FURTHER DATA ON THE FREQUENCY, RISK FACTORS AND ECONOMIC IMPACT OF SHEEP COCCIDIOSIS IN THE CANARY ISLANDS

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Abstract

Species of the genus *Eimeria* are species-specific parasitic protozoa, monoxenes found in the gastrointestinal tract of poultry and mammals. Coccidiosis affects the health and profitability of ruminants and, therefore, causes serious economic losses, since it can affect all the animals in the farm depending on the way they are managed. The aim of this study was to analyze the prevalence of ovine coccidiosis in farms on the island of Gran Canaria and to identify, by means of a questionnaire, the risk factors and the economic impact of this parasitic disease. Fecal material was collected from lambs and adult ewes of seven farms of the island. Oocyst quantification was performed for each sample and identification of the different species of ovine *Eimeria* was carried out. During the visits to the farms, surveys were made to the farmers and veterinarians responsible for each farm in order to obtain useful information about them and to interpret the parasitological results. The results obtained show the presence of *Eimeria* spp. in all the farms sampled and also the predominance of *E. ovinoidalis*. The prevalence of the parasite was variable, being the level of infection of lambs higher than adults in all farms. However, very few clinical signs were seen in both age groups. Management conditions were correlated with the parasitological data obtained, such as the size of the farm or the breeding system, as well as the lack of knowledge of the farmers about coccidiosis, since only one of the farms studied was using anticoccidial treatments. The data obtained here are a further contribution to previous studies performed in this area and would allow to obtain more consistent conclusions in order to find effective management strategies against ovine coccidiosis, including the prevention of resistance to anticoccidials.

Key words: *Eimeria* spp., sheep, survey, anticoccidials.

Índice

1. Introduction

Coccidiosis is an important parasitic disease with a worldwide distribution that affects different hosts, such as poultry, swine, sheep, goats and cattle (Chapman, 2014). It leads to a decrease in animal welfare, as well as economic losses for farmers.

Coccidiosis due to *Eimeria* spp. can give rise to a clinical picture based on dehydration, diarrhea, decreased growth, weight loss, and death (Chartier and Paraud, 2012).

1.1*.* Aetiology*: Eimeria* spp.

Eimeria spp. belongs to the phylum Alveolata, subphylum Apicomplexa, class Coccidea, order Eimeriida, and family Eimeriidae (Deplazes *et al*., 2016). The genus *Eimeria* is composed of 1,800 species of obligate intracellular parasites that affect vertebrates (Walker *et al*., 2013), including small ruminants.

1.2. Parasites's life cycle

The genus *Eimeria* has a monoxene life cycle and is transmitted by the fecal-oral route. Sporulated ocysts of *Eimeria* spp. contains four sporocysts, within which there are 2 haploid sporozoites (Fayer, 1980). After ingestion of sporulated oocysts, infection begins (Walker *et al.*, 2013) due to the rupture of the oocyst wall by mechanical and chemical actions, giving leading to the release of sporozoites. Free sporozoites will invade the enterocytes, where they will reproduce asexually and generate merozoites (Wacha *et al*., 1971). Several generations of merozoites will be elicited, and then the sexual phase of the cycle occurs, which comprises three phases:

-Gametocytogenesis: production of gametocytes from merozoites.

-Gametogenesis: production of haploid microgametes and macrogametes from gametocytes.

-Fertilization of macrogametes by microgametes, which gives rise to the production of diploid oocysts (Walker *et al.*, 2013).

After oocysts leave the host via, they will divide by meiosis to produce infectious sporozoites. The prepatent period of the cycle varies among different species of *Eimeria*.

1.3. Epidemiology and immunology

In ovine coccidiosis, initial infection can occur from dams, although the most important source is young animals infected for the first time shedding huge numbers of oocysts. Lambs are more susceptible to the parasite than adult sheep, leading to a high multiplication rate and rapid spread of the disease (Gauly *et al*., 2004). Increasing age of lambs leads to a decrease in the multiplication rate of the parasite, indicating the development of a specific immune response (Reeg *et al*., 2005).

Several management factors in indoor lamb husbandry increase the rate of disease transmission, such as poor hygiene and overcrowding. In contrast, artificial feeding seems to prevent the infection. In extensive management systems, ricks factor for infection include a high population density and a reduction in the availability of pasture stand out (de Souza *et al*., 2015). In this case, symptoms will observe with a marked seasonality, mostly in spring.

The immunity against the parasite is clearly related to the susceptibility of the animal to infection. Thus, although initial infection may originate from adult sheep, the infected lambs (with high rates of parasite multiplication and shedding of oocysts into the environment) are the main source of infection. As a result of the specific immunity acquired after exposure to the parasite, the animals became more resistant to the parasite and fewer oocysts are shed. However, immunity may not be absolute, so animals continue hosting small numbers of coccidia for live (Zanetti Lopes *et al.,* 2013).

Some factors such as inadequate nutritional intake, weaning, diet changes, transport stress, climatic changes or concomitant infections seen to play a negative effect on acquired immunity against infection. Furthermore, some authors report that females are more susceptible to *Eimeria* infection than males during pregnancy, parturition and lactation (Chartier and Paraud, 2012; Carrau *et al*., 2016).

Regarding to its geographical distribution, coccidiosis has a worldwide distribution although there are variations in prevalence by regions. Seasons with high humidity and temperature increase the accumulation of sporulated oocysts.

1.4. Pathogenesis and pathology

In general, most of sheep *Eimeria* species arise the small and/or large intestine, with the exception of *E. gilruthi* (which is localized in the rumen) induce a disease whose evolution would depend on factors such as parasite-dependent factors such as species, infective dose and host-related factors such as physical condition, stress, genetics and previous contact with the parasite (Jolley and Bardsley, 2006).

Among the parasite-related factors that may also affect the course of infection are the species. In this regard, *E. ovinoidalis* and *E. crandallis* are recognized as major pathogens (Joachim *et al*., 2018), while *E. ahsata* and *E. bakuensis* are considered minor pathogens (Deplazes *et al*., 2016).

As a consequence of the proliferation of the parasite, destruction of the enterocytes occurs, as well as ulceration of the mucosa and atrophy of the villi. These morphological alterations modify the function of these enterocytes and intestinal motility and intercellular signaling. All this will result in malabsorptive diarrhea and retention of electrolytes and nutrients in the lumen of the intestines, along with the passage of fluids into the large intestine into the large intestine. Eventually, enteritis occurs affecting the *lamina propria* and sometimes the submucosa (Jolley and Bardsley, 2006). Regeneration of the damaged epithelium and recovery of the animal is usually slow and the animal may be symptomatic for months.

E. ovinoidalis is highly pathogenic and primarily causes lesions in the terminal ileum, cecum, and proximal colon, resulting in thickened and edematous areas. Its pathogenicity seems to be related to the digestive microflora of the animal, since lambs born by caesarean section and reared sterile conditions have fewer clinical symptoms than lambs with a normal microflora, when they are infected with *E. ovinoidalis*.

E. crandallis is also highly pathogenic, causing primary lesions in the ileum resulting in villous atrophy due to first- and second-generation meronts and loss of crypts due to epithelial damage. Furthermore, it is also responsible for diffuse hyperplastic lesions, with thickened and folded mucosa.

1.5. Clinical signs

The acute clinical pattern resulting from *Eimeria* spp. infections includes varying degree of yellow to dark yellow diarrhea that may contain blood and intestinal tissue, fever, abdominal pain, anorexia, emaciation, and dehydration (Khodakaram Tafti and Mansourian, 2008). Fluids and electrolytes losses lead to increased hematocrit (Hashemnia *et al.,* 2014), while nutrient loss, and mucosal lesions cause weight loss.

Subclinical coccidiosis can also lead to reduced growth, affecting feed conversion rates (Aitken, 2007). The recovery time of the animal will be determined by the severity of the intestinal damage and the rate of crypt re-epithelialization.

1.6. Diagnosis

Coprological methods

Eimeria spp. can be detected by direct observation of feces in animal with a high parasitic load, however, concentration methods are generally required for oocyst observation (Vadlejch *et al*., 2011) such as flotation with saturated sodium chloride solution. To make a correct diagnostic it needs to provide information on epidemiology and clinical signs, as well as post-mortem examinations (de Waal, 2012).

To determine the species involved, the recommended procedure is the culture of feces using a 2.5% potassium dichromate (to prevent a fungal contamination) for 7-10 days at 20°C - 27°C. Once the oocysts have been sporulated, speciation is carried out by morphometric criteria.

In addition to identification, a quantitative coprological analysis should be performed to determine the parasite load. For this purpose, the McMaster method can be used. Parasite loads of 50,000 OPG (oocysts per gram of feces), if pathogenic species predominate, may be responsible for clinical manifestations. The definitive diagnosis requires a differential diagnosis of intestinal pathogens (Hidalgo Argüello and Cordero del Campillo, 1999; Chartier and Paraud, 2012; Andrews, 2013).

Molecular methods

More recently, great efforts are being carried out to develop molecular methods for the identification and determination of parasite loads, such as the quantitative PCR method, based on the amplification of a fragment of a specific 18S rRNA locus and subsequent sequencing. However, this is a procedure that is not very widespread beyond research laboratories.

1.7. Therapeutic treatment

When an outbreak of coccidiosis occurs, all animals should be treated, both those showing symptoms and apparently healthy whether or not they are sick. The treatment can be applied through drinking water or food, and in cases where it is not possible, it will be applied individually.

Among the drugs used, sulfonamides, amprolium, ionophores and triazines stand out (Witcombe and Smith, 2014; Paula *et al*., 2018). The most commonly used triazines in the treatment of ovine coccidiosis are trazuril and diclazuril. According to several studies, toltrazuril has greater efficacy against *Eimeria* spp. than diclazuril, presenting decreased excretion of oocysts, less duration of diarrhea and weight gain. Although, toltrazuril requires much higher doses (20 mg/kg toltrazuril vs. 1 mg/kg diclazuril) (Le Sueur *et al*., 2009; Taylor et al., 2011; Diaferia *et al*., 2013).

After the treatment of all the animals, the isolation of those animals with diarrhea should be carried out to control environmental contamination. Lambs should be treated and moved as soon as possible to clean, dry, uncontaminated pastures or pens to avoid reinfection (Taylor *et al*., 2007; Andrews, 2013).

As explained above, some animals must be individually treated, especially when they are very sick. In these cases, in addition to treatment for coccidiosis, we must give adjuvant support treatments and apply antibiotics to prevent secondary bacterial infections (Sudhakara Reddy *et al*., 2015).

1.8. Control and prevention

Regarding management, a proper cleaning of the facilities (even using a blowtorch) before lambing can reduce the occurrence of coccidiosis in lambs. Other measures to follow are the periodic change of beddings to provide the animals with clean and dry beds (Foreyt, 1990; Taylor *et al*., 2007; Saratsis *et al*., 2013; Lopes *et al*., 2014; Engidaw *et al*., 2015). Nutritional management through the intake of colostrum and vitamin supplements by newborns, rearing of animals of similar age and avoid overcrowding are other measures that can provide good results. (Taylor, 1995; Alzieu et al., 1999). In outdoor conditions, during the grazing period, the age composition of the herds, as well as the duration of pasture rotations should be established to avoid environmental contamination.

Chemoprophylaxis is essential in the control of coccidiosis, since management measures are not enough to prevent this disease. Various anticoccidial drugs has been used in drinking water or food as a preventive method (Platzer *et al*., 2005; Le Sueur *et al*., 2009; Taylor et al., 2011; Saratsis *et al*., 2013; Odden *et al*., 2017;). Among these drugs, decoquinate stands out, which is usually administered to sheep 28 days before parturition (0.5 mg/kg of body weight/day) and to lambs at 2 weeks of age (1 mg/kg of body weight). This drug cannot be administered to sheep whose milk will be destined for human consumption (Taylor *et al*., 2012; Andrews, 2013). Other drugs that could be used are clopidol, robenidine, and sulfonamides.

Effective Anticoccidial drugs against all stages of the parasite are also used (in addition to treatment purposes) in a metaphylactically approach to promote the induction of immunity against the parasite. However, long-term intensive use of these drugs could leads to the emergence of appearance of resistant *Eimeria* spp. strains, although fortunately the number of reported cases is low at the moment (Odden *et al., 2017*; Joachim *et al*., 2018).

As alternative methods to prevent the ovine coccidiosis, several studies have shown that the use of dietary supplements such as essential fats, natural antioxidants, and herbal or medicinal plant extracts with anti-inflammatory, antioxidant, or immune-stimulating products could be effectives (Rawdon *et al*., 2006). Plants carrying tannin compounds as sainfoin (*Onobrychis viciifolia*) or sericea lespedeza (*Lespedeza cuneata*) promote the control of coccidiosis when applied to sheep from before parturition to weaning or to lambs (Saratsis *et al*., 2012; Burke *et al*., 2013; Saratsis *et al*., 2016).

Regarding the development of vaccines against coccidiosis in sheep, only some preliminary trials have been carried out, so the use of vaccines in sheep does not seem feasible in the short term (Catchpole *et al*., 1993; Ruiz *et al*., 2014).

2. Objective and hypothesis

The aim of this study was to contribute to previous research carried out by the Parasitology Unit of the ULPGC on the prevalence of ovine coccidiosis in different geographical areas of Gran Canaria Island as well as to analyze, by means of a questionnaire, its relationship with risk factors and the economic losses produced in this livestock species. All the information collect would be of interest to design strategies of control to minimize the risk of clinical coccidiosis and their economic impact of this parasitic infection in each particular sheep farm.

3. Material and methods

3.1. Study area (farms)

This study was carried out on seven sheep farms on the island of Gran Canaria. These farms are located in different geographical areas of the island and show variations in terms of climatic conditions, flock size, breeding systems, management and even in the protocols used for the control of different parasitic infections.
The seven farms studied belong to the municipalities of Ingenio (G8 and G9), Moya (G10), Gáldar (G11 and G12), Valsequillo (G13) and Valleseco (G14) (Figure 1).

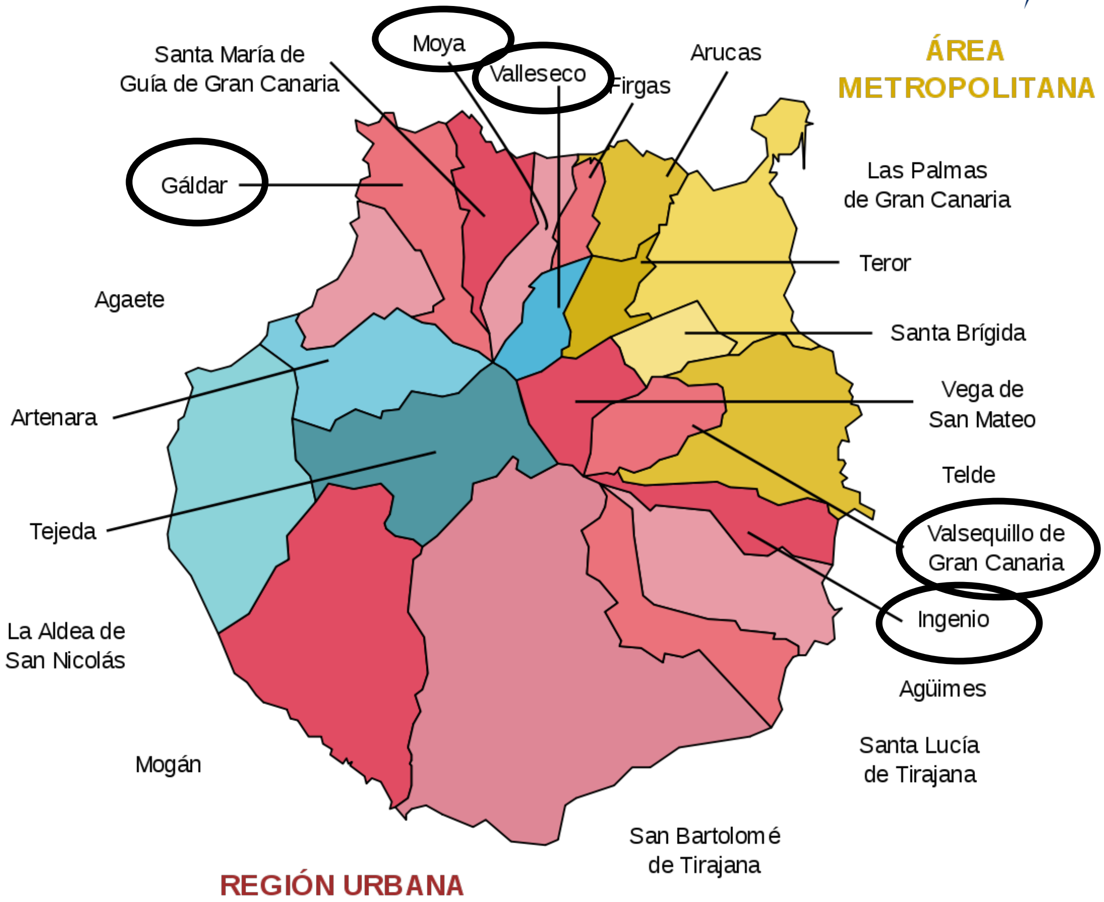

Figure 1. Geographical distribution of the seven farms sampled

In relation to the isoclimatic characteristics of each of the areas where the farms are located, three different isoclimate conditions are found

- Gáldar and Valsequillo present a dry steppe climate with dry summers, as well as average average temperature and rainfall.
- Moya and Valleseco have a mild temperate climate warm and dry summers and mild winters with 400 mm annual mean rainfall.
- Ingenio have a dry desert climate with low rainfall, average temperatures and dry summers.

3.2. Collection and preparation of samples

On each farm, 10 faecal samples were collected from adult ewes, as well as 8 from lambs. Between one and three months of age before they have not received any antiparasitic treatment. Samples were collected directly from the rectum and were used for qualitative and quantitative coprological analysis.

Samples were collected in latex gloves and storage at 4 ºC during transport to the laboratory. For preservation, a 2,5 % potassium dichromate solution was added to each sample and kept at 4 ºC until they were processed. In this study, 70 fecal samples from adults and 56 from lambs were collected and analyzed.

3.3. Farm characteristics questionnaire

In order to obtain as precise data as possible, questionnaires were carried out jointly by the farmers and the veterinarian responsible for the farm. These questionnaires consisted of 21 questions, covering topics such as: farm data, animal groups, facilities, type of farm regime, management and control practices, and treatment against coccidiosis, among others.

After completion of the questionnaires, the data collected were associated with the parasitological results obtained during the study, thus providing an additional information on main risks of ovine coccidiosis in Gran Canaria and the economic losses associated with *Eimeria* infection in this species.

3.4. Faecal sample preparation

From each sample, 1 g of feces were taken and placed in a pre-weight Eppendorf tube to which 3 drops of potassium dichromate 2% were added to prevent fungal growth. The samples were then homogenized and stored at 4ºC.

Each sample was identified with the following code:

- Farm: each farm was labeled with a "G" and a number from 8 to 14.

- Age of the animal: adult or lamb, were identified with the letter A or C respectively, followed by the number of the animal among the total of its category for each farm. Thus the first lamb of the first farm sampled was labelled with the following code: G8C1.

3.5. Coprological analysis

Two different analyses were performed on each of the samples: a quantitative analysis of oocyst excretion (oocysts per gram = OPG) using a modified McMaster method, and a qualitative analysis to identify the different *Eimeria* species base on morphometrical criteria.

To perform the McMaster method, the faecal samples were diluted in 30 ml of a saturated NaCl solution (d= 1.2 g/ml). After homogenization, these samples were placed using a Pasteur pipette in each of the McMaster chambers (with a total volume of 0.30 ml.

Oocyst counts were done under the microscope at a 100x magnification. The final results were obtained multiplying by 100 the count obtained in the two chambers and expressed in oocysts per gram of feces (OPG). For each sample, counts were corrected according the exact weight of the sample and. If necessary, a additional dilution was made (to facilitate the counts in samples in which the number of oocysts was very high, dilutions of 1/10 or 1/100 of the starting suspension were made).

Qualitative coproscopic analysis (focused on speciacition through the identification of the oocysts of *Eimeria* spp.), were performed from the suspension used for oocyst counts, but using cameras as shown in Figure 2, which allows the observation of the sample at a 400x magnification. The identification of 20 oocysts per sample was carried out following the morphometric criteria listed in Table 1. Measurements were performed using a micrometer eyepiece.

Figure 2: Home-made chamber used in the qualitative analysis of the samples**.**

Eimeria spp.	Morphological criteria
E. pallida	The smallest of the eleven *Eimeria spp.* taken into account, closely followed by *E. parva*. More spherical than ellipsoid shape, with no polar cap and micropyle. Based on the bibliography, the following threshold was stated: *E. parva* was considered to have a longitudinal diameter between 22.5-13 µm, and *E. pallida* below 13 µm, additionally to the previously mentioned shape description.
E.parva	Combined criteria with *E. pallida*.
E. marsica	Ellipsoid shape with relatively straight lateral walls, and presence of polar cap and micropyle (these structures were sometimes so small, that we could only perceive a thickening of the polar/anterior wall). Longitudinal diameter between 22-15 µm.
E. ovinoidalis	The oocyst has no polar cap and the shape is variable, more ellipsoid -like the previous mentioned species-, but relatively spherical compared with other longer species. Its micropyle, present even without polar cap, was particularly remarkable, as a black notch within the polar wall. If a n absent -polar cap oocyst bigger than 22.5 µm, and incompatible with *E. faurei's* characteristic shape was seen, it was considered *E. ovinoidalis* even if the micropyle was not evident. Its size range is considered to be between 25 -17 µm.
E. weybridgensis/ ***E. crandallis***	Combined because of their morphological similarities and overlapping size ranges. Even sporulated, a solid criterion about their differences was not appreciable. With both polar cap and micropyle, and a more spherical shape than other polar capped species, they were considered to be under 27.7 µm sized (longitudinal diameter) and not smaller than 20 µm)
E. faurei	The biggest no polar cap *Eimeria* spp. found in this study. With an evident micropyle, the oocyst, between 37 -28 µm of size, has a very particular shape: the posterior side is significantly wider than the anterior/polar side/wall, "similar to a pear".
E. granulosa	With polar cap and micropyle, this species is considered to be between 35 -22 µm, which overlaps with the majority of polar capped species. However, *E. granulosa* shows a singular shape, as it has a wider anterior side, with a relatively large micropylar cap and polar granules very large.

E. bakuensis	It could be considered a bigger version of *E. marsica*, as it also shows an ellipsoid shape with straight lateral walls, and, in this case, an evident polar cap and micropyle. In case the straight shape was not clear enough, the oocysts were classified according to its size (considered to be between 32-27 µm).
E. ashata	The second largest oocyst, considered to be beyond 32 µm at least (described to be between 37- 29 µm). The main conflict at classifying this species was on its comparison with *E. bakuensis* (with whom size overlaps) if the mentioned straight walls for *E. bakuensis* were not evident. By contrast, *E. ashata* oocysts were considered to have relatively curved longitudinal sides.
E. intricata	The largest among the *Eimeria* species found in this study. It's hardly mistaken, as it's not only much larger than other species (a mean size of 48 µm), but also it has much thicker and striated walls, and it's the only *Eimeria* species with a brown colour.

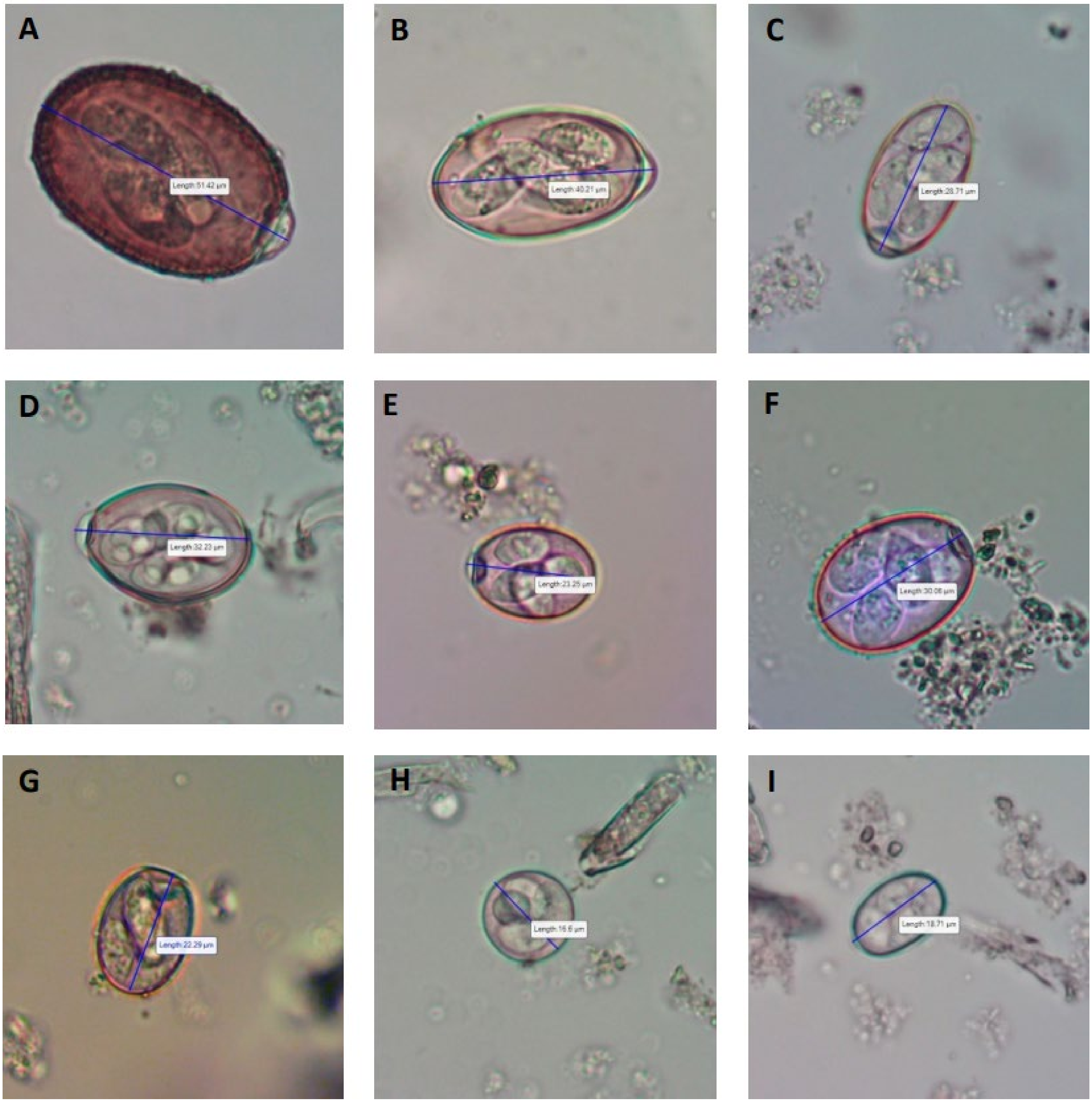

Figure 3: Examples of the *Eimeria* species found in the study. **A:** *E. intricata*; **B:** *E. ashata*; **C:** *E. bakuensis*; **D:** *E. granulosa*; **E:** *E. crandalis*; **F:** *E. faurei*; **G:** *E. ovinoidalis*; **H:** *E. parva*; **I:** *E. marsica*

3.6. Statistical analysis of the results.

OPG value were transformed to log OPG plus one [log(OPG+1)] to obtain a normal distribution. From these data, the mean OPG score of the adult and lamb groups of each farm as well as the standard deviation and standard error of the mean (SEM) were determined. The mean values of the farms and the overall mean were compared by one-way ANOVA and Mann-Whitney rank sum test (SigmaPlot 15.5).

Chi-square was used to compare the frequencies of different *Eimeria* species. All calculations were performed for each age group (adults and lambs) and using overall data. To identify risk factors for ovine coccidiosis on each farm, the parasitological data were analyzed together with the information collected through the questionnaire, such as management information. OPG and pathogenic *Eimeria* and frequency of *E. ovinoidalis* were compared using Microsoft Excel® software using dynamic graphs for management and parasitological data.

4. Results

4.1. Parasitological analysis

4.1.1. Faecal oocyst counts

On all farms sampled, large variations in individual OPG *Eimeria* counts in both ewes and lambs were observed, resulting in high standard deviation and non-normalized distributions. Therefore, faecal oocyst counts are represented as Log (OPG+1).

OPG counts in **adult sheep** ranged from zero oocysts per gram for the lowest frequency on farms G4 and G6, to 6246.3 oocysts per gram on farm G5 for the highest frequency. The overall mean for adult ewes was 2047.1 OPG. Although the large difference between the maximum and minimum results was notable, most of the flocks studied showed quite similar results, with only G4 and G6 farms showing lower OPG values than the rest of the farms (1279.8 and 977.6 OPG, respectively).

The statistical analysis based on Log (OPG+1) data (Fig. 4) allows us to determine that G4 and G6 farms showed significantly lower counts than most of the other farms (G1, G2 and G5) with significant values ranging from $P < 0.05$ to $P < 0.01$, while mean oocyst counts were similar between farms G1, G2, G3, G5 and G7.

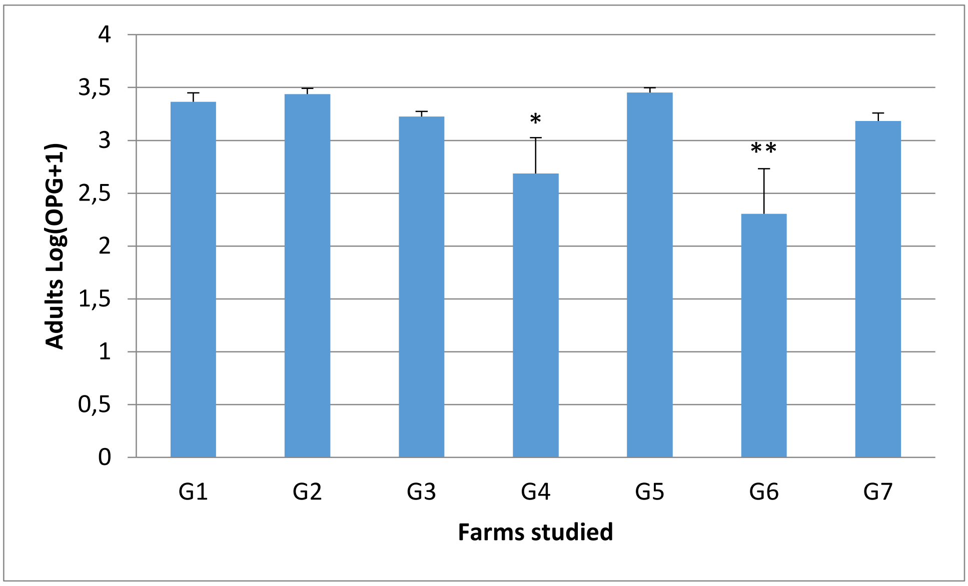

Figure 4: Faecal oocyst counts found in adult sheep in the different farms (G1-G7). Data are represented as mean values of Log (OPG+1) ± SEM (standard error of the mean). * ($P < 0.05$), ** ($P < 0.05$).

As for **lambs**, the maximum OPG count was detected on farm G1 (1250745.8 OPG), while the lowest mean OPG value was found on farm G5, where 3 animals had no faecal oocysts. The overall OPG counts of lambs from the seven farms gave a mean value of 225165.3 oocysts per gram of feces.

Log (OPG+1) in lambs gave results without large variations among farms G1, G2, G3 and G7, as shown in Fig. 5. However, some variations were observed: farm G5 showed significantly lower oocyst counts than the other farms ($P < 0.001$). In addition, farms G4 and G6 also showed lower OPG counts than mean values found in farms G1, G2, G3 and G7 ($P < 0.05$ - $P < 0.01$).

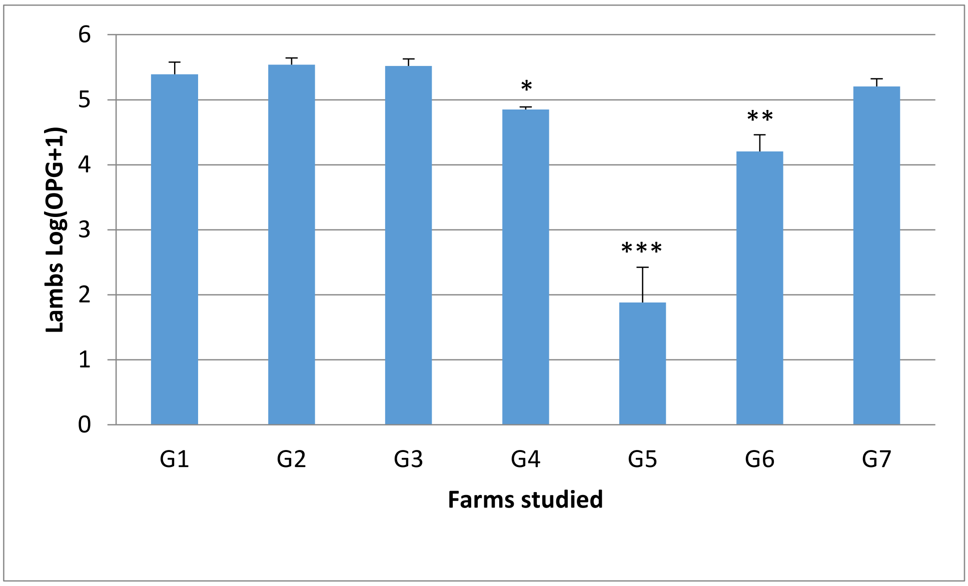

Figure 5. Faecal oocyst counts found in lambs in the different farms (G1-G7). Data are represented as mean values of Log (OPG+1) ± SEM (standard error of the mean). * (*P* < 0.05), ** (*P* < 0.01), *** (*P* < 0.001).

In all farms included in the study, the mean OPG values of lambs were significantly higher than those of ewes. Consequently, the total count of all lambs analyzed in the study was significantly higher (*P* < 0.001) than that found in adult animals, as shown in Fig. 6.

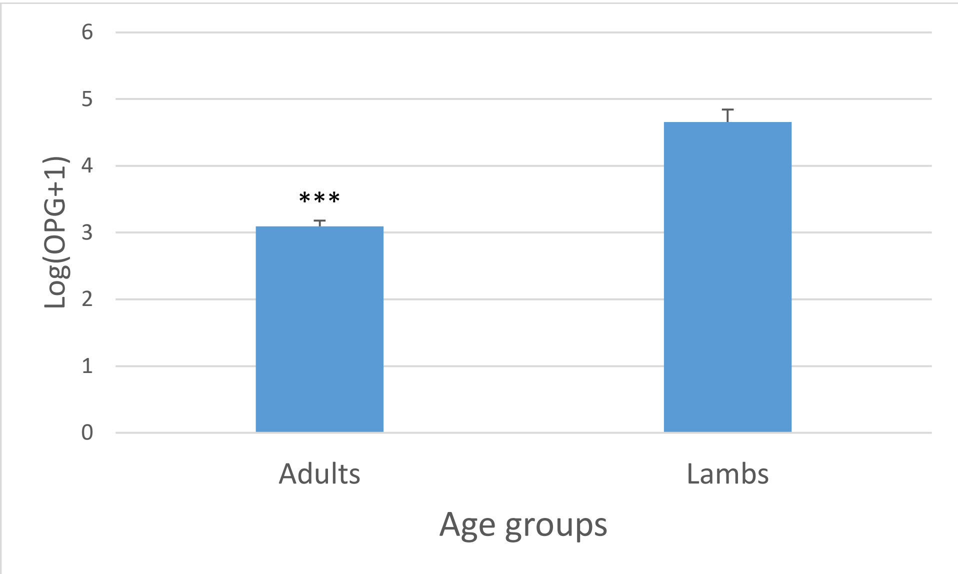

Figure 6. Total faecal oocyst counts found in adult ewes and lambs on all farms combined. Data are represented as mean values of Log (OPG+1) ± SEM (standard error of the mean). *** (*P* < 0.001).

4.1.2. Faecal oocyst speciation

During the speciation process of faecal samples, a total of 2286 oocysts were studied. The frequency for each species was analyzed between farms and age groups (Fig. 7), as well as the proportion of pathological/non-pathological *Eimeria* spp. (Fig. 8).

The most prevalent species for **adult sheep** were *E. ovinoidalis* (27.6%, 347 of 1259 oocysts classified), *E. faurei* (22%, 277/1259) and *E. parva* (21.2%, 267/1259). Intermediate frequencies were also found for *E. weybridgensis/crandallis* (17.2%, 216/1259), while *E. granulosa* (5.4%), *E. bakuensis* (2.6%), *E. ashata* (2.4%), *E. pallida* (0.6%), *E. intricata* (0.6%) and *E. marsica* (0.4%) were minor species in this age group.

In **lambs**, speciation of stool samples showed that also *E. ovinoidalis* was the most prevalent *Eimeria* species (28.8%, 296/1027), followed by *E. weybridgensis/crandallis* (17%, 175/1027), *E. faurei* (14.3%, 147/1027) and *E. parva* (12.3% (126/1027). *E. granulosa* and *E. bakuensis* showed similar frequencies (10.9% and 9.5%, respectively), and that of the remaining *Eimeria* species were even lower (Fig. 5), with *E. intricata* having the lowest frequency (0.1%, 1/1027).

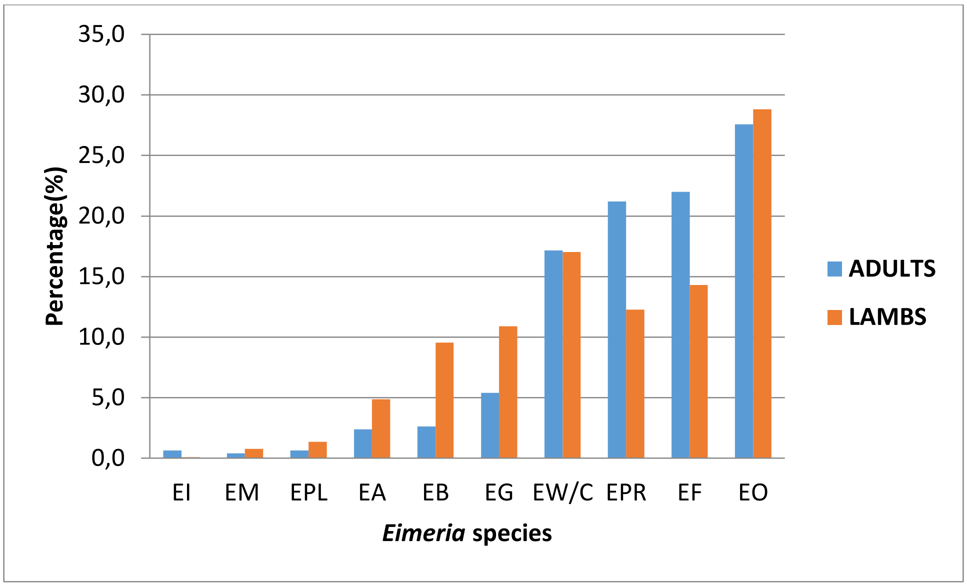

Figure 7: Overall percentage of occurrence of *Eimeria* spp. in faecal samples of the two age groups. EA: *E. ashata*; EB: *E. bakuensis*; EW/C: *E. weybridgensis/crandallis*; EF: *E. faurei*; EG: *E. granulosa*; EI: *E. intricata*; EM: *E. marsica*; EO: *E. ovinoidalis*; EPL: *E. pallida*; EPR: *E. parva*.

Differences in the frequency of *Eimeria* species found in the 7 farms samples in the study were observed (*P*<0.01), but only those that are pathogenic will be described in detail.

In **adults**, *E. ovinoidalis* was found in all farms studied. Its lowest frequency was found on farm G5 with a percentage of 21.5% and the highest frequency was 34.0% on farm G3. **Lamb**s also showed the presence of *E. ovinoidalis* on all seven farms, with minimum (19.4%) and maximum (49.4%) frequencies found in farms G3 and G1, respectively.

E. weybridgensis/crandallis showed different distribution than *E. ovinoidalis* in **adults**, having the lowest frequency with 3.3% in farm G2 and the highest with 27.4% in farm G4, thus showing lower overall frequency values than for *E. ovinoidalis*. As for **lambs**, the lowest (3.9%) and the highest (29.7%) frequencies for EW/C where found in farms G6 and G5, respectively.

Finally, *E. bakuensis* had a lower frequency than the other two species and less variation between farms, with a minimum frequency of 0% for farm G2, and 8.4% for farm G4 in **adults**. **Lambs** also showed a more homogeneous distribution with the lowest percentage in farm G1 with 3.8% and the highest in G6 with 17.6%.

Interestingly, taking into account the overall count between pathogens and non-pathogens (Fig. 8), it could be observed that lambs presented, in more cases, a higher proportion of pathogenic species, specifically, in five of the seven farms. Young animals belonging to farm G5, with 86.5% of pathogenic species, showed the highest rate of pathogenic *Eimeria* species. The adult ewes, however, did not have such high differences in the frequency of pathogenic species, with farm G4 having the highest proportion (64.2%).

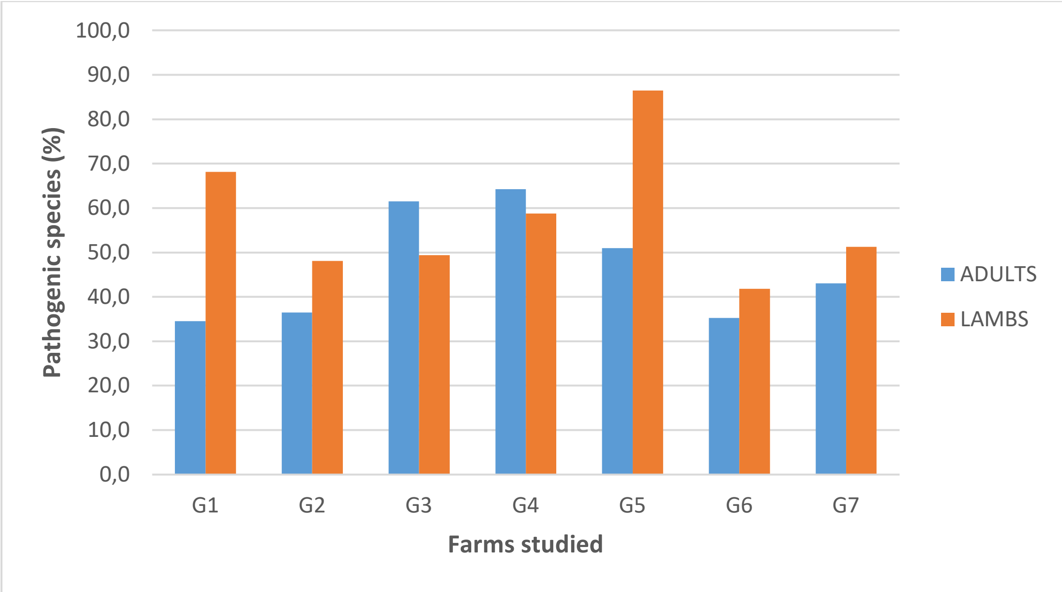

Figure 8: Proportions of pathogenic species among the farms studied.

4.2. Questionnaire analysis

4.2.1. Farm characteristics and management data

Five of the seven farms were considered small (30-200 animals) and only two were of medium size (200-600). The animals on all farms were fed a commercial mix (usually containing alfalfa pellets, wheat, oats, corn and barley), dry grass and, in some occasions, fresh alfalfa (3/7) or fresh pasture grass (2/7).

All the farms studied were dairy farms and the breed reared in all cases was the Canarian Wool Sheep. Six of the farms produced cheese, having similar facilities among them. About 40% of the farms lacked enough pens and therefore did not divide their animals into groups, and one of the seven farms did not have a lazaretto to isolate sick animals.

The 85.7% of the farms studied had only one main lambing period per year, while the G6 farm had two main calving periods per year, one in Autumn and other in Spring. All farms practiced natural lactation.

Semi-intensive and semi-extensive systems (42.8% and 42.8% of the farms, respectively) were the main regime systems, with the practice of transhumance and grazing in about 50% and 100% of the farms, respectively. In only one farm, the animals were reared under an extensive system (14.3%).

Regarding the hygienic care of the facilities, four farms cleaned the accumulated manure weekly, another monthly, another once a year and the last one never removed the manure. Disinfection was applied weekly in 57.1% of the farms sampled, annually in 14.3%, and in 28.6% no disinfection was ever accomplished. In four of the farms, parasitological examination was not carried out previously, and in the other three, it was usually performed annually.

In only one farm no vaccination was applied, however, in the rest of the farms, vaccination against different diseases was applied, ranging from vaccination against 4 diseases in one farm with 30-200 animals to vaccination against 1 disease in four of the farms. Treatment against coccidia was only applied on one farm (G6) and toltrazuril was the anticoccidial used. This drug was applied monthly at 2-8 weeks of age and the time used was less than two hours. On this farm, the application of toltrazuril was reported to be very effective.

4.2.2. Parasitological knowledge

As for coccidiosis, only one of the seven farmers was aware of it and knew about *Eimeria* spp. On the other hand, five of the seven farms gave little importance to the parasite and did not relate *Eimeria* spp. as the cause of diarrhoea or other clinical signs. Only one farm showed awareness and took the disease seriously.

4.2.3. Economic impact

Clinical signs associated with coccidiosis (diarrhea, dehydration, weight loss, death of lambs, etc.) were rarely or never observed in the majority of the farms.

About 43% of the farms spend more than 200€ per year on antiparasitic treatments, one of them dedicated to anticoccidials. Three farmers reported spending between €100 and €200 per year, and only one farmer spent between €10 and €50 on parasite control.

4.3. Correlation between questionnaire and parasitological data

The information obtained from the questionnaires was used to compare different elements of management, clinical signs of coccidiosis or parasite knowledge with main parasitological parameters: oocyst counts per gram, percentage of pathogenic species and frequency of *E. ovinoidalis*, both in adults and lambs. Only the most relevant associations in which a clear relationship between parameters was observed will be detailed below.

4.3.1. OPG accounts correlations (Fig. 9)

Regarding the "farm size" parameter, smaller farms with 30-200 animals had higher **adult** OPG counts than medium-sized farms. The exception was farm G6, which, despite being small in size, had the lowest adult OPG counts in the study (500-1000 oocysts per gram). This farm was also the one with the highest "number of births per year" (2 births/year). Interestingly, the G6 farm was the farm which removed manure less frequently, but the only one that gave a high importance to coccidial infection and, accordingly, routinely applied anticoccidial treatments. As for the rearing system of the farm, only that farm whose regime was extensive did not exceed a count of 1001-2000 OPG. On another hand, clinical signs had no significant relationship with oocyst counts in ewes. The rest of the parameters also showed no correlation with OPG counts in adults, so no further conclusions could be drawn.

As with adults, "farm size" was inversely proportional to **lamb** OPG counts. Also, the only farm with 2 lambing units per year (the largest) had the lowest lamb OPG counts. In contrast to adults, a farm with a semi-extensive regime had the lowest lamb OPG counts. Likewise, farms that did not separate by flocks had the highest OPG counts in lambs. On the other hand, the farm G6 that only disinfected annually had lower OPG counts than those farms that disinfected more frequently. As shown for adults, the only farm that treated for coccidia had the lowest lamb OPG counts in the study.

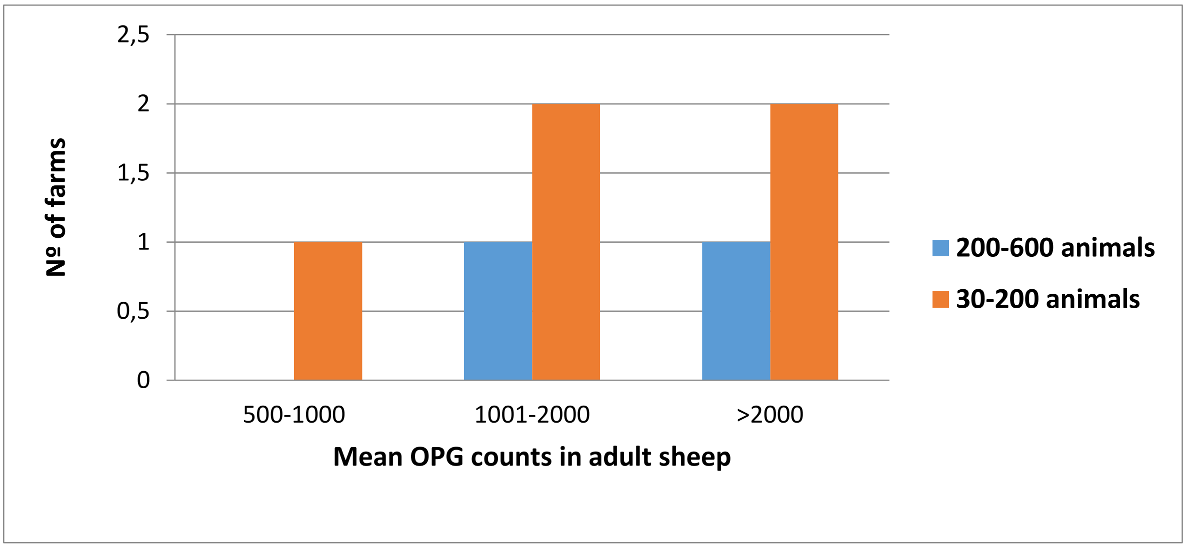

Figure 9: Graph of pivot tables compared in order to analyze the correlation between the questionnaire and parasitological data (e.g. OPG counts in adult ewes versus farm size).

4.3.2. Pathogenic Eimeria spp proportion

The percentage of pathogenic *Eimeria* spp. in **adults** showed no correlations with management, clinical signs or anticoccidial treatments. On reference to the percentage of pathogenic *Eimeria* spp. in **lambs**, an association to the anticoccidial treatment, as well as the importance against parasites and coccidia in particular, was found.

4.3.3. E. ovinoidalis frequency

Particularly in lambs, the frequency of *E. ovinoidalis* showed an association to the use of anticoccidials, observing a higher percentage of *E. ovinoidalis* in those farms not treating against coccidia.

The rest of the survey parameters did not show significant associations with the parasitological data and, therefore, have not been presented.

5. Discussion.

The **general results** obtained in this investigation show the existence of *Eimeria* spp. in each of the 7 farms studied. There were individual variations both in adults and lambs though and, actually, no faecal oocysts were detected in some animals. On the other hand, it has been observed that the most frequently found *Eimeria* species out of a total of 11 was *E. ovinoidalis*, which is considered the most pathogenic in sheep. Overall, *Eimeria* spp. oocysts were found in 95.2% of the samples studied in adults and 95.7% in lambs. Although the infection rate in lambs was, in general, higher than in adult ewes, clinical signs associated with coccidiosis were scarce in both age groups. Comparisons with a previous study carried out on the island of Gran Canaria (Cruz, 2021), showed that the frequency of coccidiosis in adults was lower (70%), however, in lambs it was almost 100%. In this other study, *E. weybridgensis/crandallis* was the most frequently observed and, as we found in our study, clinical signs of coccidiosis were not evident.

In relation to epidemiological studies performed in other locations, the **frequency** found in this study for *Eimeria* infections was generally greater (Yan *et al.,* 2014; de Souza *et al.,* 2015; Macedo *et al.,* 2019; Hassanen *et al.,* 2020; Macedo *et al.,* 2020; Trejo *et al.,* 2020). Interestingly, even with lower prevalence, some studies did report higher clinical infection ratios (Lakew and Seyoum, 2016) compared to those found here based on questionnaires. The present study had a OPG record of 1.25×10^6 OPG, with 10 animals having values over 500 thousand OPG. These peak and also mean values are, in general, lower than those found by Cruz (2021), and in other studies performed in Turkey, Australia and Greece (Saratsis *et al.,* 2011; Yan *et al.,* 2014).

Most authors agree in that clinical signs of coccidiosis are more common and prominent in young **age groups** (Saratsis *et al*., 2011; Lakew and Seyoum, 2016; Hassanen *et al.,* 2020) and, accordingly, lambs consistently have significantly higher oocyst shedding ratios compared to adults, which is in agreement with results obtained in the present study (mean values are almost 100 times greater in lambs *vs* adults). Ruminants begin to present certain immunity against coccidiosis from their first year of life (Keeton and Navarre, 2018) and from this age onwards they may hold *Eimeria* infections asymptomatically. The continuous contact of the ruminant with

Eimeria oocysts causes a constant immunological stimulation and a limited proliferation so it creates an equilibrium with the host that explains an asymptomatic state. However, when the animal presents stress or concomitant diseases, clinical signs may appear (Platzer *et al*., 2005). Therefore, many authors claim that the increased excretion of oocysts and the prominent clinical symptomatology in lambs is due to the lack of immunity in this age group (de Souza *et al.,* 2015; Alcala *et al.,* 2020; Bangoura and Bardsley, 2020; Sirbu *et al*., 2020).

Different frequencies have been reported in different studies for sheep ***Eimeria* species**, but most of them include *E. ovinoidalis* among the most frequently found in agreement to our results. For instance, in one study performed in Paraná (Carneiro *et al*., 2022), the authors reported a dominant prevalence for *E. ovinoidalis*, but with a higher percentage (98.1%) than in our survey. Likewise, in a UK investigation (Al-Neama *et al*., 2021), the most prevalent species was *E. ovinoidalis* together with *E. bakuensis*. Finally, an epidemiological study conducted in Algeria (Meradi and Bentounsi, 2021) showed that *E. intricata* was the most frequently observed (50.3%). High percentages of *E. ovinoidalis* and *E. weybridgensis/crandallis* were also found in that study, which was related to the occurrence of diarrhoea in lambs. In contrast, as referred above, *E. weybridgensis/crandallis* was the most frequent *Eimeria* species in the previous study carried out in Gran Canaria (Cruz, 2021), with *E. ovinoidalis* being the fourth in frequency.

In the UK study referred before ((l-Neama *et al*., 2021), the season of the year affected the prevalence of *E. ovinoidalis*, increasing infection in summer and winter compared to autumn. In contrast, a study in Kazakhstan (Yan *et al*., 2022) revealed that the highest oocyst counts per gram in sheep occurred in spring, followed by autumn, and the lowest in summer and winter. In the present work, a transversal sampling has been done, so the influence of the season can’t be evaluated, but there are studies showing that the prevalence of the different *Eimeria* species may be correlated with the **geographical distribution** due to their adaptation to climatic conditions. Nevertheless, as reported by Cruz (2021), no consistent association were found between frequency or intensity of *Eimeria* infections with the different climatic / geographical areas sampled in our study.

The **surveys** performed in the present work were conducted to identify risk factors of coccidiosis in sheep farms in Gran Canaria through the evaluation of the correlations or associations between the different parameters in the questionnaire (in particular, management conditions) with the parasitological results obtained. The identification of risk factors would help farmers to improve possible management strategies and thus to obtain better yields in the farms.

As mentioned above, all the farms sampled consisted of wool sheep, so it is not known whether there are variations among **breeds** in terms of resistance to coccidia. On the other hand, studies reveal that the pelibuey breed has a higher resistance to certain parasites such as gastrointestinal nematodes (Palomo-Couoh *et al*., 2017), so lower OPGs than those obtained for wool sheep would be expected. However, such resistance is not only breed-dependent, but also has an individual genetic component (Gauly *et al.,* 2001; Jacobson *et al*., 2020) the selection of which could be effective in research to control the presence of coccidia in sheep. In relation to this genetic component, as previously mentioned, there are individual variations within the same farm in terms of OPG values, even in flocks of the same age and under the same management and environmental conditions.

Regarding **farm size**, no clear association between farm size and parasite load has been observed, although smaller farms seem to have the highest oocyst counts in both adult ewes and lambs. These results are contrary to those expected, as higher infection rates are commonly observed in bigger farms due to overcrowding and excrement accumulation (de Souza *et al.,* 2015; Carrau *et al.,* 2018; Bangoura and Bardsley, 2020). This could indicate that smaller farms have inadequate management and poor hygiene measures because they usually have few workers, little knowledge about parasites and reduced space for pens where excrement may eventually accumulate. Interestingly, only one of the smaller farms (G6) had the lowest OPG counts of the entire study in both lambs and adult ewes. The particularities of this farm will be detailed below.

In this study, farms with extensive/semi-extensive **husbandry systems** had the lowest parasite loads in both adults and lambs. There was no significant correlation though, but these results could be explained by a dilution effect over the oocysts excreted through faeces when animals are not restricted to the same pens the whole day (de Souza *et al*., 2015; Lakew and Seyoum, 2016; Carrau *et al.,* 2018).

In relation to the husbandry system, **grazing** and **transhumance** were not observed as relevant factors in the prevalence of coccidian, even though these rearing practices are both closely related to the extensive or semi-extensive systems. In particular, the influence of transhumance on parasite load requires further investigation since, although this management practice implies a dilution effect of the parasitic dissemination elements in the farm of origin, it is possible that an overpopulation of animals may occur in the common pasture area. The latter may also imply that certain parasites can be transmitted from one herd to another (Hinney *et al.,* 2020). Interestingly, on a single farm where both practices were performed, the lowest prevalence of *E. ovinoidalis* was observed compared to the other farms.

Hygiene is a factor with great importance to take into account among management practices, since *Eimeria* spp. is transmitted via faecal-oral, so the accumulation of excrements increases its prevalence (Lakew and Seyoum, 2016; Macedo *et al.,* 2019; Alcala *et al.,* 2020; Jacobson *et al*., 2020; Macedo *et al.,* 2020). Paradoxically, in this study, the farms where cleaning was less frequent presented, in general, lower coccidial counts than those farms where cleaning was weekly. This could indicate inadequate management practices that favour the coccidiosis rate in spite of cleaning, such as overcrowding, inadequate use of cleaning products, etc. On the other hand, the percentage of pathogenic *Eimeria* species in adults showed a high proportion in the farm that was cleaned only once a year; however, in lambs, the highest percentages were found in those farms where cleaning was carried out weekly. Therefore, although hygiene is one of the key points in *Eimeria* spp. control, additional studies are needed to prove its importance in sheep in the Canary Islands in the context of other management practices.

Feeding is a practice with a high effect on the animal's health, and can modify its resistance to parasite infections. In addition, studies have affirmed that there are useful plants to treat some parasitic infections (Chartier and Paraud, 2012; Keeton and Navarre, 2018). In this study, all farms used commercial feed-mix, so no clear conclusions could be drawn. However, other studies have shown that farms that used crop by-products had lower OPG counts in adults, as well as a lower proportion of pathogenic *Eimeria* spp. in adults (Cruz, 2021).

The **lactation system** also was the same in all the farms, therefore, it was not possible to make comparisons among natural and artificial lactation feeding. However, some authors state that the longer the adult-lamb relationship is, the greater the exposure to the parasite can be for lambs (Carrau *et al.,* 2018; Macedo *et al.,* 2020). On the other hand, the transference of passive immunity from ewes to lambs is expected to be higher in natural lactation feeding, although this feature needs further investigation in ruminant coccidiosis (Torres *et al.,* 2021).

In relation to the number of **lambing**, only one farm usually has two lambing per year (G6). The existence of several farrowing per year may correlate with a greater accumulation of oocysts in the pens, which could translate into a higher prevalence (Gauly *et al.,* 2001; de Souza *et al*., 2015; Macedo *et al.,* 2019; Alcala *et al.,* 2020; Jacobson *et al*., 2020). However, contrary to expectations, OPG counts and percentages of pathogenic *Eimeria* species in this farm were the lowest in the study in both adult ewes and lambs.

Vaccination favours the immune response in the host, thus, promoting the host's resistance to different diseases. Thus, an immunocompetent animal is less likely to suffer a clinical disease. This hypothesis could not be demonstrated in our study, probably because vaccines were administered only to adults, and ewes are usually immunocompetent for coccidial infections.

The use of **anticoccidial drugs** presented an inversely proportional association to the OPG values. Of the 7 farms, only farm G6 treated against coccidia and it is there where the OPG values were the lowest found in the study. The anticoccidial treatment would have reduced the parasite load despite the fact that a higher load of oocysts in the environment would be expected in this farm due to the low frequency of manure removal (annual) and the existence of two lambing periods per year. In addition, the percentages of pathogenic species in adult ewes and lambs were low, which supports the efficacy of toltrazuril against pathogenic species (Odden *et al.,* 2018; Odden *et al.,* 2019). However, some of the farms that were not treated against coccidia had a low proportion of *E. ovinoidalis*, so it is difficult to reach a definitive conclusion.

Clinical signs of coccidiosis were almost never observed, except for occasional evidence of weight loss and diarrhoea. Interestingly, cases of diarrhoea were detected in the only farm that treated for coccidiosis (G6), indicating that the farmer is aware of the disease and relates the symptomatology to coccidiosis. In addition, knowing the consequences of this parasitosis, the farmer applies effective treatment and, probably, some other improved management conditions. Interestingly, in the farms with higher OPG values the farmers never referred to the existence of diarrhoea or other symptoms, which could indicate that there is a high percentage of subclinical disease. The absence of clinical coccidiosis could be also corroborated during the faecal sampling of ewes and lambs and it is in agreement to previous data reported by Cruz (2021).

As for clinical signs, deaths were only rarely observed. This finding was found on a farm with the highest OPG values, but a conclusive association could not be demonstrated.

Surprisingly, most of the farmers participating in the surveys were not familiar with the term "coccidiosis" and, in general, they did not associate this disease with the clinical symptoms. Neither did they know *Eimeria* spp. except for only one farm where both terms were known and where a lot of importance was given to parasites and coccidiosis in particular. This general misinformation is largely due to the high proportion of subclinical disease, which makes farmers less concerned about disease prevention.

Coccidiosis is a globally distributed disease, so *Eimeria* spp. infections have a major economic impact, reaching up to $140 million annually in economic losses (Bangoura and Bardsley, 2020). In our study, because clinical symptomatology was rarely observed, the main **economic losses** would have been due to the subclinical presentation of the disease. Subclinical coccidiosis may result in a reduction of the levels of production on farms mainly due to delayed growth rates of lambs. If clinical disease was present, seizure of carcasses and organs that do not meet acceptance criteria may occur at slaughter.

All farms treat sheep against parasites and it has been found that the economic expenditure is not proportional to the size of the farm: some larger farms spend less money per month (€100-200) than other small farms (>€200) on deworming.

However, only one farm treats for coccidia. This farm has 30-200 animals and spends 100-200 € monthly on toltrazuril for the treatment of coccidiosis in 2-8 week old lambs. Anticoccidial treatment in this farm was referred to produce economic benefits despite the costs of treatment, veterinary work and farm workers.

6. Conclusions

- Infection by *Eimeria* spp. is extremely frequent in sheep herds of the Gran Canaria Island (almost 100% of the animals infected), with intensities of infection particularly high in lambs.

- The high rates found for *E. ovinoidalis,* which is considered the most pathogenic *Eimeria* species in sheep, should be taken into account to prevent clinical coccidiosis, particularly in young animals.

- Based on questionnaire data, the anticoccidial treatment with toltrazuril seems to be effective to maintain acceptable levels of coccidian in the farms, although combined improvement of management practices would be advisable.

- Probably because the limited number of farms included in the questionnaire survey, clear associations between management practices and parasitological data could not be demonstrated in most cases, so further studies are need for this purpose.

- Interestingly, most of the farmers were not aware about coccidiosis, which would be a drawback for the establishment of appropriate preventive measures for the control of coccidiosis in these farms

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