

**Naturally occurring
Eimeria infection in
Iberian ibex (*Capra
pyreinaca*) from
North East, Spain
(Aragón)**

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**NATURALLY OCURRING EIMERIA
INFECTION IN IBERIAN IBEX (*CAPRA
PYREINACA*) FROM NORTH EAST, SPAIN**

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ABSTRACT

Species of the genus *Eimeria* are considered specific parasitic protozoa that mainly affect the gastrointestinal tract of a wide variety of mammalian species, causing clinical or subclinical diseases with important consequences for the population. The aim of this study was to analyse the prevalence of *Eimeria* spp. in *Iberian ibex* (*Capra pyrenaica*) in the different provinces of Aragón, and in some populations of stray-feral goats within the same area. A total of 182 samples faecal samples were collected from adult and young animals. The coprological analysis of each sample was carried out based on the quantification of oocysts and the identification of the different species of *Eimeria*. The results obtained showed the presence of *Eimeria* spp. in approximately 70% of the samples and the two *Eimeria* species more frequently found were *E. ninakohlyakimovae* and *E. arloingi*. The prevalence of the parasite was variable depending on the sampling area, with Huesca being the province with a higher level of oocyst excretion. Young *Iberian ibex* showed slightly higher OPG counts than adults in Huesca but, in the other two provinces no differences were observed between adults and young animals in terms of faecal oocyst load. Except for some morphological distinct *Eimeria* oocysts exclusively found in *Iberian ibex*, the frequency, the intensity of infection and the most abundant *Eimeria* species were approximately the same in stray-feral goats and in *Iberian ibex*. The data obtained in the present study have been a contribution to previous publications in Spain about parasites in *Capra pyrenaica*, allowing for a more solid and consistent conclusion.

Key words: Iberian ibex, Provinces of Aragón, Coprological analysis, *Eimeria* spp.,



1. INTRODUCTION

The Iberian ibex is the only native wild caprine species in the Iberian Peninsula (*Capra pyrenaica*), which was originally distributed throughout the Iberian range and in southwestern of France (Pérez *et al.*, 2002). Historically, four subspecies of Iberian ibex have been recognized based on coat colour and horn morphology. However, during the last two centuries, Iberian ibex suffered a considerable population decline due to the progressive destruction of its natural habitat, overgrazing, epidemics diseases, and uncontrolled hunting (Fandos, 1991), which resulted in its extinction in the northernmost areas of its native range, in the Pyrenees (Herrero *et al.*, 2021).

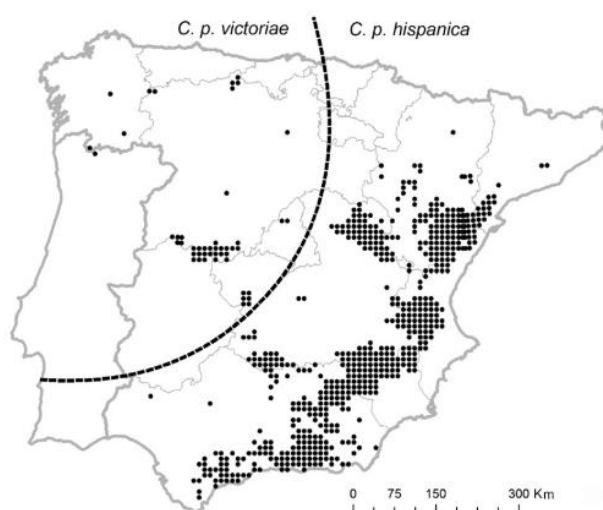


Figure 1. Estimated distribution of the two subspecies of Iberian ibex in the Iberian Peninsula in 2011. The discontinuous line represents the distribution between *Capra pyrenaica victoriae* in the northwest and *Capra pyrenaica hispanica* in the south and east of Spain (Acevedo and Real, 2011).

Currently, only two subspecies of Iberian ibex are located in the Iberian Peninsula, *Capra pyrenaica victoriae* and *Capra pyrenaica hispanica* (Acevedo and Real 2011). The other two subspecies have become extinct, *Capra pyrenaica pyrenaica* in the beginning of 21st century and *Capra pyrenaica lusitanica*, at the end of the 19th century (Cabrera, 1914; Granados *et al.*, 2001; Pérez *et al.*, 2002). *Capra pyrenaica victoriae* occurs in the central Iberian mountains (Sierra de Gredos) (Acevedo and Real, 2011). In order to strengthen its conservation status, this subspecies has been reintroduced into other locations, such as Las Batuecas, La Pedriza, Riaño, Ancares, Invernaderio, Val d’Aran and Serra do Xurés, from where it moved to Portugal and France



(Pyrenees), afterwards (Moço *et al.*, 2006; Herrero and Pérez, 2008; Acevedo *et al.*, 2009). On the other hand, *Capra pyrenaica hispanica* occupies the arc of mountains of the Mediterranean coast, from Cádiz to the Pyrenees. This subspecies has been also introduced in some places such as Montserrat, where its reinforcements were developed (Herrero *et al.*, 2021).

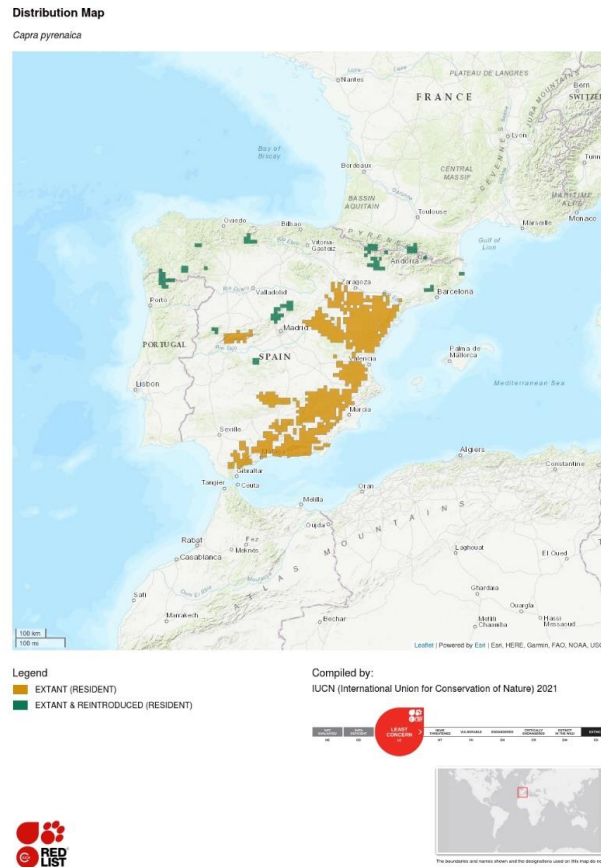


Figure 2. Recent distribution (2021) of the Iberian ibex in the Iberian Peninsula.

The main populations of Iberian ibex remain in their usual locations (yellow squares) as also shown in Fig. 1. In addition, *Capra pyrenaica victoriae* has been reintroduced in the south of France, Catalonia, and in the north of Portugal (from Galicia), which implies that the population is expanding (green squares) (Herrero *et al.*, 2021).

According to Acevedo and Real (2011), the Iberian ibex is mainly distributed in the southeast and northwest of the Iberian Peninsula (Fig.1). However, a recent study (Herrero *et al.*, 2021) has reported an updated distribution of the subspecies, describing its reintroduction and expansion in several territories (Fig.2).

Up to date, some studies reported that the increasing presence of domestic livestock and exotic ungulates in the ibex's range was the main threats to the conservation of



Iberian ibex subspecies, such as *Capra pyrenaica hispanica*, as it is a risk factor for cross transmission of pathogens between wild and domestic caprine (Acevedo *et al.*, 2005a; Gortázar *et al.*, 2006; Richomme *et al.*, 2006; Walker and Morgan 2014). Therefore, it is essential to monitor the health of livestock and wildlife to identify threats and manage their populations (Richomme *et al.*, 2006; Fonseca *et al.*, 2017). Some of these major threats to the Iberian ibex population include parasitic infections.

1.1 Parasitic infections in the Iberian ibex

Parasitic infections can affect goat population viability, due to these infections represent a relevant hazard for these animals, thereby triggering important economic losses in domestic goats under different breeding systems and being some of the major threats to wild goats (Manfredi *et al.*, 2010, Lambertz *et al.*, 2018). Because of the interaction between wild and domestic goats, some studies focusing on parasitic infections in wildlife have revealed a wide diversity of parasites in the Iberian Peninsula. In the Iberian ibex, some species of gastrointestinal nematodes, lungworms, coccidia and cestodes have been described. Several cases of sarcoptic mange and oestrosis have been also reported in *Capra pyrenaica* (Bernal, 2020).

1.1.1 Gastrointestinal parasites, lungworms and cestodes

Previous studies in *Capra pyrenaica* have identified several species of trichostrongylid nematodes included into the genera *Teladorsagia*, *Ostertagia*, *Trichostrongylus*, *Nematodirus* and *Marshallagia*, where *Teladorsagia* spp. were the most common gastrointestinal nematode in Iberian ibexes (Pérez *et al.*, 2003; Cardoso *et al.*, 2021). Bronchopulmonary nematodes of the family Protostrongylidae (*Muellerius*, *Neostrongylus*, *Cystocaulus* and *Protostrongylus*) and intestinal cestodes of the family Anoplocephalidae (*Moniezia expansa* and *M. benedeni*) were also identified in Iberian ibexes (Luzón *et al.*, 2008; Santiago-Moreno *et al.*, 2010).

1.1.2 Sarcoptic mange

In Spain, sarcoptidosis has severely affected population of Cantabrian chamois, *Rupicapra pyrenaica parva*, in the north-west (Fernández Morán *et al.*, 1997) and Iberian ibex, *Capra pyrenaica* (Fandos, 1991; Pérez *et al.*, 1997; Leon Vizcaino *et al.*, 1999) and Barbary sheep, also known as aoudad, *Ammotragus lervia* (González Candela *et al.*, 2004) in the south; in some cases with mortalities exceeding 90% (Fandos, 1991; Leon Vizcaino *et al.*, 1999). This parasitic infection is classified as a



multi-categorical variable based on lesions that differentiate infected from non-infected animals (Pérez *et al.*, 2003; Serrano *et al.*, 2007; Alasaad *et al.*, 2008; Pence and Ueckermann, 2022).

In southern Spain, specifically in the Sierra Nevada Natural Area a group of rangers was trained to monitor the sarcoptidosis affecting wild goats using binoculars and telescopes during a monthly program of fieldwork (Granados *et al.*, 2008). Then, each animal was classified into a specific category based on a visually estimated percentage of affected skin. This protocol is very similar to the methodology used in north-west Spain for monitoring sarcoptic mange in a population of Cantabrian chamois, *Rupicapra pyrenaica parva* (González-Quirós and Solano, 2009). However, the usefulness of these mange categories has never been specifically validated.

1.1.3 Oestrosis

Oestrosis is one of the most prevalent parasitosis affecting Iberian ibex, *Capra pyrenaica* (Arias *et al.*, 2014). *Oestrus* spp. larvae develop inside the nasal cavities and frontal sinuses of domestic and wild hosts (Zumpt, 1965). *Oestrus* spp. parasitizes sheep and goats worldwide, having been described in Asiatic ibex (*Capra ibex sibirica*), argali, bighorn sheep and European mouflon (Grunin, 1957; Capelle, 1966; Wetzel & Bauristhene, 1970; Moreno *et al.*, 1999). Later, according to Minar (1985) and Pérez (1996) *Oestrus caucasicus* was found to parasitize Asiatic ibex in Mongolia and Iberian ibex; *Capra pyrenaica* in southern Spain. On the other hand, *Oestrus ovis*, have been reported in sheep and domestic goats in the Iberian Peninsula. Therefore, *Oestrus ovis* and *Oestrus caucasicus* are the two most frequent species affecting domestic and wild goats and sheep in Spain.

1.2 Coccidiosis in domestic goats and in the Iberian ibex

Coccidiosis is an important protozoan infection caused by species of the genus *Eimeria*, which causes several economic losses and, in turn, leads to population decline, resulting from the high mortality rate among goat kids, reduction of productivity and delayed weight gains of parasitized animals (Lima, 2004; Ruiz *et al.*, 2012).

1.2.1 *Eimeria* species and geographical distribution in domestic goats

Eimeria infections are highly common in a large variety of mammalian host species in which young animals are highly susceptible to infection, causing clinical or subclinical disease with significant population consequences (Foreyt 1990; Daugschies and



Najdrowski 2005). Despite the great variety of host, *Eimeria* species are considered highly host-specific, so cross infection is not possible.

The occurrence of *Eimeria* in small ruminants is worldwide (Chartier and Paraud, 2012).

Sixteen *Eimeria* species have been described in goats (Terrones, 2018), wherein the most prevalent are shown in Fig. 3. For instance, in Europe, in temperate areas, the most prevalent are *E. ninakohlyakimovae* and *E. arloingi* (Yvoré *et al.*, 1981). In semi-arid areas of Gran Canaria, *E. ninakohlyakimovae* (30%), *E. arloingi* (28.6%) and *E. alijevei* (20.5%) have been reported (Ruiz *et al.*, 2006), whereas in midwestern United States of America, the most frequent are *E. arloingi* (98.8%), *Eimeria christenseni* (58.2%), *E. ninakohlyakimovae* and *E. parva* (33.3%) (Lima, 1980).

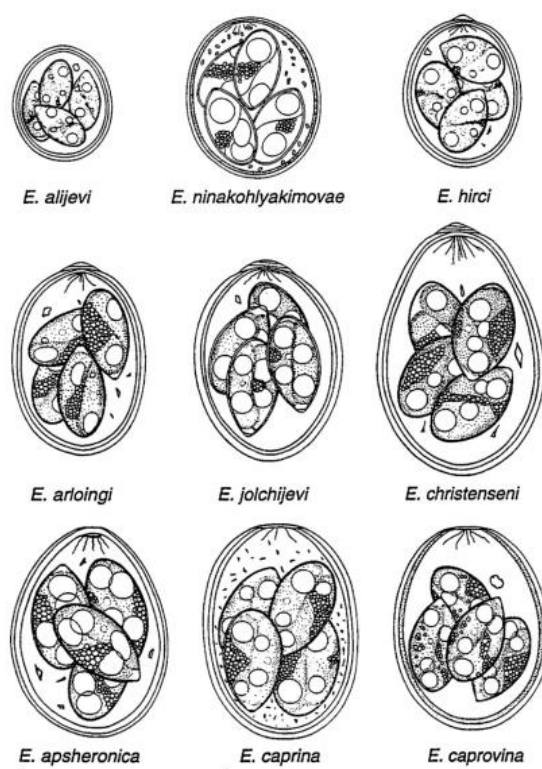


Figure 3. Sporulated oocyst of the most prevalent *Eimeria* species in goats (Chartier and Paraud 2012).

1.2.2 Geographical distribution of *Eimeria* species in the Iberian ibex

The Iberian ibex are mostly parasitised by the genus *Eimeria*, where its prevalence in some studies ranges from 43% in Salamanca to 74% in Andalucía, in southern Spain (Pérez Jiménez, 2005; Ramajo *et al.*, 2007). In addition, higher excretion rates have



been described in Iberian ibex in northwestern Spain due to favourable conditions for *Eimeria* survival compared to the southern of the Iberian Peninsula. However, coccidia showed a higher mean oocysts per gram in domestic goats than in Iberian ibex (Cardoso *et al.*, 2021).

In the Sierra de Gredos, located in the north-west of Spain, six species of *Eimeria* have been identified in *Capra pyrenaica victoriae*; *E. intricata*, *E. parva*, *E. faurei*, *E. gonzalezi*, *E. ovina*, and *E. crandalli* (Bernal *et al.*, 2020).

On the other hand, in the Sierra de Guadarrama National Park, which extends southwest–northeast of Madrid and Segovia, the most prevalent *Eimeria* species were *E. christenseni* with values ranging from 55% to 60%; followed by *E. arloingi-cylindrica* complexes and *E. caprina-caprovina* (Araujo *et al.*, 2020).

1.2.3 Life cycle of *Eimeria* spp.

The life cycle of this protozoa requires a single host and includes an exogenous phase (**sporogony**) and an endogenous phase (**schizogony and metogony**) (Soulsby, 1982).

As it is shown in Fig. 5, after the ingestion of oocysts by the host, sporozoites (Fig.4) penetrate epithelial cells of the small intestine to transform into a schizont. Afterwards, two or more schizogonies (asexual multiplications) take place in the small or large intestines depending on the *Eimeria* spp. Finally, the resulting merozoites penetrate the epithelial cells of the large intestine, leading to the sexual phase or gamogony, which leads to the production of gamonts, gametes and then non-sporulated oocyst, which are released in the faeces (Foreyt, 1990).

Non-sporulated oocyst, sporulate in the environment to become infective (Fig. 4) (Ernst and Benz 1986; Shirley *et al.*, 2005, Bangoura and Dauschies 2018). Oocyst sporulated within 2-7 days, depending on the *Eimeria* species and environmental conditions such as humidity, oxygen, and temperature. Although sporulated oocyst are resistance in the environment, extreme desiccation, elevated temperatures, and exposure to direct sunlight have been shown to promote rapid deterioration of oocysts, which are lethal conditions for oocysts (Foreyt, 1990).

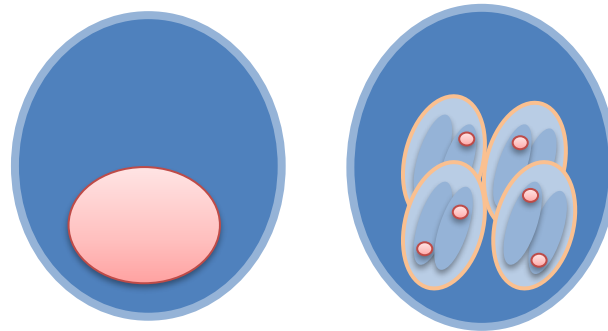


Figure 4. Non-sporulated oocysts of *Eimeria* spp. (left) and sporulated oocyst (right), characterised by two sporocysts and four sporozoites each.

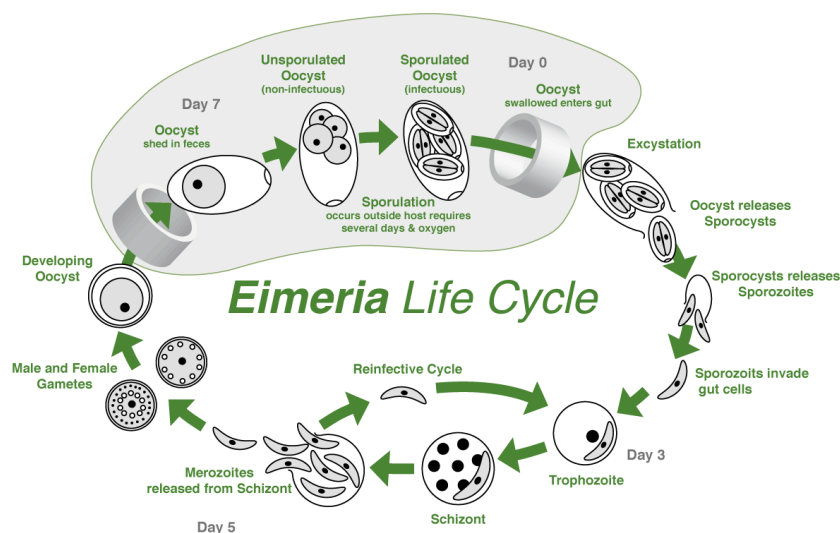


Figure 5. *Eimeria* life cycle (López-Osorio *et al.*, 2020)

E. ninakohlyakimovae and *E. arloingi* are the most pathogenic species in goats, causing destruction of intestinal epithelial cells and a massive change of the natural microflora (Yvoré *et al.*, 1985; Koudela and Bokova, 1998). In particular, *E. ninakohlyakimovae* develops the first schizogony in the ileum around 10 days post-infection, the second schizogony in the crypt cells of the cecum and colon (12 days post-infection) and finally, the gamogony occurs in the large intestine (13 days post-infection), with a prepatent period of 15 days (Vieira *et al.*, 1997).

1.2.4 Incidence and risk factors of coccidiosis in goats

Coccidiosis is a clinically limiting disease that occurs worldwide. Two conditions can lead to clinical coccidiosis, massive ingestion of sporulated oocysts due to a highly infected environment and significant asexual multiplication in the host. Moreover,



factors such as stress, poor conditions of hygiene, weaning, transportation, among others, can deplete immunity in the host, thus aggravating the infection (Chartier and Paraud, 2012).

1.2.5 Pathogenesis of coccidiosis in goats

In coccidiosis, the intensity of oocyst excretion is of great importance, as it is the main source of infection for other individuals, with young animals being the major excretors of oocysts.

In clinical coccidiosis, symptoms are usually recorded among the animals around 2-4 weeks after weaning. The main symptom in sheep and goats is diarrhoea; the faeces may be watery with clumps of mucus and the colours may change from brown to dark yellow (Koudela and Bokova, 1998).

We can also find animals with weight loss and dehydration, and the general condition of the animal worsens due to decrease appetite. In some cases, coccidiosis can even be characterised by sudden mortality without preceding digestive signs, especially in young animals between 2-4 months old (Chartier, 2009).

In subclinical coccidiosis, goat kids show impaired of growth, so prophylactic administration of anticoccidial treatments before and after weaning is very important (Foreyt, 1990).

In general, in small ruminants *Eimeria* spp. causes a catarrhal enteritis in jejunum, ileum, caecum and proximal colon that can be congestive and haemorrhagic with mucus and fibrin (Koudela and Bokova, 1998; Khodakaram Tafti and Mansourian, 2008). Also, the mucosa and submucosa show significant oedema and small greyish-white lesions, which appear to resemble mottled nodules about 1-2mm in diameter (Koudela and Bokova, 1998)

1.2.6 Diagnosis

The appearance of certain digestive symptoms such as diarrhoea, impairment of growth, sudden mortality during the weaning period, and poor hygienic conditions suggest a possible coccidiosis.

As *Eimeria* spp. has a faecal-oral transmission, a coproscopic examination is necessary, based on McMaster's technique with NaCl or MgSO₄, flotation and sedimentation technique (Yvoré *et al.*, 1987). *Eimeria* spp. can be identified from



sporulated oocysts, whose diagnostic criteria include the size, shape, and the presence of characteristic elements such as polar cap, micropyle, appearance of the oocyst wall, colour, among others (Eckert *et al.*, 1995).

Moreover, during necropsy, examination of intestine allows visualisation of possible pathognomonic lesion of coccidiosis.

1.2.7 Treatment

In general, a preventive treatment is preferred to therapeutic treatment as this reduces both the multiplication of the parasite and disease burden in affected animals (Conlogue *et al.*, 1984; Gräfner *et al.*, 1985; Mundt *et al.*, 2005a). If any case, it is of great importance to start treatment as soon as possible, (Chartier and Paraud, 2012). To this end, anticoccidials drugs can be used to treat livestock with clinical or subclinical coccidiosis problems (Dauguschies and Najdrowski, 2005). These include *sulfonamides* which are antibiotics whose viability is both for preventive and therapeutic use, as they have a direct inhibitory effect on both *Eimeria* schizonts and sexual stages (Bangoura *et al.*, 2022); *amprolium*, a coccidiostat that inhibits the first asexual stages of *Eimeria* and causes degeneration of schizonts (Ryley and Wilson, 1976; Danforth and Anderson, 1989); *ionophores* which are anticoccidial compounds whose efficacy is based on inhibition of sporozoites and extracellular merozoites activity (Mehlhorn *et al.*, 1983, Noack *et al.*, 2019); and *triazines* (toltrazuril, diclazuril) coccidiocidal drugs that act immediately on early and late stages of internal development, and do not require long treatment periods (Haberkorn and Schulz, 1981).

1.2.8 Control

Due to coccidiosis importance, it is essential to carry out exhaustive control and prevention procedures.

Firstly, the control of *Eimeria* infections in wildlife is relatively complicated, so control measures are limited to domestic animal populations. However, the application of antiparasitic treatments in captive wild animals has been described, as it prevents the introduction of infected animals and, consequently, the spread of the disease to the rest of the population (Villanúa *et al.*, 2006). Other reason for introducing antiparasitic treatments is to reduce the incidence of coccidiosis and the exceeding mortality in wildlife (Foreyt *et al.*, 2004). However, it is good to know that anticoccidial treatments



can lead to the selection of parasite strains resistance to these compounds, so regular monitoring of treated animals is necessary (Foreyt, 1990).

In summary, control of *Eimeria* does not aim to eliminate the pathogen, which is almost impossible, but to reduce the disease to acceptable levels, leading to a reduction of the disease while the host is still able to establish a protective immune response (Bangoura and Bardsley 2020).

2. OBJECTIVE

The general objective of the present study was to assess parasitization by *Eimeria* spp. in Iberian ibex (*Capra pyrenaica*) in northwestern Spain.

Specific objectives:

- 1) To evaluate the frequency and intensity of parasitization
- 2) To compare between adult and juvenile populations.
- 3) To determine differences between provinces / geographical location
- 4) To compare with stray-feral goats sharing the same habitat.

3. MATERIALS AND METHODS

3.1 Study area and distribution

This study was carried out during July and August 2021 in Spain, specifically in the region of Aragon (41° N 1° W), covering a total area of 47 719,2 km², of which 15 636,2 km² belong to the province of Huesca, 17 274,3 km² to the province of Zaragoza and 14 808,7 km² to the province of Teruel.

Aragon is located in the center of the Ebro depression, being surrounded by two high mountainous areas, the Pyrenees to the north and the Iberian system to the south (Peña *et al.*, 2004).

Aragón is included in the continental Mediterranean climate, with cold winters and hot, dry summers. However, the orographic characteristics of the territory impose a varied range of climatic environments, from the extreme aridity of the central lands of the Ebro to the permanent snows of the highest peaks of the Pyrenees (Peña *et al.*, 2004).



The altitudinal variations in the aragonese territory determine a wide range of thermal values. In summer, temperatures in the Ebro Valley are above 24°C, while in the Pyrenean peaks they are below 12°C. On the other hand, in winter, as the average altitude increases towards the Pyrenees and the Iberian System, the temperature oscillates below 6°C, being below 0°C in these regions (Atlas Climático de Aragón).

Furthermore, precipitation is characterized by high variability and the presence of long periods of drought. The precipitation regimen is divided into two maximum rainfall periods in spring and autumn, separated by minimums in summer and winter. In summer, the average annual rainfall is less than 150 mm, which leads to a significant water deficit and high aridity, whereas in autumn there is a generalized increase in precipitation throughout the territory, with rainfall exceeding 100 mm in a period of less than 24 hours (Atlas Climático de Aragón).

The habitat is heterogeneous: in the higher areas there are forests with pines, firs, beeches, oaks, thickets, and meadows, while in the Ebro valley, holm, oaks, and junipers are predominant.

As shown in Figure 6, the samples were collected in the Sierra de Guara (42°17'19" N 0°13'46" W), located at 25 km northwest of Huesca and covering an area of 80,000 ha, specifically in Vadiello reservoir (726595/4680537 724520/4680221 724236/4680259) and Vadiello road (724354/4679293), Calcón reservoir (728926/4678931) and mount Aruabón (729191/4682869 729017/4683038 728410/4683395).

Moreover, some samples were collected in Mezalocha (41°25'34"N 1°05'00"W), a municipality of Campo de Cariñena in the province of Zaragoza; specifically, samples were collected at the Mezalocha reservoir and the Carrascoza ravine (660314/4584844 660367/4586666).

Finally, samples were also taken in different areas of the province of Teruel: Sierra de Palomera (651876/4495841), Castelfrío (675948/4481222), Mosqueurela (717305/4472648 717272/4472154 718312/4470486), Villarlengo (708941/4502409 708417/4502439), Ejulve (711488/4523530 712380/4523463) and Montalbán (696354/4519687 695251/4519719).



Figure 6. Sampling sites in Aragon. Provinces: **A.** Huesca ($42^{\circ}08'24''\text{N}$ $0^{\circ}24'32''\text{W}$), **B.** Zaragoza ($41^{\circ}39'00''\text{N}$ $0^{\circ}53'00''\text{W}$), **C.** Teruel ($40^{\circ}20'37''\text{N}$ $1^{\circ}06'26''\text{W}$).

3.2 Animals and samples

Faecal samples were collected on the ground from 186 Iberian ibex, 54 from young animals and 128 from adult animals (Fig. 7). Only isolated faecal depositions presumed to be from a single animal were collected, making sure that they were as fresh as possible. In the collection of faeces from young animals, only faecal pellets less than half the size of those from adults were selected.

About 2-5 grams were collected with latex gloves and then, kept at a room temperature in flasks containing 2% potassium dichromate. The exact weight of the faecal material was recorded for further estimation of the parasitic load. All samples were shaken daily for at least 7 days to ensure complete sporulation. Afterwards, the samples were maintained at 4°C until parasitological analysis were performed. All samples were analysed in the Parasitology Unit of the University of Las Palmas de Gran Canaria (Department of Animal Pathology).



LOCATION	ADULTS ANIMALS	YOUNG ANIMALS
PRESA VADIELLO	14	8
MONTE ARUABÓN	6	3
CARRETERA VADIELLO	9	6
PRESA CALCÓN	8	8
VILLANUEVA HUERVA	7	6
MONTE EMBALSE MEZALOCHA	10	2
SIERRA DE PALOMERA	8	9
CERRO DE CASTELFRÍO	10	0
MONTE DE MOSQUEURELA	20	0
MONTE DE VILLARLUENGO	10	0
MONTE DE EJULVE	13	6
MONTE MONTALBÁN	13	6
TOTAL	128	54

Figure 7. Samples of adult and young animals according to their location.

As shown in Figure 7, a total of 74 faeces samples of adult animals were collected in the province of Teruel, with the highest number of samples collected in Monte Mosqueurela, while the lowest number was collected in the province of Zaragoza. On the other hand, samples from young animals were higher in the province of Huesca, with a total of 25 samples.

3.3 Coprological analysis

Each sample was analysed using the flotation technique combined with the McMaster technique to verify and quantify the presence of *Eimeria* oocysts.

First, the entire sample was mixed and homogenized with saturated saline solution in a 30 ml tube. Then, the corresponding dilution was performed according to the grams of the sample (1-3 gr: dilution $\frac{1}{2}$; >3 gr: dilution $\frac{1}{4}$). The content of the tube was transferred to a glass which was completely filled and then, the object-glass was gently dropped on the liquid surface as shown in Figure 8 and kept for 40 minutes until the flotation technique was completed.



While the flotation was being completed, the McMaster technique was performed, which consisted of taking the previously made dilution and using a Pasteur pipette fill both compartment of the count cell (Fig.9), and then, proceeding to count them by microscope examination.

As shown in the Figure 9, each compartment has 6 grids (in green) in which oocysts float on the surface. Thus, counting begins in one of these grids and continues in the following ones, without counting oocysts outside the grids.

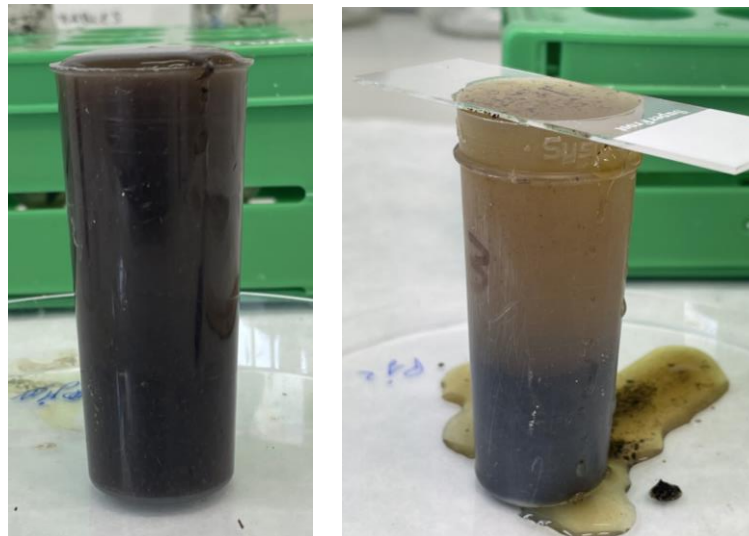


Figure 8. Flotation method.



Figure 9. Compartment of the count cell. Each compartment has a surface area of 10 x 10 mm, with a space between object-glass and coverslip of 1.5 mm (Thienpont *et al.*, 1979).

During the microscopic visualization of the flotation samples, longitudinal and transverse measurements of the oocysts were recorded, as well as the corresponding photographs for subsequent speciation analysis, for which a maximum of 20 oocysts were taken as a reference for each individual sample. *Eimeria* oocysts were visualized in an Olympus microscope (Olympus CH40) and associated camera (Motican BTW)



by using a x40 objective. The program Moti Connect was employed for measurements and picture recording according to keys reported previously (Levine and Ivens, 1986; Alyousif *et al.*, 1992; Soe and Pomroy, 1992).

3.4. 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.

4. RESULTS

4.1 Faecal oocysts counts

Global differences between breeds and age range

Eimeria oocysts were detected in 91.30% of samples from stray-feral adult goats, and in 71.43% of samples from young goats, while in Iberian ibex the frequency was 73.33% and 70% for adults and young individuals, respectively.

OPG counts in adult stray-feral goats ranged from zero oocysts per gram for the minimum value to 5405.41 oocysts per gram for the maximum value, with an overall mean of 1545.65 OPG. In adult Iberian ibex faecal oocyst counts ranged also from zero oocysts per gram as the minimum to 51428.57 oocysts per gram as the maximum value, with an overall mean of 774.59 OPG.

On the other hand, in young stray-feral goats the OPG counts ranged from zero to 3454.55 oocysts per gram for the maximum, with a mean of 1174.97 OPG, while in Iberian ibex ranged from zero to 120000.00 oocysts per gram for the maximum value, with a mean of 3997.21 OPG.

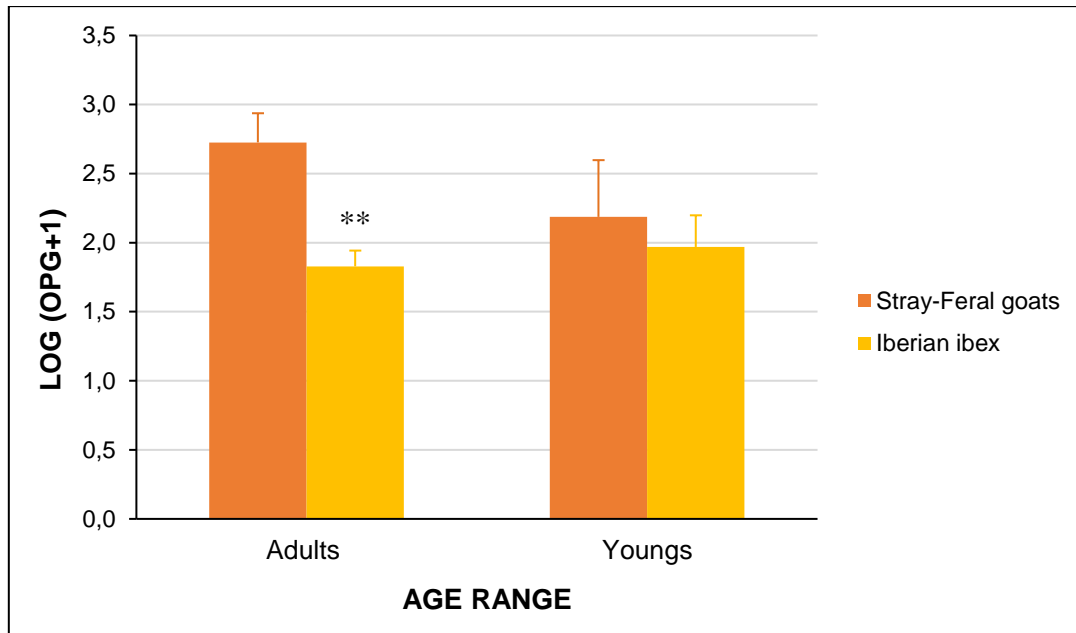


Figure 10. Global oocysts per gram (OPG) in adults and young stray-feral goats and *Iberian ibex*. Data are represented as mean values of the logarithm of the OPG plus one (Log [OPG+1]) \pm SEM (standard error of the mean). ** ($P < 0.01$).

As shown in Figure 10, the statistical analysis based on Log (OPG+1) data showed that in the young goats group there was almost no significant differences, due to the huge standard deviation, although mean oocyst counts in the stray-feral goats were slightly higher when compared to those of the Iberian ibex. By contrast, adult stray-feral goats showed significantly higher values than adult Iberian ibex ($P < 0.01$). When comparing oocyst counts between adult and young animals, no significant differences could be proven in any of the two breeds of goats.

Differences according to breed and age range in Huesca

As faecal samples of stray-feral goats were only taken from the province of Huesca, at two different locations (Monte de Aruabón and Presa de Calcón), an additional analysis in comparison to samples from Iberian ibex from this province was performed. Oocysts were detected in 91.30% of samples from stray-feral adult goats, and in 71.43% of samples from young goats, while 100% of the adult and young Iberian ibex were positive for *Eimeria* oocysts.

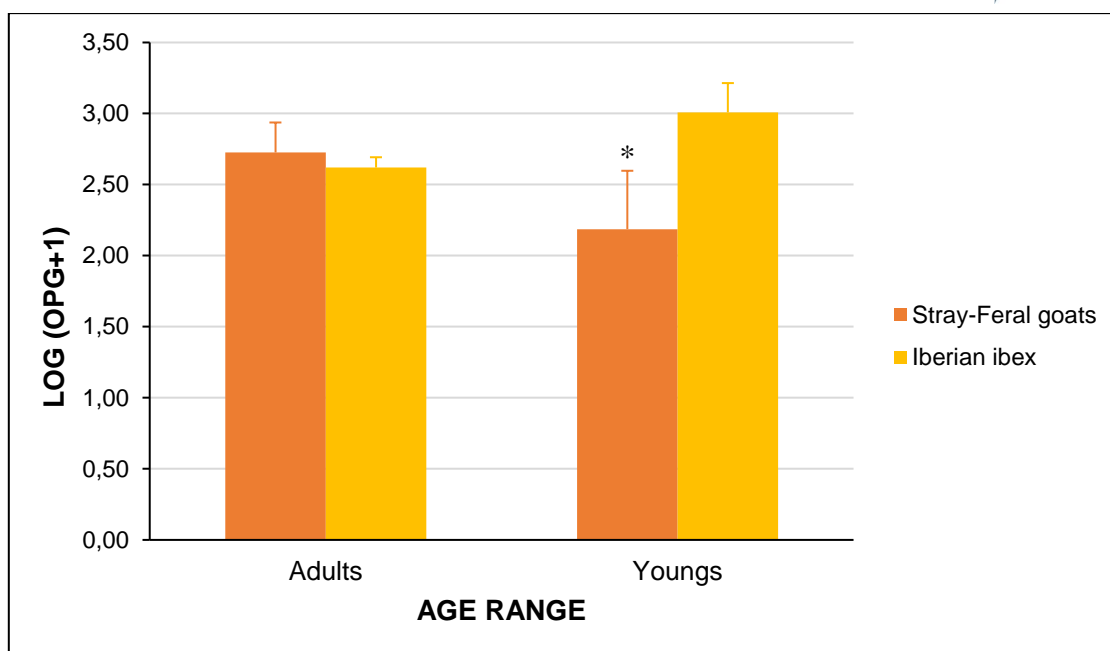


Figure 11. Total oocysts per gram (OPG) in adults and young stray-feral goats and Iberian ibex in Huesca locations. Data are represented as mean values of the logarithm of the OPG plus one ($\text{Log} [\text{OPG} + 1]$) \pm SEM (standard error of the mean). * ($P < 0.05$).

OPG counts in adult stray-feral goats ranged from zero as the minimum to 5405.41 as the maximum, while in youngs ranged from zero to 3454.55 as the maximum. On the other side, in adult Iberian ibex oocysts per gram ranged from 170.21 to 1032.26, whereas in youngs the faecal counts varied from 111.11 to 18421.05 as the maximum.

The statistical analysis based on $\text{Log} (\text{OPG}+1)$ data in Figure 11, showed almost no differences in adult individuals between the two populations; however, young Iberian ibex showed a higher value compared to stray-feral goats ($P < 0.05$). On the other hand, adult Iberian ibex had higher oocyst counts than young individuals, while the opposite was found for stray-feral goats; however, no statistical differences could be proven in any of the two breeds of goats.

Differences according to province in Iberian ibex

Oocysts were detected in 100% of samples from the province of Huesca, in both adults and young Iberian ibex. On the other hand, in the province of Zaragoza, oocysts were detected in 47.05% of adult samples, and in 62.5% of young samples, while, in Teruel they were detected in 74.32% of adult samples and in 57.14% of young samples.



In the province of Huesca, OPG counts in adult Iberian ibex ranged from 170.21 oocysts per gram as the minimum value to 1032.26 oocysts per gram as the maximum value, with a mean of 489.10 OPG, while, in Zaragoza OPG counts ranged from zero to 1375.00 oocysts per gram for the maximum, and a mean value of 282.29 OPG. Finally, in Teruel, OPG counts oscillated between zero oocysts per gram to 51428.57 oocysts per gram as the maximum value, with a mean of 941.70 OPG.

As for young Iberian ibex in Huesca, OPG counts ranged from 111.11 to 18421.05 oocysts per gram, with a mean of 2907.89 OPG. In contrast, OPG counts in Zaragoza and Teruel varied between zero for the minimum value to 1714.29 and 120000.00 for the maximum, having mean records of 443.37 and 5921.66, respectively.

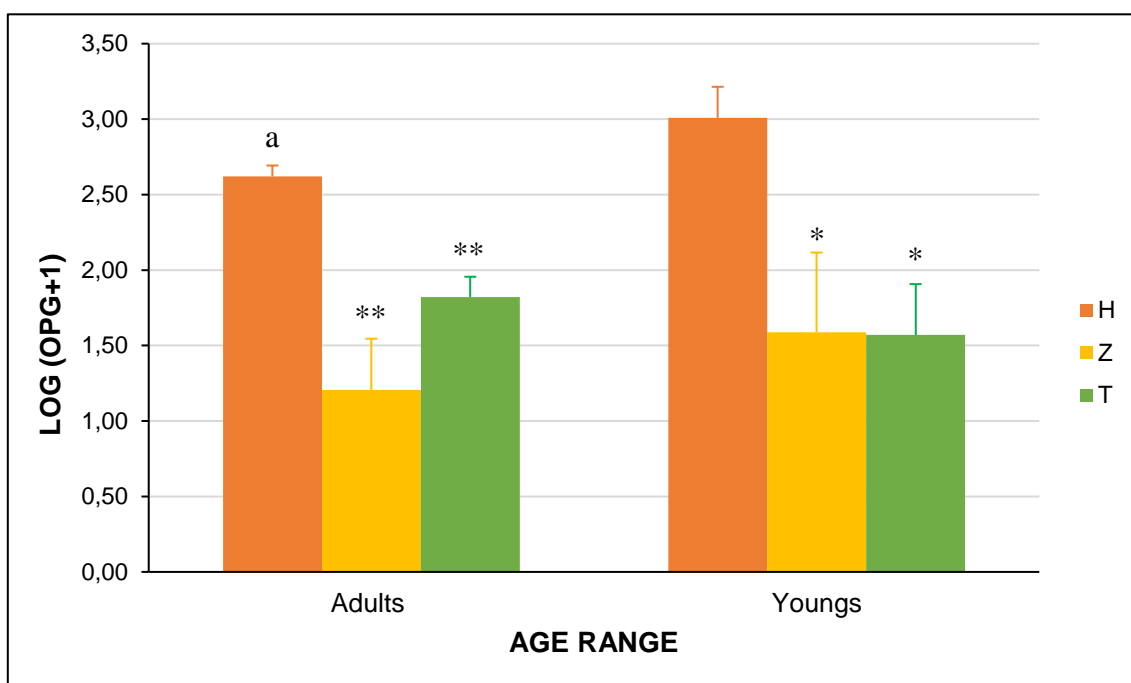


Figure 12. Total oocysts per gram (OPG) in adults and youngs in the different provinces of Aragon: Huesca (H) Zaragoza (Z) and Teruel (T). Data are represented as mean values of the logarithm of the OPG plus one ($\text{Log} [\text{OPG} + 1]$) \pm SEM (standard error of the mean). * ($P < 0.05$), ** ($P < 0.01$) for province comparisons; ^a ($P < 0.05$) for age range comparisons.

Faecal oocyst counts in adult Iberian ibex in the province of Huesca showed a higher value compared to Zaragoza and Teruel, being lower in Zaragoza ($P < 0.01$) (Fig.12). As for young Goats, Huesca also presented a higher value for OPG counts ($P < 0.05$) than those from Zaragoza and Teruel. On the other hand, both adult and young Iberian Ibex showed similar values in the provinces of Zaragoza and Teruel.



When comparing OPG counts between adult and young Iberian ibex in the three provinces, significant differences were not found both in Zaragoza and Teruel. On the contrary, adult goats had slightly higher mean values than young animals in Huesca ($P < 0.05$).

Differences according to location in stray-feral goats

Oocysts were detected in 85.71% of adult stray-feral goat samples at Presa de Vadiello and in 100% of young samples, whereas at Carretera de Vadiello, oocysts were detected in 100% of adult samples and in 33.33% in young samples.

Adults from Presa de Vadiello (PRV) presented OPG counts between zero to 5405.41 oocysts per gram as the maximum value, while young animals ranged from 720.00 to 3454.55 oocysts per gram. On the other hand, adults from Carretera de Vadiello (CTA) showed counts from 150.94 oocysts per gram to 1960.78 for the maximum, whereas young animals ranged from zero to 333.33 oocysts per gram.

Log (OPG+1) in adult goats showed very similar values in both locations, however young individuals presented higher values at the Presa de Vadiello (PRV), while in Carretera de Vadiello (CTA) the young goats had significantly lower OPG counts ($P < 0.01$). As for the age range, no differences were found in Presa de Vadiello; in contrast, adults from the Carretera de Vadiello had significantly higher faecal oocyst counts than young goats ($P < 0.01$).

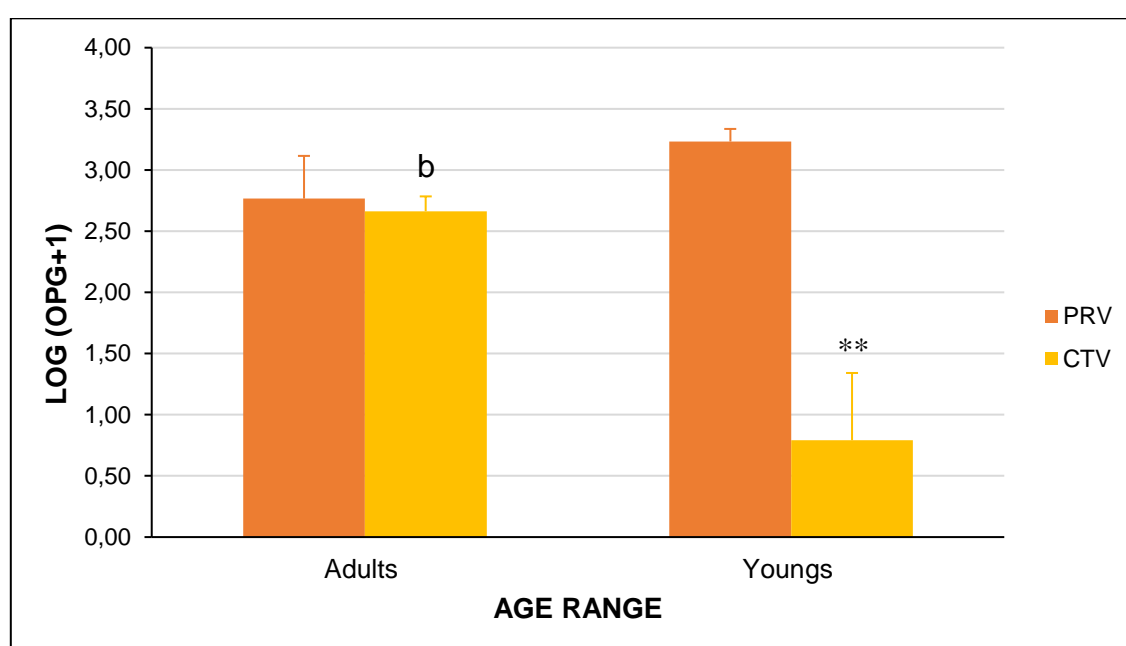




Figure 13. Global oocysts per gram (OPG) in adults and youngs stray-feral goats in two locations of Huesca: Presa de Vadiello (PRV) and Carretera de Vadiello (CTV). Data are represented as mean values of the logarithm of the OPG plus one (Log [OPG + 1]) \pm SEM (standard error of the mean). ** ($P < 0.01$), for breed comparisons; ^b ($P < 0.01$) for age range comparisons.

Differences according to location in Iberian ibex

As shown in Figure 14, the statistical analysis based on Log (OPG+1) data indicated that MEM and CAS had significantly lower oocyst counts in adult Iberian ibex than most of the other sampling locations (MTA, PRC, VLH, PAL) ($P < 0.001$), while mean oocysts count were similar between MTA, PRC, VLH, PAL, MOS, VIL, EJU and MON. The maximum OPG count was detected in MON in the province of Teruel, with a record of 51428.57 oocysts per gram of faeces.

Log (OPG+1) in young Iberian ibex resulted in large variations among locations as shown in Fig. 15. MTA and PRC showed higher oocyst counts compared to the other sampling locations. Some differences were observed among the other locations, but without statistical significance. The maximum OPG count was detected in MON in the province of Teruel with 120000.00 OPG.

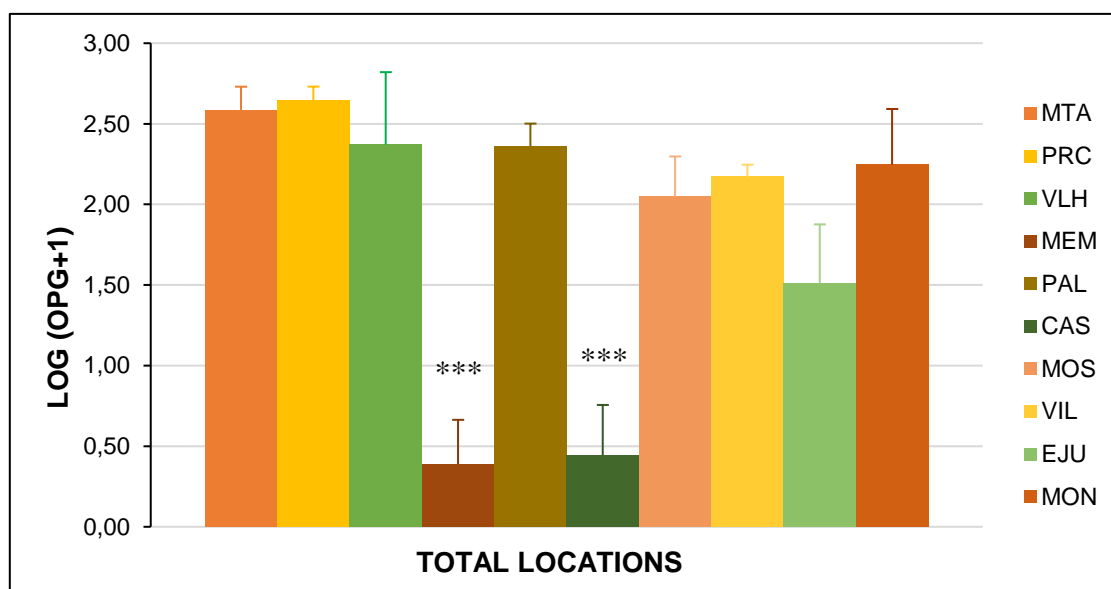


Figure 14. Faecal oocysts counts found in adults *Iberian ibex* in the different sampling locations of Huesca: Monte Aruabón (MTA), Presa de Calcón (PRC); Zaragoza: Villanueva de Huerva (VLH), Monte-Embalse Mezalocha (MEM) and Teruel: Sierra de Palomera (PAL), Cerro de Castelfrío (CAS), Monte de Mosqueurela (MOS), Monte de Villarluego (VIL), Monte



de Ejulve (EJU) and Monte Montalbán (MON). Data are represented as mean values of the logarithm of the OPG plus one ($\text{Log [OPG + 1]} \pm \text{SEM}$ (standard error of the mean). *** ($P < 0.001$).

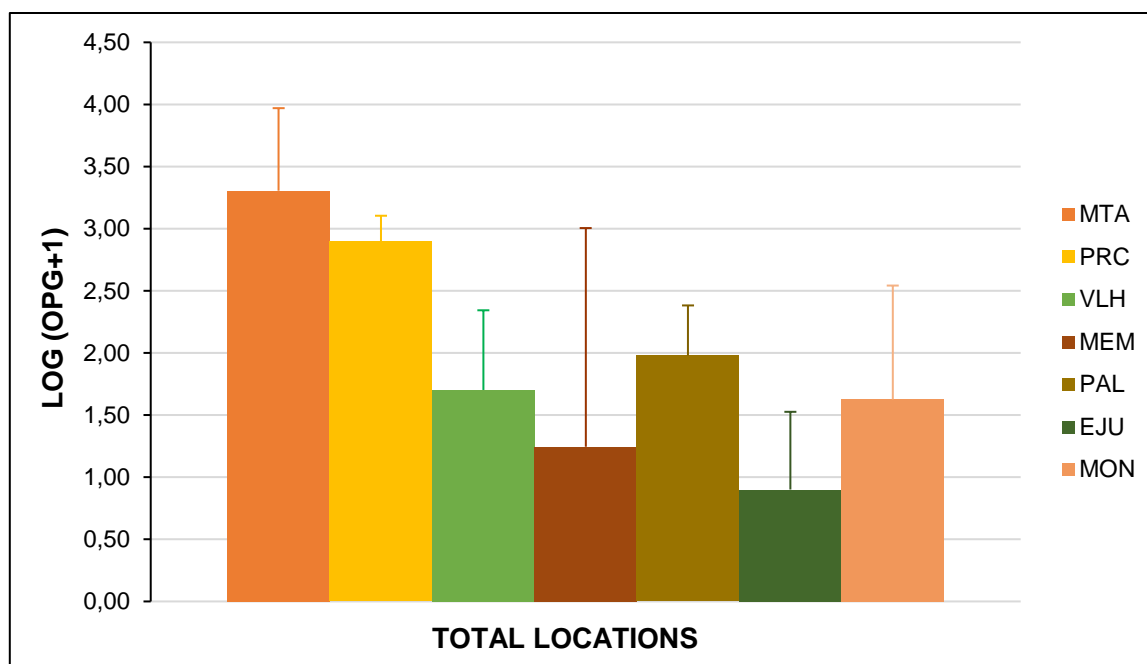


Figure 15. Faecal oocysts counts in youngs *Iberian ibex* in the different locations of Huesca, Zaragoza and Teruel. Data are represented as mean values of the logarithm of the OPG plus one ($\text{Log [OPG+1]} \pm \text{SEM}$ (standard error of the mean). * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

3.2. Faecal oocysts speciation

Global frequencies for Eimeria species in Iberian ibex

A total of 946 oocysts, 658 from adult Iberian ibex and 288 from young Iberian ibex, were studied during the speciation process of faecal samples. The frequency of each species was analysed according to breed and provinces.

As shown in Figure 16, the most frequent species for adult Iberian ibex were *E. arloingi* (30.1%) and *E. ninakohlyakimovae* (28.4%), followed by *E. alijeivi* (10.3%), *E. christenseni* (8.4%), *E. caprina* (8.2%), *E. hirc* (7.3); while *E. pallida* (4.0%), *E. caprovina* (2.6%), *E. jolchijevi* (0.5%) and *E. aspheronica* (0.3%) were minor species in this age group.

In young Iberian ibex the most frequent species were also *E. arloingi* (22.2%), *E. ninakohlyakimovae* (21.9%), *E. christenseni* (19.8%) and *E. alijeivi* (18.1%), followed



by *E. caprina* (13.9%); finally, *E. caprovina* (2.4%), *E. pallida* (1.0%) and *E. jolchijevi* (0.3%) were minor species.

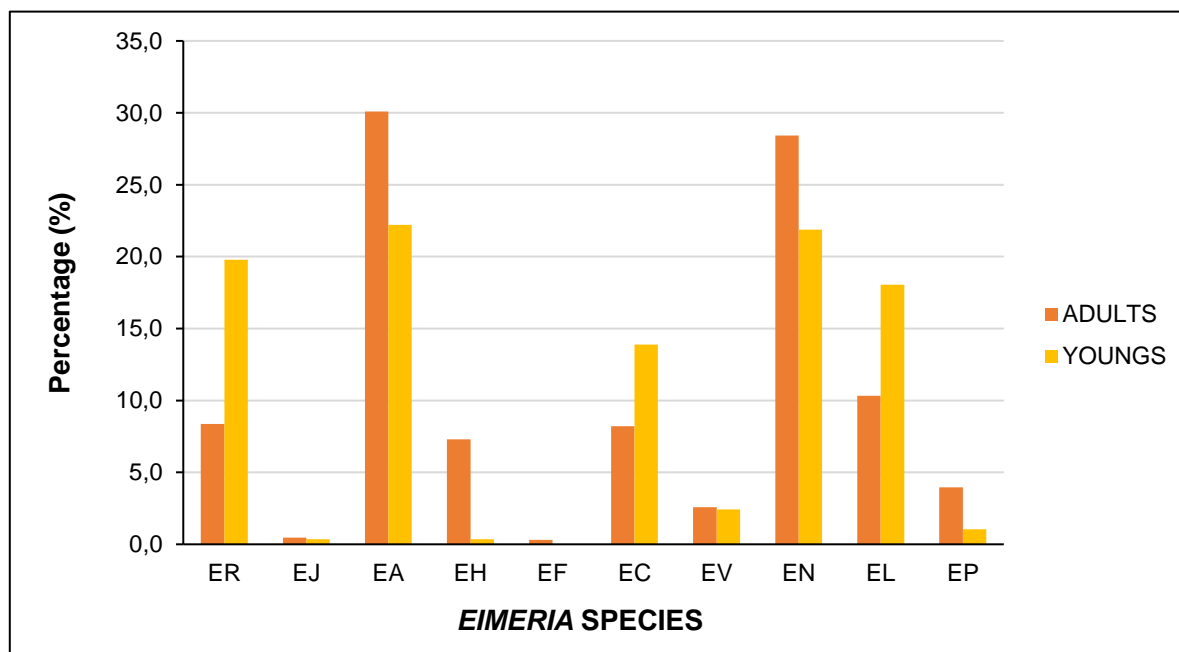


Figure 16. Percentage of occurrence of *Eimeria* spp. in faecal sample of adult and young Iberian ibex. ER: *E. christenseni*; EJ: *E. jolchijevi*; EA: *E. arloingi*; EH: *E. hirc*; EF: *E. aspheronica*; EC: *E. caprina*; EV: *E. caprovina*; EN: *E. ninakohlyakimovae*; EL: *E. alijeви*; EP: *E. pallida*.

Frequencies for Eimeria species in Iberian ibex and stray-feral goats in Huesca

On the other hand, the frequency of *Eimeria* spp. was analysed in adult and young stray-feral goats (Fig.17) and Iberian ibex (Fig.18) within the province of Huesca.

As shown in Figure 17, the most frequent species in adult stray-feral goats were *E. ninakohlyakimovae* (29.1%) and *E. arloingi* (23.7%), followed by *E. caprina* (18.6%) and *E. christenseni* (13.8%), whereas for youngs the most frequent was *E. ninakohlyakimovae* (35.0%). Also, species with a moderate frequency were detected in young animals, such as *E. arloingi* (16.5%), *E. hirc* (13.6), *E. christenseni* (11.7%), *E. caprina* (11.7%) and *E. alijeви* (10.7%).

As depicted in Figure 18, the most frequent *Eimeria* spp. in adult Iberian ibex were *E. ninakohlyakimovae* (43.4%) and *E. arloingi* (32.7%), followed by *E. alijeви* (13.3%).

As for young Iberian ibex, the most frequent species coincides with those of the adults, being *E. ninakohlyakimovae* (27.1%) and *E. arloingi* (26.2%) the most frequently found.

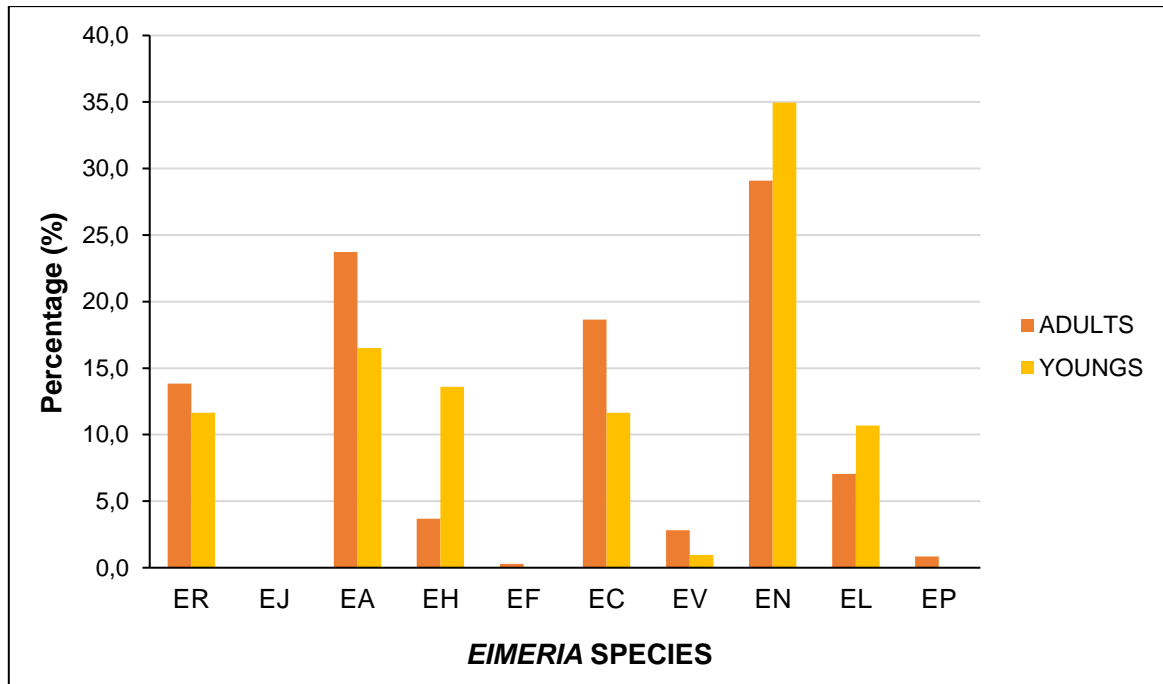


Figure 17. Percentage of occurrence of *Eimeria* spp. in adult and young stray-feral goats in the province of Huesca.

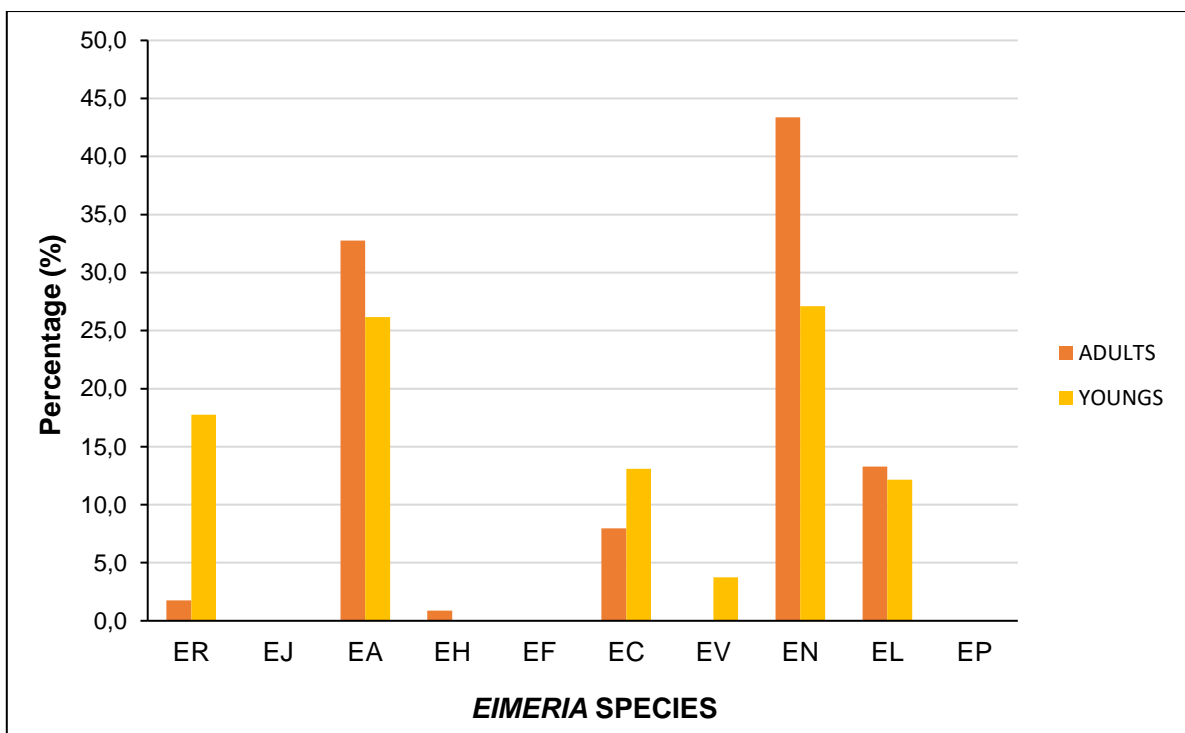


Figure 18. Percentage of occurrence of *Eimeria* spp. in adult and young Iberian ibex in the province of Huesca.

As shown Figure 17 and 18, the two most frequent species both in adults and young animals in the two breeds were *E. ninakohlyakimovae* and *E. arloingi*.



Frequencies for *Eimeria* species in Iberian ibex according to provinces

The frequency of *Eimeria* spp. was analysed comparing data from the provinces of Aragon, both in adult and young Iberian ibex.

For adults in the province of Huesca the most frequent species were *E. ninakohlyakimovae* (43.4%) and *E. arloingi* (32.7%), followed by *E. alijevi* (13.3%). The same frequency schedule was observed in Zaragoza: *E. ninakohlyakimovae* (33.1%), *E. arloingi* (29.9%) and *E. alijevi* (13.4%). Finally, in Teruel the most frequent were *E. arloingi* (29.4%) and *E. ninakohlyakimovae* (23.0%), then *E. christenseni* (11.2%), *E. caprina* (8.9%) and *E. alijevi* (8.6%).

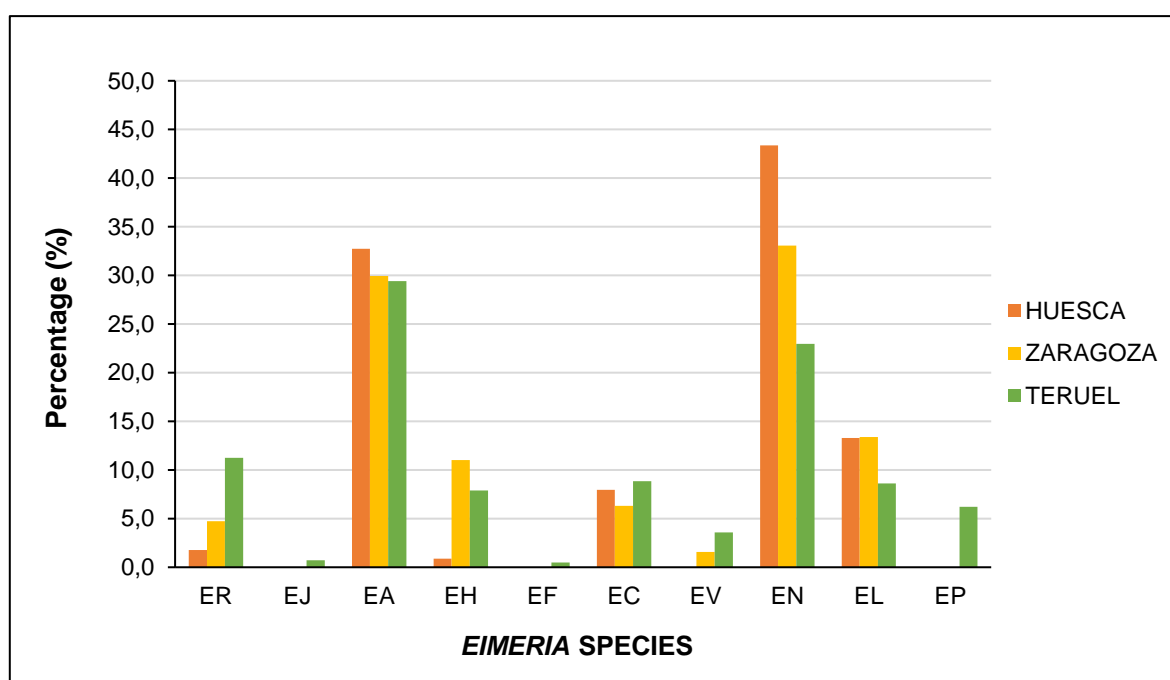


Figure 19. Percentage of *Eimeria* spp. in adults in *Iberian ibex* in the different provinces of Aragon.

For young animals in the province of Huesca the most frequent species were *E. ninakohlyakimovae* (27.1%) and *E. arloingi* (26.2%), followed by *E. christenseni* (17.8%), *E. caprina* (13.1%) and *E. alijevi* (12.1%). In Zaragoza, the most frequent species were *E. christenseni* (57.1%) and then *E. arloingi* (23.8%) and *E. caprina* (11.9%). Whereas, in Teruel the most prevalent were *E. alijevi* (28.1%), followed by *E. ninakohlyakimovae* (23%), *E. arloingi* (18.7%), *E. caprina* (15.1%) and *E. christenseni* (10.1%).

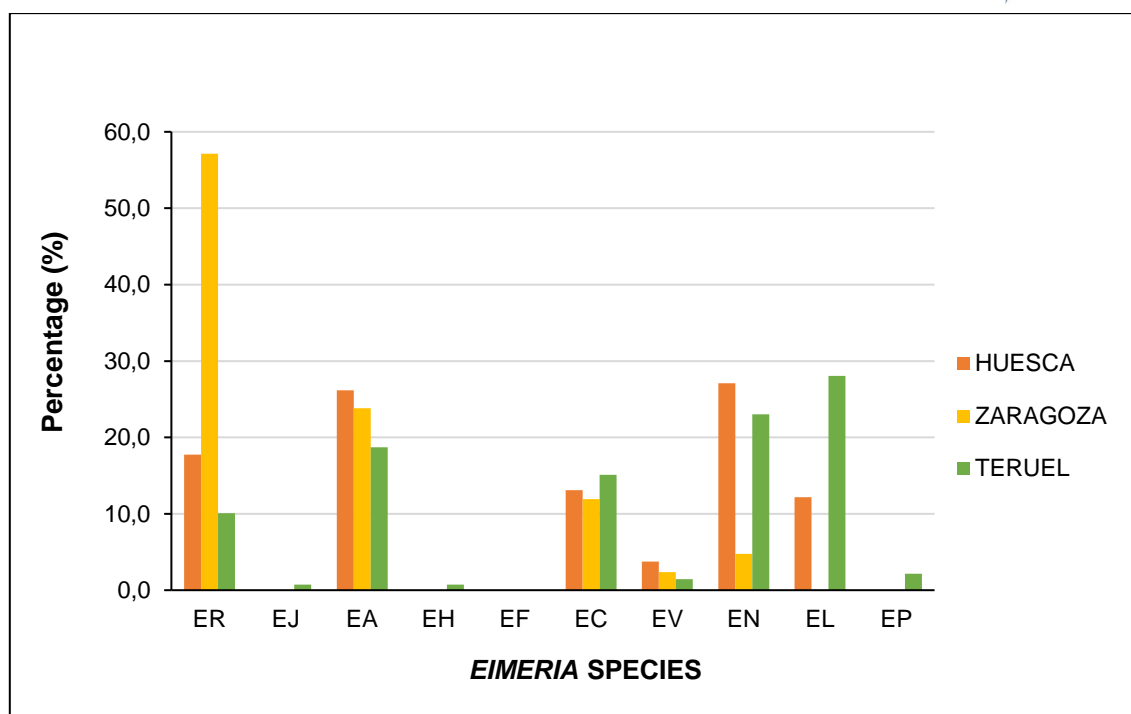


Figure 20. Percentage of *Eimeria* spp. in youngs in *Iberian ibex* in the different provinces of Aragón.

5. DISCUSSION

Though the undergoing expansion and partial recovery of the *Capra pyrenaica* in Aragón, studies in Iberian ibex health status in this region are scarce. Therefore, the present study estimates the *Eimeria* oocyst excretion levels in populations of adult and young animals established in different locations of Huesca, Zaragoza and Teruel. Stray-feral goats were also analyzed in order to compare different populations of goats established in the Guara Natural Park (Huesca).

In the present study, the global prevalence of *Eimeria* infection detected on adult ibexes (around 70%) were similar to the results described in previous surveillances carried out in this wild ruminant in Iberian Peninsula (Pérez Jiménez, 2001; Ramajo *et al.*, 2007; Luzón *et al.*, 2008; Santiago-Moreno *et al.*, 2010), even though it was higher (around 90%) in ibexes sampled in Western Spain (Barba, 2015; Calero-Bernal *et al.*, 2020). It is important to mention that all these studies have been performed in adults. In order to know the incidence of *Eimeria* infection in wild populations of Iberian ibex, we considered adult and young animals for sampling collection due to the importance of knowing the health status of these two age ranges.



In this study, adult stray-feral goats showed a higher prevalence of *Eimeria* infection than adult Iberian ibex, a result that is similar to previous data published for Iberian ibex and domestic goats that inhabits the Gerês-Xurés Transboundary Biosphere Reserve (GXTBR) in north-western Iberian Peninsula (Cardoso *et al.*, 2021). Social behavior differs between one species of small ruminant and the other. In Iberian ibex, animal density is much lower because these animals prefer high-slope areas and higher elevations of difficult access and sexual segregation is very evident most of the year, except during the rutting season. Moreover, due to Iberian ibex is adapted to the habitat and to extreme natural conditions for generations, natural breeding may have selected specimens less susceptible to different diseases due to the development of a more efficient immune response. On the other hand, the higher *Eimeria* spp. prevalence in stray-feral goats could be associated to higher animal densities and higher parasitic load in the field.

Regarding to the global intensity of *Eimeria* infection in Iberian ibex, estimated by OPG, it was moderate to low in adult and young animals, with less than 2×10^3 OPG. These results were similar to previous data published for domestic adult goats naturally infected with *Eimeria* spp. from different farms located in Gran Canaria (Ruiz *et al.*, 2006) and for Iberian ibex inhabits the GXTBR in north-western Iberian Peninsula (Cardoso *et al.*, 2021). However, other parasite disease surveillances in Iberian ibex established in Tejeda y Almirajara National Game Reserve (Málaga) and in Gredos Mountain range (Western Spain) have shown variation in the intensity of *Eimeria* infection in these wild ruminants, with mean OPG values that range from 2×10^2 to 7×10^3 OPG (Luzón *et al.*, 2008; Santiago-Moreno *et al.*, 2010; Calero-Bernal *et al.*, 2020). These differences in mean oocyst excretion could be due to diverse factors such as location of the study areas and a lower sample size on two of the studies (Luzón *et al.*, 2008; Santiago-Moreno *et al.*, 2010).

The similarities between faecal oocyst counts between adult and young ibexes could be due to young goat kids were almost immunocompetent (3-4 moth old) at sample collection. In agreement to this hypothesis, the highest OPG counts was found in a young ibexes smaller than the mean and still being with its mother (the size of the faecal pellet of this animal was accordingly smaller). In domestic goat, the development of the immunity against *Eimeria* spp. build up after weaning period and increase with age (Ruiz *et al.*, 2006).



In order to compare two local breeds in the Guara Natural Park (Huesca), different populations of stray-feral goats and Iberian ibexes sharing the same geographical area were sampled. The higher faecal oocyst counts detected in young ibexes in comparison to young stray-feral goats might indicate a higher susceptibility of these animals to *Eimeria* infection. On the other hand, previous publications have highlighted the importance of the animal immunity in the development of the coccidiosis that is a life threatening disease in goats before weaning (Ruiz et al., 2006). So, a possible explanation of differences in oocysts excretion between the two breeds may be associated with the real age of the animal sampled, with ibexes that could have birthed a few weeks later than the young stray-feral goats analyzed in this study.

Faecal collection in ibexes from Huesca, Zaragoza and Teruel allowed the detection of significant higher oocyst counts in ibexes from Huesca, independently of age range. One explanation of these results could be related to the weather conditions in Guara Natural Park (Huesca), with temperatures range between 27°C and 14°C in summer and forest in the north, that provides favourable conditions of temperature and humidity for the survival of *Eimeria* spp. oocysts in the field. Furthermore, these climatic conditions may allow the development of a longer life cycle of *Eimeria* spp. than the one developed in Zaragoza and Teruel. Very hot and dry summer in Zaragoza y Teruel, when the sample collection took place, could have reduced the viability of oocyst in the ground. In agreement, previous studies in southern Spain also show lower oocyst shedding intensity in Iberian ibex (Luzón et al., 2008; Santiago-Moreno et al., 2010), compared to those found in western Spain although, in this case, the eight-fold higher densities of ibexes in that area could have been a risk factor (Calero-Bernal et al., 2020).

Unlike other parasites, including phylogenetically close Apicomplexa such as *Cryptosporidium*, the species of the genus *Eimeria* are characterized by being species-specific, i.e., each host has its own *Eimeria* species. In fact, there are studies showing that there are no cross-infections between *Eimeria* species in hosts as phylogenetically close as sheep and goats. It would be expected, therefore, that the *Eimeria* species of Iberian ibex (*Capra pyrenaica*) would be very similar to those of the domestic goat *Capra hircus*. Accordingly, in the present study 9 of the species of *Eimeria* species described in different epidemiological investigations on coccidiosis in different breeds



of domestic goats in Spain, Europe and other parts of the world (Ruiz *et al.*, 2020) have been documented.

Most of the *Eimeria* species found here have been previously described in epidemiological studies carried out in different geographical areas of the Iberian Peninsula. Thus, Pérez *et al.*, (1996) reported *E. christenseni*, *E. ninakohlyakimovae* and *E. parva* (*E. pallida*) in Sierra Nevada (South Spain), and later surveys have (Antón *et al.*, 2002; Pérez *et al.*, 2006) have documented the presence of *E. christenseni*, *E. jolchijevi*, *E. arloingi*, *E. hirci*, *E. aspheronica*, *E. caprina*, *E. caprovina* and *E. ninakohlyakimovae* in *Capra pyrenaica* from the same geographical region. More recently, Araujo *et al.* (2020) described the presence of three *Eimeria* species in *Capra pyrenaica victoriae* at the National Park of Sierra de Guadarrama (Spain): *E. christenseni*, *E. arloingi-cylindrica* complexes and *E. caprina-caprovina*. For an overview, see the review by Valldeperes *et al.*, (2023). Herein, *E. alijevei* has been reported for the first time.

Interestingly, Calero-Bernal *et al.*, (2020) reported 8 *Eimeria* species in Iberian ibex (*Capra pyrenaica victoriae*) in Western Spain, *E. arloingi*, *E. intricata*, *E. ninakohlyakimovae*, *E. parva*, *E. faurei*, *E. gonzalezi*, *E. ovina* and *E. crandallis*, 6 of which (*E. intricata*, *E. parva*, *E. faurei*, *E. gonzalezi*, *E. ovina*, *E. crandallis*) corresponding to species of *Eimeria* traditionally considered specific from sheep. These “sheep species” referred by Valldeperes *et al.*, (2023) in their review as *Eimeria* sp., suggesting that are not common *Eimeria* species from goats. Here also, in the morphological identification of the *Eimeria* species, some oocysts similar to *E. arloingi* but smaller and wider were found in some individuals and, occasionally, *E. faurei*- and *E. granulosa*-like oocysts were documented (data not shown). Whether they are new species or species adapted from sheep deserves further investigation.

In general, the two most abundant *Eimeria* species in the present study were *E. ninakohlyakimovae* and *E. arloingi*, which is the rule in most of the epidemiological studies performed in domestic *Capra hircus* worldwide (Ruiz *et al.*, 2020). This is in agreement to previous studies in *Capra pyrenaica* (Pérez *et al.*, 1996; Antón *et al.*, 2002; Pérez *et al.*, 2006). Pérez *et al.*, (1996) also reported high frequency for *E. christenseni* which, in our study was also within the most abundant *Eimeria* species.



The nine *Eimeria* species found in this study were present both in adult and young animals, and the differences in frequency were not significant, except for *E. christenseni*, which was observed in higher frequency in faecal samples from young Iberian ibex when compared to adults. This is in agreement to previous observations showing that this *Eimeria* species may be the most abundant in goat kids from Majorera breed (Ruiz *et al.*, 2012; Torres *et al.*, 2022).

All the *Eimeria* species were found in the three provinces of Aragón and their frequency did not vary from one province to the other, indicating that the distribution of the different species is not strongly influenced by climatic or geographic conditions.

Finally, the different *Eimeria* species and their frequency were approximately the same both in stray-feral goats and Iberian ibex, which suggest that the evolution of the species within the two host was parallel and that there has probably been an exchange of oocysts between breeds through history in animals sharing the same geographical areas. All the *Eimeria* species were found in the three provinces of Aragón and their frequency did not vary from one province to the other, indicating that the distribution of the different species is not strongly influenced by climatic or geographic conditions.

Finally, the different *Eimeria* species and their frequency were approximately the same both in stray-feral goats and Iberian ibex, which suggest that the evolution of the species within the two host was parallel and that there has probably been an exchange of oocysts between breeds through history in animals sharing the same geographical areas.

6. CONCLUSIONS

First. The frequency of *Eimeria* spp. infection is relatively high (around 70%) in the populations of Iberian ibex from the North East of Spain (Province of Aragón).

Second. The intensity of parasitization was moderate to low, corresponding to the normal values observed in adult animals in other goat breeds worldwide.

Third. Probably because young goat kids were already almost immunocompetent (3-4 months old), differences in the intensity of infection between adults and young animals were barely observed.



Forth. A total of nine *Eimeria* species were found both in adult and young goat faecal samples analysed from Iberian ibex, with *E. ninakohlyakimovae* (the most pathogenic *Eimeria* species for goats) being the most frequently observed.

Fifth. The frequency and the intensity of parasitization in Iberian ibex and stray-feral goats sharing the same geographical area was approximately the same, suggesting that both goat breed are equally susceptible to *Eimeria* infection. Roughly, the same *Eimeria* species and frequencies were recorded as well.

Sixth. The highest intensity of *Eimeria* infection was observed in Huesca, the province located more in the North, probably because weather conditions in this area, less dry and hot than Zaragoza and Teruel, may favour the persistence of the oocyst in the environment.

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