

Research Article

Postparturient Rise in the Excretion of *Eimeria* Spp. in Manchega Dairy Sheep

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Abstract

The present study was carried out on the occurrence of a postparturient rise of *Eimeria* spp. in breeding ewes grazing in natural pastures. The research was performed in a dairy flock in the municipality of Sangonera, Murcia (Southeast Spain). A total of 216 faecal samples recovered from 20 ewes and 35 lambs of the Manchega breed were analyzed between December 2014 and March 2015. The analyses to quantify oocysts per gram of faeces (OPG) and to identify *Eimeria* species were conducted at the Veterinary Faculty of the University of Murcia. Nine species of *Eimeria* were identified: *E. ovinoidalis* (39.8%), *E. parva* (22.1%), *E. weybridgei* (13.5%), *E. crandallii* (9.4%), *E. ahsata* (4.6%), *E. pallida* (3.6%), *E. bakuensis* (3.5%), *E. granulosa* (3.4%) and *E. faurei* (0.1%). *Eimeria ovinoidalis*, *E. parva* and *E. weybridgei* were the most frequent species but only *E. ovinoidalis* showed highest oocyst shedding after parturition ($P < 0.05$). Significant differences in OPG counts were found between before and after lambing weeks ($P < 0.05$). The detection of several *Eimeria* species, in particular *E. ovinoidalis*, suggests that *Eimeria* spp. probably have an important pathogenic potential in dairy sheep in Murcia. Moreover, the presence of a postparturient rise in *Eimeria* oocyst shedding indicates that ewes may play an important epidemiologic role in the study area, and the design of control measures will reduce infection of lambs, lower mortality and increase the productivity of the flocks.

ABBREVIATIONS

OPG: Oocysts Per Gram

INTRODUCTION

As multi-purpose animals, sheep (*Ovis aries*) can produce meat, milk and wool under very diverse conditions. Although sheep's principal purpose is meat production, milk has become really important in some countries. From the last 30 years, the global sheep population has persisted at around one billion animals, where Europe and Central Asia share a 12% of the world ovine population with over 244 million heads [1,2]. In this sense, Mediterranean countries are leaders in small ruminant production in Europe, and Spain produces about 20% of these animals, rearing about 16.5 million head of sheep (12.6 million ewes). In fact, sheep production represents the 8% of the total livestock production in Spain [2].

Spanish sheep production is divided into two principal sectors: meat and milk products, each of them with its particular conditions associated to the breed's adaptation to the territory.

Dairy sheep production is almost totally carried out in extensive systems where sheep are kept on pasture for different grazing times [3]. This system includes two breeding seasons per year: one during winter (December – February) and the second during autumn (September – October). In the first month after lambing, farmers combined lambs suckling period with milking until lambs are weaned.

Coccidiosis is a protozoan disease caused by a monoxenous apicomplexan parasite of the genus *Eimeria* observed in almost all sheep-rearing countries of the world [4]. Its presence has been reported worldwide [5,6].

In sheep, the endogenous development of *Eimeria* life cycle occurs in epithelial and endothelial cells of the small and large intestine, including both sexual and asexual phases [7]. The severity of the disease is commonly related to the *Eimeria* species infecting the host, the infectious dose (number of sporulated oocysts ingested), and the immune status and age of the host at the time of infection, among other factors. Despite young animals are particularly vulnerable [8], adult sheep also shed *Eimeria* oocysts almost continuously.

Nowadays, 11 named species of intestinal coccidia are known to infect sheep including *E. ahsata*, *E. ovinoidalis*, *E. bakuensis* (*ovina*) and *E. crandallis* which are considered the most pathogenic species [4]. This disease has become a substantial problem, principally in farms with increased stocking densities and reduced availability of pasture for sheep [8]. The economic importance of coccidian infections in high sheep producer countries has been previously documented [9]. Typically, these economic losses are a direct consequence of the reduction in weight gains or animals being static in their growth [10]. Salisbury and Whitten [11] reported that coccidiosis caused thriftiness and losses among 4-6 months old lambs. The principal clinical sign is profuse diarrhoea related to a loss of gut absorptive capacity combined with watery and mucous faeces, followed by an important dehydration and weight loss [7]. Concomitantly, a decrease of appetite worsens general condition of the animals. However, it is common to find *Eimeria* spp. oocysts in faecal samples of animals with absence of clinical signs. Subclinical coccidiosis is usual in adult animals, whose main sign is just an impairment of growth [12]. Subclinical coccidiosis can quickly progress to clinical, especially under induced stress conditions such as dietary changes, long travels, extreme temperatures, weather conditions, environment changes, severe concomitant infections, nutritional status, peripartum and other factors [8,13].

The periparturient rise (PPR) of parasitic stages shedding has been worldwide studied. The phenomenon is well recognized in ewes [14] and has also been described in cattle [15]. Previous studies have suggested that the increase in faecal nematode egg output are due to the temporary impairment of the immune response of the periparturient ewes [16,17], resulting in the maturation of arrested parasites and in the establishment of newly acquired parasites. Although the mechanisms responsible for the decrease in the immunity of the ewes remain unknown, evidence suggests that their nutritional status may affect it [18], as well as hormones, stress and production management can also directly affect sheep and increase the parasite excretion [19].

The present study was conducted to evaluate the intensity of oocyst excretion, total and of each *Eimeria* species, during eight weeks at peripartum period in adult dairy sheep and their lambs, in an extensive flock from southeastern Spain.

MATERIAL AND METHODS

Study area

The current study was conducted in an extensive dairy sheep farm from Cuevas del Norte (Murcia, Southeastern Spain, 37° 54' 39.114", -1° 15' 5.871") during December 2014 to March 2015. The climate of that region is hot subtropical semi-arid with Mediterranean influences, with mild winters and hot summers. At the study time, the mean temperature in Cuevas del Norte was 17.5 °C, the mean rainfall was 313 mm, and the mean relative humidity was 62.8% [20].

Animals

The studied flock was constituted by 500 animals of Manchega breed. Excretion patterns of *Eimeria* oocysts in ewes and their lambs were studied at the Faculty of Veterinary Medicine, University of Murcia. Twenty ewes in late pregnancy

period were chosen and their faecal samples evaluated. They had been treated with 5 mg/kg body weight of fenbendazole ten weeks before starting the study, and fed with a balanced diet of alfalfa and mixed concentrate with water *ad libitum* during the period of the study.

Thirty five lambs were born from these 20 ewes from 6 December 2014 and 27 February 2015. Unfortunately, eight lambs died during the study and one ewe also died after the 4th study week. Therefore, by the end of the study deaths had reduced the numbers to 19 ewes and 27 lambs.

Faecal samples ($n = 216$) were taken from the rectum of all ewes ($n = 20$) from 3 weeks before lambing until 4 week after the start of lambing and later from 35-27 of their lambs every week ($n = 92$). Lambs were weaned 1 week before the end of the study.

Laboratory procedures

Each sample was carefully identified and stored in plastic bags. Samples were transported to the Faculty of Veterinary Medicine (University of Murcia), where they were kept at 4°C until coprological analyses were performed.

At the laboratory, all faecal material was firstly analyzed using a qualitative flotation technique with Sheather's solution as flotation fluid ($\rho = 1.27$). Positive *Eimeria* spp. samples were quantitatively analyzed in order to calculate the oocysts output of each sample by a McMaster technique [21]. After OPG were calculated, each sample was mixed in a pool with all faecal samples collected during the same periparturient week. To identify isolated *Eimeria* species, oocysts were allowed to sporulate by suspending them in 2% (w/v) potassium dichromate ($K_2Cr_2O_7$) in Falcon® bottles. For this purpose, faeces were thoroughly mixed with tap water and the suspension was poured through a wire mesh screen with an aperture of 80 mm into a 2000 ml glass beaker, allowed to settle for 12 hours, after which supernatant was discarded. The sediment was mixed with 4% potassium dichromate in 1:1 proportion, so the final solution would be 2% (w/v) and introduced into the bottles, which were checked and moved to infuse oxygen until the sporulation of the *Eimeria* oocysts was completed. Sporulated oocysts were examined at 400× magnification with an optical Nikon eclipse 50i microscope coupled to a digital camera. One hundred oocysts were randomly selected and identified according to their morphometric characteristics following Péllerdy [4].

Statistical analysis

Faecal oocyst counts were transformed into $[\log (OPG + 1)]$ to obtain a normal distribution. One way or two-ways analysis of variance for repeated measures was used for statistical evaluation. Results were analyzed with post-hoc Tukey's.

In addition, post hoc Bonferroni pair wise mean comparisons test was used between each pair of *Eimeria* species during periparturient weeks. Differences were considered statistically significant at $P < 0.05$. All analyses were performed using Graph Pad Prism®[22].

RESULTS

Eimeria oocysts were found in 94.4% ($n = 119/124$) of

the ewes faecal samples. Nine different *Eimeria* species were identified in the present study, such as *E. ovinoidalis* (39.8%), *E. parva* (22.1%), *E. weybridgeensis* (13.5%), *E. crandallis* (9.4%), *E. ahsata* (4.6%), *E. pallida* (3.6%), *E. bakuensis* (3.5%), *E. granulosa* (3.4%) and *E. faurei* (0.1%) (Table 1).

Oocyst shedding evaluation started at 3rd week before parturition and was already high at this moment. It decreased 4-fold 2 weeks before birth and then increased 2-fold one week before the start of parturition (Figure 1). After, oocyst levels increased more than 10-fold reaching a peak at 2 weeks post-partum. Oocyst shedding after parturition was significantly higher than before that moment ($P < 0.05$). However, there were no differences when weeks were compared.

Most of the infected sheep (90.3%; $n=112$) showed oocysts counts of less than 1,000 OPG, and the intensity of infection range was low (mean number of oocysts shed: 245 ± 419.9 ; range: 13-2,833 OPG). The highest OPG values were recorded during second week after birth ($536.5 \text{ OPG} \pm 823.30 \text{ OPG}$) associated to the presence of clinical signs, which were detected in 78.9% of ewes ($n=15/19$). Those clinical manifestations included mucous diarrhoea, mild dehydration, inappetence and weakness.

Eimeria parva was predominant at the 3rd week before partum, decreased at the 1st week after birth, and increased again from the 2nd until the 4th week after partum. In contrast, *E. ovinoidalis* increased gradually along the study period. Furthermore, *E. weybridgeensis* and *E. crandallis* decreased gradually from the 3rd week before partition and remained at low shedding levels until the end of the survey. Additionally, *E. bakuensis*, *E. pallida*, *E. ahsata* and *E. granulosa* showed similar shedding values along the study. *Eimeria pallida* maintained low shedding values, showing a brief peak at the 3rd week after birth. Finally, *E. faurei* was found only during the 4th week after birth.

Significant differences were detected when each *Eimeria* species oocysts shedding was weekly compared. However, only *E. ovinoidalis* showed higher OPG counts after birth ($P < 0.05$) whereas other species had not significant differences in OPG before and after birth weeks (Figure 1). In most of the weeks, seven or more species of *Eimeria* were simultaneously identified. In all cases, oocysts from *E. ovinoidalis*, *E. pallida*, *E. crandallis*, *E. parva*, *E. weybridgeensis*, *E. ahsata*, and *E. bakuensis* were recovered, although the percentages of each species varied among weeks.

Eimeria oocysts were found in lambs from the 3rd week after birth in 100% of the analyzed animals, and excretion continued increasing along the study. So, from the 3rd week after birth until the 5th week oocysts shedding increased 15-fold. The intensity of excretion ranged from 33 to 4,233 OPG ($285.2 \pm 609 \text{ OPG}$). As happened when ewes were analyzed, most of the faecal samples (91.3%) contained less than 1,000 OPG. However, the morbidity was higher than in ewes, showing acute clinical symptomatology with profuse watery diarrhoea affecting up to 83.7% ($n = 77/92$) of the animals during this three weeks period. Mortality was high, 22.8% ($n = 8/35$) of the lambs died because of dehydration or concomitant infections, frequent complications of coccidiosis. Significantly high differences ($P < 0.05$) were found between the oocysts shedding counts when comparing lambs' and ewes'

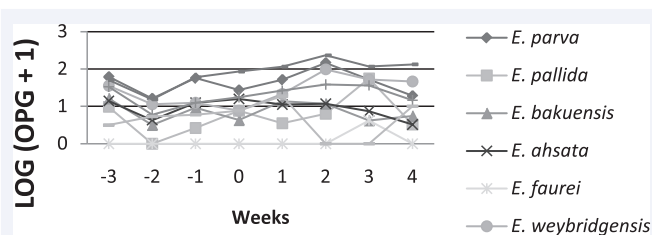


Figure 1 *Eimeria* spp. oocysts excretion from the 3rd week before partum to the 4th week after partum, expressed in [log (OPG+1)] (0= week of parturition).

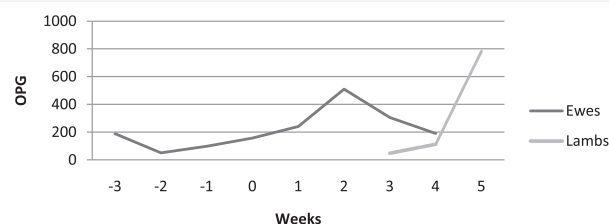


Figure 2 *Eimeria* spp. oocysts shedding of ewes and lambs during peripartum time (0 = week of parturition).

weekly records. However, no significant differences were found between oocysts shedding of lambs among the weeks (Figure 2). It should be underlined that shed oocysts had abnormal morphology in lambs and could not be identified.

DISCUSSION

This is the first study to describe the natural evolution of *Eimeria* infection in ewes during periparturient time in Murcia. In accordance to other authors, prevalence of *Eimeria* in our study overmatches 90% [6,23].

As previously described [5], moderate OPG counts were found in this survey. *Eimeria* infections have a high degree of homologous resistance to a challenge after prior exposure [24,25] as reflected in the low oocysts shedding of adults as a consequence to the establishment of an effective immune status in older sheep after primary exposure [7,8]. Lambs oocysts excretion was lower than those described in other studies performed in Spain [26]. Some authors affirmed that maternal factors have a direct influence in the immune reaction of young animals, and newborn lambs lack almost completely specific antibodies unless they get supply via colostrum. Thus, colostrum might protect lambs against coccidia, as was demonstrated in *E. crandallis* [27]. In our study, lambs were receiving milk directly from their mothers since their birthday and ingestion of colostrum was allowed. In the same way, previous studies reported that management practices have also a direct effect on oocysts shedding: low hygienic conditions, higher stock densities in confinement and semi-confinement conditions, together with no administration of antiparasitic treatments are the management factors mainly associated to a heavy coccidian infection [28,29]. The studied animals were kept under extensive breeding and had good hygienic conditions despite no coccidial treatment was used in the farm.

Although animals showed a low oocysts output, they can play a reservoir role of infection in the flock. Windon [30] suggested that even few individuals can contribute to the environmental contamination of the herd, and that they could be the reason for the permanent reinfection of animals. Also, the high oocysts shedding in young animals population would allow the maintenance of *Eimeria* in the farm. Finally, adult ewes do not seem to excrete large numbers of oocysts around the time of lambing, as it has been shown for digestive nematode eggs [31].

Nine of the eleven *Eimeria* species described in sheep [4] were found in the surveyed farm. This record coincided with the species that were previously reported in Central Spain [26]. However, it was lower than the one registered in North-West Spain [32], where up to ten species were found. The most frequent species in the present study was *E. ovinoidalis* (39.8%), in accordance with prevalences found in previous studies [24,26]. *Eimeria ovinoidalis* predominance has been associated to dry arid climates such as the climate in Murcia [33]. In contrast, *E. ahsata* and *E. bakuensis* frequencies were 4.6% and 3.5% respectively, much lower than the records from Northwestern Spain [32]. In the same way, *E. parva* (22.1%) and *E. weybridgeensis* (13.5%), were less frequent [26]. *Eimeria pallida* (3.6%) were not described in previous studies in Central Spain [26], and *E. granulosa* frequency (3.4%) was similar to the one previously reported in León, Northern Spain [34].

The PPR of oocysts shedding has been previously studied in ruminants, but with conflicting results. Most authors have observed a PPR in oocysts shedding [14,35] but Platzer *et al.*, [29] fail to do so. In our study, a PPR in oocysts shedding was observed in Manchega dairy sheep; the phenomenon began 2 weeks before parturition and lasted for at least 3 weeks after lambing. Moreover, significantly higher OPG counts were observed after birth in the studied ewes, during the postparturient period. Nonetheless, FEC (Faecal Egg Count) peak of nematodes have been worldwide reported in periparturient ruminants [36]. Similarly, other authors reported the elimination of *Cryptosporidium parvum* oocysts and *Giardia* sp. cysts during the periparturient time in sheep [37]. Differences found between weeks and oocysts shedding in our study and previous records could be attributable to physiology and life cycle of each studied parasite and its natural development along peripartum in ewes.

The mechanism responsible for the PPR in oocyst excretion remains unclear, although it could be associated to the activation of inhibited parasitic stages [15]. Some authors have previously reported a decrease of the immunity over the periparturient period, called periparturient relaxation of immunity (PPRI) [16]. There are several theories about the origin of PPRI including hormones production around parturition, stress at parturition, lack of antigenic stimulation and hormonal depression of immunity [36]. Although each of these factors probably influences the extent of the PPRI, its direct cause has not been identified. Recently, it has been found that an increase in metabolizable protein supply to periparturient ewes reduced to some extent the effects of the PPRI of *Teladorsagia circumcincta* [19]. Moreover, Chartieret al. [33] defined an association between PPRI and variations in hormonal concentrations in dairy goats, supporting a possible role for prolactin in host immunity. More studies

suggested that further factors are involved in the PPRI, such as the combination of glucocorticosteroids along with prolactin [38]. Besides, Catchpole et al. [39] confirmed that steroid hormones, progesterone and estrogen increase dramatically during late pregnancy, but their role remain unknown, if any, in the PPR of ewes.

Partum is described as a highly stressful event. In our study, ewes were under high stress conditions because of the combination of milking production, lambs suckling, and the well-described partum stress. In this farm, sheep milking starts two weeks after birth and continued for two weeks more, simultaneously with suckling. This stressful period matches the peak of oocysts excretion, indicating the relation between stress and immune status.

Frequency of *Eimeria* species varied among the weeks, and differences in their occurrences were observed over the study. Despite differences found during the weeks in the *Eimeria* species, only *E. ovinoidalis* showed statistically significant PPR. *Eimeria parva*, *E. weybridgeensis*, *E. bakuensis* and *E. crandallis* had also a peak at the 2nd week after parturition, at the same moment as the higher OPG counts of *E. ovinoidalis* were registered. *Eimeria ovinoidalis*, *E. crandallis* and *E. bakuensis* are known to be the most pathogenic species in sheep [4]. The predominance of *E. ovinoidalis* and *E. crandallis* oocysts might be associated to the high level of proliferation of these species when compared to other sheep coccidian [39]. Both species replicate in the ileum, reduce fluid absorption and cause diarrhoea. *Eimeria ovinoidalis* can seriously damage large intestine mucosa due to the combination of gamonts formation with intense leukocyte reaction [40]. Also, *E. crandallis* sporozoites infect epithelial cells of the jejunum crypts [41]. Moreover, despite common subclinical coccidiosis in adults, indicating a host-parasite equilibrium [42], mild clinical signs of ewes during the 2nd week after birth might be a consequence of the infection with former pathogenic strains simultaneously. During the study, lambs showed acute coccidian symptomatology and high morbidity. Although species could not be identified, *E. crandallis* and *E. ovinoidalis* were probably the most frequent ones since they are directly related to this kind of symptomatology in lambs [7].

The morphological study of ovine *Eimeria* oocysts showed a great variation in oocysts sizes while other characteristics persisted almost constant [6]. It is known that oocyst size is not necessarily constant. For example, morphometric changes could be derived from the state of patency, as demonstrated for *E. necatrix* [43]. Also, other authors suggested that the mean dimensions of *E. crandallis* tended to decrease as the patency progressed [41]. Despite the morphological changes, all oocysts excreted by ewes could be identified based on morphometric characteristics.

According to previous researches, the same morphological changes also occurred in *Eimeria* oocysts shed by lambs during this study. Oocysts shapes were irregular and subspherical, and the margin of the micropyle was thickened. However, in all cases oocysts sporulation was completed. Different theories could explain this process: (i) a variation in oocyst morphology occurred as a result of heavy infections [44], or (ii) changes are associated to the immune response of the host [45]. Consequently, morphometric identification of oocysts can be used when the

variation of oocysts' characteristics is scarce.

CONCLUSION

Our results showed that postparturient rise of *Eimeria* spp. oocysts in ewes is a real phenomenon in semi-arid Mediterranean areas during breeding time, lasting at least from 1st to 3rd week after birth, being associated to the increase of *E. ovinoidalis* oocysts shedding. Therefore extensive sheep flocks from Murcia region would beneficiate with well-designed control programs and administration of anticoccidial treatments before breeding seasons, which could prevent huge economic losses especially due to low production performances such as lower milk production or lambs' poor weight gains or death.

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