			0,20
Upper 95% CL			87
Lower 95% CL			**13**
Drench effectiveness			**Resistant**

Table 17 - Summary results for Farm R.

Percent Fecal Egg Count Reduction (FECR)	
Drench	**Closantel plus Mebendazol**
All species	66
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	59
Sp. *Haemonchus*:	66
Sp. Other:	77
Drench Effectiveness	
Drench	**Closantel plus Mebendazol**
All species	**Resistant**
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	**Resistant**
Sp. *Haemonchus*:	**Resistant**
Sp. Other:	**Resistant**
Lower CL for Percent FECR	
Drench	**Closantel plus Mebendazol**
All species	13
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	-5
Sp. *Haemonchus*:	13
Sp. Other:	41

In Farm M, where the anthelmintic chosen was also closantel plus mebendazole, the FECRT showed a percentage reduction of 79%, which means that anthelmintic resistance is present. On Table 18, the calculation for drench effectiveness on Farm M is shown and on Table 19, the summary results for the most prevalent genera are shown. In this particular farm, additionally to *Haemonchus contortus* and *Trichostrongylus* spp. there were also *Oesophagostomum* spp.*, Chabertia ovina, Bunostomum* spp. and *Strongyloides papilosus* L3 found after coprocultures. *Haemonchus contortus* and *Trichostrongylus* spp., were not found in the second faecal culture of the treated group.

Table 18 - Drench effectiveness in Farm M.

Farm M (Combined Species)

Drench	Pre-Test	Control	**Closantel plus Mebendazole**
Number	8	6	8
Arith. Mean	3700	2233	475
Var (FEC)	51883571	8363667	358571
% Reduction			**79**
Var (Reduction)			0,48
Upper 95% CL			95
Lower 95% CL			**9**
Drench effectiveness			**Resistant**

Table 19 - Summary results for Farm M.

Percent Fecal Egg Count Reduction (FECR)	
Drench	**Closantel plus Mebendazole**
All species	79
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	100
Sp. *Haemonchus*:	100
Sp. Other:	39
Drench Effectiveness	
Drench	**Closantel plus Mebendazole**
All species	**Resistant**
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	**Susceptible**
Sp. *Haemonchus*:	**Susceptible**
Sp. Other:	**Resistant**
Lower CL for Percent FECR	
Drench	**Closantel plus Mebendazole**
All species	9
Sp. *Ostertagia*:	
Sp. *Trichostrongylus*:	95
Sp. *Haemonchus*:	96
Sp. Other:	-160

In table 20, a brief summary of the anthelmintics used, target species, percentage reduction and consequently drench effectiveness according to the Excel spreadsheet created by Angus Cameron is shown.

Table 20 - Summary of drench effectiveness by farm.

Farm	**Drench**	**Species**	**% Reduction**	**Drench effectiveness**
C	Eprinomectin	Goat	100	Low Count
L	Fenbendazole	Mixed	48	Resistant
Q	Fenbendazole	Mixed	84	Resistant
R	Closantel plus mebendazole	Sheep	66	Resistant
M	Closantel plus mebendazole	Sheep	79	Resistant

4. DISCUSSION

The main objectives of this study were to characterize the presence and level of parasitism of small ruminants in nine farms located in the region of Lisbon and Tagus Valley, as well as the presence and level of anthelmintic resistance cases in a study performed in five of the nine farms. In farm C, which consists in a dairy goats' farm (intensive production system), the anthelmintic chosen was eprinomectin (Eprinex® Pour-on). In the mixed (both sheep and goats) farms L and Q (extensive production system) the anthelmintic chosen was fenbendazole (Panacur® 2,5%) and in the farms R and M (only sheep in extensive production system) the anthelmintic chosen was an association of mebendazole and closantel (Seponver® Plus). The dairy goats' farm C was located in the district of Setúbal, Farm F in the district of Lisbon and the remaining farms were located in the district of Santarém.

Starting with the presence of parasites, a total of 205 fecal samples were collected and analysed from both sheep and goats. Out of 97 sheep and 108 goat faecal samples, 183 were positive for GI parasites, giving an overall presence of 89.27%, where 76.85% of the tested sheep were positive for at least one GI parasite and 92.78% of the tested goats were positive for at least one GI parasite. Tábuas (2013), in a study performed in Alentejo Central, reported 80% adult sheep and 73,33% adult goats positive for GIN, which was the opposite detected in the present study. However, Crespo & Jorge (1999), in a study performed in sheep in Alentejo and Ribatejo, reported a prevalence of GIN of 56,67% in the first region and a prevalence of 72,09% in the second. Lastly, Pedreira et al. (2006), in a study performed in Galiza (Spain), found 100% prevalence of GI parasites in 49 sheep farms, which may indicate similar results between studies and regions. There was a significant difference in the occurrence of GI parasites between sheep and goats, probably justified by the identical way these species are dewormed, usually underdosing goats who have a higher metabolic rate and usually require a higher dose rate for drugs (1.5-2 times the recommended dosage for sheep). However, most of the anthelmintics used in goats have not been licensed for this specie and correct dosage rates are barely known, which leads to an equal treatment as the one for sheep (Papadopoulos 2008, Várady et al. 2011). Goats were found to have mostly *Strongyloides papillosus* eggs (58.33%) and *Eimeria* spp. oocysts (50.93%), followed by eggs of the Strongyle type (17.59%). On the other hand, sheep were found to have mainly eggs of Strongyle type (84.54%), followed by *Eimeria* spp. oocysts (12.37%), eggs of *Strongyloides papillosus* (3.09%) and *Moniezia expansa* (2.06%). On overall, the most frequent parasites were the ones from strongyle type (88.88%), followed by *Eimeria* spp. (66.66%), *Strongyloides papillosus* (55.55%) and lastly *Moniezia expansa* (11.11%), results that are in agreement with the study performed by Guerreiro (2009), comparing the level of parasitism of small ruminant farms between the Alentejo and Andaluzia regions. It was also reported that in the Alentejo region, the most prevalent parasites were the ones from strongyle type, while in Andaluzia the most prevalent parasites found were *Eimeria* spp. oocysts. The different results found in Alentejo compared to Andaluzia were justified by the type of management, since in the first region all the animals had access to pasture, similarly to the results from the present study, where sheep were found to be infected mostly by strongyle type eggs and at a less level, with *Eimeria* spp. oocysts and goats were found to be more infected with *Eimeria* spp. oocysts and less strongyle type eggs than sheep. Anastácio (2011) performed a study evaluating the gastrointestinal parasitosis in sheep in the same region as the present study and the most frequent parasites found were also from strongyle type such as *Trichostrongylus* spp. and *Teladorsagia* sp. The parasites from the genera *Strongyloides* spp. were found at a minor percentage in adults but were the most frequently found nematode in lambs. This might indicate that the strongyle type population in Benavente county remains similar as it was 8 years ago, with a slight increase in *Strongyloides papillosus* population, which according to Bowman (2014), usually causes only a moderate and asymptomatic infection. In the present study, the mean EPG in sheep was 2028.87 with a range between 0 and 21300 and the mean OPG was 47.42 with a range between 0 and 2500. As for goats, the mean EPG was 606.02 with a range between 0 and 5850 and the mean OPG 109.26 with a range between 0 and 850. In Tábuas (2013) study, the mean EPG in adult sheep was 154,29 and in young animals 122,1 and the mean EPG in adult goats was 167,61, values substantially inferior to the mean EPG values of the present study. Chikweto et al. (2018), in a study performed in Grenada, West Indies, also found a higher percentage of positive goats (98%) compared to sheep (88%), and a higher EPG mean in sheep and higher OPG mean in goats. In that study, goats were mostly positive to strongyle type eggs (89%) and *Eimeria* spp. oocysts (76%), followed by *Strongyloides* sp. (32%) and *Moniezia* spp. (16%). In the present study, sheep were found to have essentially strongyle type eggs (84.54%), less Eimeria spp. oocysts (12.37%) and a similar low occurrence of *Strongyloides papillosus* (3.09%) and *Moniezia expansa* (2.06%), results only analogous to Chikweto et al. (2018) study in the occurrence of Strongyle type eggs, since Coccidia presence in Grenada was substantially higher (75%). Eke et al. (2019), in a study performed in an abattoir in Nigeria, found similar results, with 63.2% of sheep and 75.0% of goats positive for GI parasites, with an overall prevalence rate of 69.64%. Tramboo et al. (2015) reported an overall prevalence of GI helminths of 77% in ovine population in the Bugdam district of

Kashmir Valley (India). In general, the current results are in accordance with the results from the referred studies, except for the *Strongyloides papillosus* which was found at a higher rate.

The level of parasitism for both species was classified in “low” if EPG count was less than 500, “moderate” if EPG count was between 500 and 1500 and “high” if EPG count was higher than 1500. In the present study, 5 out of 9 farms had more than 50% of the animals ranked in the low level of infection category (Farms F, BO, C, B and L). Farms M, P and Q had all 3 types of classifications with similar proportions, rounding 35% each. Farm R had 75% of the animals ranked in the high level of infection category. These results from farms F, BO, C and B may be explained by the fact that these animals (dairy goats in farms C and B, lambs in farm BO and both sheep and goats in farm F) are raised indoors, with no access to pasture, reducing the probability of infection. Farm L consisted in a mixed flock with mostly sheep in extensive production, pertaining to a small holder without a big area of pasture. This flock is dewormed every six months by the assistant veterinarians and by the time of the faecal collection, six months had passed since last treatment. Farms M, P and R consist in sheep flocks and farm Q comprises both sheep and goats at the same proportion. All these animals are raised in an extensive production system, pertaining to small holders with limited areas of pasture, which means that there is a higher number of animals per hectare, increasing the probability of contamination of the pastures and posteriorly infection. Their deworming system is the same as farm L (every six months), which according to Pedreira et al. (2006) does not cause any reduction on EPG level. Another aspect which may affect the level of parasitism is the fact that only one dose of anthelmintic is administered, which reduces the efficacy compared to an administration of two doses 12 hours apart (Fleming et al. 2006). Anástacio (2011) found similar results concerning sheep, with most of the animals with low levels of infection, but with a farm with high levels of GIN infection and Guerreiro (2009) also reported that even though most farms were positive to GI parasites, the levels of infection presented by the adult animals were low. Lagares (2008), in a study conducted in 25 small ruminant farms in the region of Cova da Beira found the majority of sheep farms with high levels of infection by GI parasites (ranked as having more than 900 EPG), with the dairy sheep in the dry period having the highest levels of infection, followed by lactating sheep and post-partum sheep. Dairy sheep farms were not evaluated in the present study, but regarding the meat purpose sheep flocks, the results were similar, with high levels of infection by strongyle type eggs and low levels of infection by *Eimeria* spp. oocysts. Lambs showed high levels of infection by strongyle type eggs and *Moniezia* spp., as well as *Eimeria* spp. oocysts. These results are similar to the ones found in the present study in the lamb’s feedlot (Farm BO), where high levels of infection by *Eimeria* spp. were also seen and where *Moniezia expansa* was identified. Furthermore, dairy goats revealed high levels of infection by strongyle type eggs but low levels of infection by *Eimeria* spp., which was not the case for the dairy farms C and B, where the infection by strongyle type eggs was low, compared to the infection by *Strongyloides* sp. and *Eimeria* spp.

Regarding the anthelmintic efficacy study, according to Coles et al. (1992, 2006), 4 out of 5 farms presented failure in anthelmintic efficacy. In farms L, Q, R and M the FECRT presented a percentage reduction of 48%, 84%, 66% and 79%, respectively. In farms L and Q (mixed flocks), the drench chosen was fenbendazole (Panacur® 2,5%) and the study revealed the lack of efficacy of this anthelmintic. Resistance to this molecule was also found by Sudan (2013) in the treated group of 10 goats in India, with a percentage reduction of 71%. Nonetheless, Tramboo (2015) reported a fenbendazole's efficacy of 99% in a group of 30 sheep in Kashmir Valley (India). These results may suggest that anthelmintic resistance is more prevalent in goats, probably due to the fact that this species is usually underdosed, promoting the selection of resistant strains. In farms R and M (sheep flocks), the drench chosen was an association of closantel and mebendazole (Seponver® Plus) and the study also revealed the presence of resistance to this anthelmintic. Even though Tramboo (2015) reported an efficacy of 98% for closantel in a group of 30 sheep in India, Furgasa (2018) showed the development of resistance against albendazole (which is structurally related to mebendazole) by GIN in sheep in Haramaya University, Ethiopia. As for Farm C (dairy goats), where the anthelmintic chosen was eprinomectin (Eprinex® Pour-on), the FECRT showed a percentage reduction of 100%. However, since the treatment and control group comprised only 5 animals each and the EGP counts from its faecal samples were low, the results from the calculation were not significant due to the low count. Nonetheless, these animals showed a low level of parasitism, even with no deworming system (they had not been dewormed in the previous years), which may indicate that this intensive and indoors production system may decrease the infection by GI parasites. Additionally, Arsenopoulos (2019) reported a high efficacy of eprinomectin in dairy ewes naturally infected by GIN in Greece. Sargison et al. (2010) reported that the field population of parasitic nematodes from a sheep flock in south-east Scotland was resistant to benzimidazoles, imidazothiazoles, and both ivermectin and moxidectin macrocyclic lactones anthelmintics. Regarding the study of AH efficacy in Portugal, Mateus (2018), in a study performed in Northern Portugal, revealed that even though the EPG level that sheep were excreting (below 100) did not require deworming actions, AHR was already present, specially to benzimidazoles. Anastácio (2011) reported a "doubtful" efficacy of netobimin and resistance to diclazuril in GIN and *Eimeria* spp. in lambs, since the FECRT resulted in percentage reduction of 91,75% and 55,38%, respectively.

The widespread and high presence of GI parasites in some farms of this study may suggest that some deworming procedures may be failing, since anthelmintic resistance has been demonstrated in 4 farms and most animals were infected with more than one GI parasite. These results are in accordance with the results from the referred studies, which unfortunately may indicate the development of MDR nematode strains in Portugal.

5. CONCLUSIONS

The experimental study performed for the present dissertation permitted the professional and personal experience both in the field and laboratory work, allowing the assessment of the parasitological situation of the region of Lisbon and Tagus Valley and mostly the pioneering results regarding anthelmintic efficacy in small ruminants in Portugal.

Results from this study revealed a widespread presence of gastrointestinal parasites in the region of Lisbon and Tagus Valley, with all the 9 farms presenting positive animals to at least one type of GI parasite. Although the majority of farms had more than 50% of their animals ranked in the “low” level of infection, there were 3 with a similar proportion of the 3 types of classification and one with 75% of the animals ranked in the “high” level of infection category. To conclude, 4/5 farms presented anthelmintic resistant nematodes: two farms against fenbendazole and other two farms against the association of closantel and mebendazole. This means that even though there was a generalized infection by gastrointestinal parasites, this infection appears to not have fatal repercussions when at low levels. However, when anthelmintic efficacy was required it was not at the levels as it was supposed to be, which announces an increase of AHR nematode strains.

The difference in the parasitism between sheep and goats may be explained by the different management and deworming systems, showing that when with access to pasture, sheep can be more resistant to GI parasites, opposed to goats that when are not allowed to feed through bushes, seem to be more susceptible to infections by GI parasites, as well as, the probability of this species remaining underdosed compared to sheep when deworming time comes.

The growing resistance of GIN of sheep and goats to several anthelmintics is becoming a serious worldwide problem. Results from the present study indicate that the GIN of the four studied Portuguese farms might be globally resistant to benzimidazoles, due to the lack of efficacy of fenbendazole and the association of closantel and mebendazole.

Controlling the increasing AHR must be an urgent ambition, in order to reduce significant complications in animal health and welfare in the future, which ends up compromising the animal production. Measures like educating the farmers, promoting rotational grazing, reducing the unnecessary and frequent usage of anthelmintics should be taken, plus, when deworming is required, an effort to apply the correct dosage according to specie and weight should be made. Other strategies like keeping some parasites in refugia, applying the FAMACHA© system, supplementation with protein in order to increase immunity and resilience to parasitism, the introduction of nematophagous fungal spores, formulation of vaccines, the use of plants with anthelmintic properties and selective breeding of animals that tolerate nematodes, must be part of the strategies to control the spread of AHR.

Currently a project denominated MERINOparasite is in development with the purpose of identifying the genetic markers associated with resistance to internal parasites in the Merino Branco breed. In the future, and since the importance of anthelmintic resistance is increasing, it would be interesting to study the level of parasitism and anthelmintic efficacy of the most used dewormers in other regions of Portugal, in order to assess the current situation of the level of AHR in the country.

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