

Natural infection by *Eimeria* spp. in a cohort of lambs raised extensively in Northeast Brazil

Infecção natural por *Eimeria* spp. em uma criação de cordeiros criados extensivamente no Nordeste do Brasil

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Abstract

The aim of the study was to examine and describe the changes in the course of *Eimeria* infection in a cohort of lambs of the mixed breed Santa Inês raised extensively on a farm in the State of Rio Grande do Norte, northeastern Brazil. The study was carried out between April and August 2008, during which period fecal samples were collected on a weekly basis from each of the 27 male lambs starting from the day after birth until the age of 90 days. Samples were analyzed under optical microscope and those testing positive for *Eimeria* oocysts were submitted to a quantitative analysis. Oocysts were detected in 17% of the lambs by week 3, but by week 6 100% of the animals were infected. Eight species of *Eimeria* were identified in the fecal samples, namely, in decreasing order of prevalence, *E. crandallis*, *E. parva*, *E. granulosa*, *E. ovinoidalis*, *E. absata*, *E. ovina*, *E. faurei* and *E. intricata*. Of the 191 fecal samples that contained *Eimeria* oocysts, only 32 (16.8%) were infected with a single species whereas 23 (12.0%) were infected with at least two species and 136 (71.2%) harbored three or more species. Although *Eimeria* coccidiosis is a complex disease owing to the mixed nature of the infective agents, an ongoing prevention program should be implemented to reduce production losses.

Keywords: Coccidiosis, *Eimeria* spp., sheep, Brazilian Northeast.

Resumo

O objetivo do estudo foi analisar e descrever as alterações no curso da infecção por *Eimeria* em uma criação de cordeiros mestiços da raça Santa Inês criados extensivamente em uma fazenda no Estado do Rio Grande do Norte, nordeste do Brasil. O estudo foi realizado entre abril e agosto de 2008, período durante o qual amostras fecais foram coletadas semanalmente de 27 cordeiros machos a partir do nascimento até a idade de 90 dias. As amostras foram analisadas em microscópio óptico e aquelas positivas para oocistos de *Eimeria* foram submetidos a uma análise quantitativa. Oocistos foram detectados em 17% dos cordeiros na terceira semana, mas na sexta semana 100% dos animais estavam infectados. Oito espécies de *Eimeria* foram identificadas nas amostras fecais, citadas em ordem decrescente de prevalência, *E. crandallis*, *E. parva*, *E. granulosa*, *E. ovinoidalis*, *E. absata*, *E. ovina*, *E. faurei* e *E. intricata*. Das 191 amostras de fezes que continham oocistos de *Eimeria*, apenas 32 (16,8%) estavam infectados com uma única espécie, enquanto 23 (12,0%) estavam infectados com pelo menos duas espécies e 136 (71,2%) abrigavam três ou mais espécies. Apesar de *Eimeria* ser uma doença complexa, devido à natureza mista dos agentes infecciosos, um programa de prevenção em curso deve ser implementada para reduzir as perdas de produção.

Palavras-chave: Coccidiose, *Eimeria* spp., ovinos, Nordeste brasileiro.

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Introduction

The exploitation of small domestic ruminants represents an important commercial activity worldwide, and most especially in countries with arid and/or semiarid climates (SIMPLÍCIO et al., 2004; LEITE, 2007). In 2002, the ovine population in Brazil was estimated at 15 million animals accounting for 1.45% of the global flock (FOOD AND AGRICULTURE ORGANIZATION, 2007) and most animals (57%) were in northeastern Brazil. In the semiarid Caatinga area of this region, the average sheep meat production is around 2.8 kg.ha⁻¹ per year, but it could be increased to between 31.4 and 71.2 kg.ha⁻¹ with more appropriate farming methods (VASCONCELOS; VIEIRA, 2007).

Although sheep husbandry has developed considerably over the years, infectious and parasitic diseases are still major challenges to production, and continue to cause significant economic losses (PINHEIRO et al., 2002). One of the most important and insidious parasitic diseases is coccidiosis. Its causative agents are obligate intracellular protozoan pathogens of the genus *Eimeria*. *Coccidia* infect the intestinal tract with clinical manifestations that vary from asymptomatic to severe diarrhea with blood and gut lining tissue in the feces. Subclinical coccidiosis is the most common form of the disease and, since it cannot be readily identified, have a significant impact on the flock's health and production efficiency. Although coccidiosis affects animals of all ages, it is more prevalent in young animals and especially in newly weaned and confined lambs. Animals aged between four and seven weeks are most susceptible to the disease and account for most clinical cases (GREGORY et al., 1980; FOREYT, 1990).

Losses may amount to US\$ 120 million when costs associated with disease burden, such as expenses with antibiotics, disinfectants, veterinary care, specialist equipment and manpower, and losses associated with reorganization of farm management and lower productivity due to parasitism and/or medication are factored in. For example, Pinheiro et al. (2002) have estimated that the cost (including manpower and drugs) of treating coccidiosis in small ruminants amounts to US\$ 8 per animal.

Since there are scarce reports of ovine coccidiosis in Brazil in general, and in the northeastern region in particular, the aim of the present study was to examine and describe changes in the course of *Eimeria* infection in lambs. The animals studied were raised under an extensive regime in a farm in Rio Grande do Norte, the fifth largest sheep-producing state in Brazil with a flock of over 488,000 animals (ANUALPEC, 2005).

Material and Methods

This study was carried out on a 1042 ha sheep farm located in Angicos, Rio Grande do Norte State, northeastern Brazil (05° 42' 00" S and 36° 14' 41" W) (IDEMA, 2007) during April to August 2008. The climate in the Angicos region is characterized by a distinct rainy (from February to July) and a dry season (from August to January) (meteorological station of Empresa de Pesquisa Agropecuária do Rio Grande do Norte). At the start of the study in April 2008, the rainfall was 212 mm per month but gradually

fell to 26 mm per month in June. The average temperature was 28 °C and the relative humidity ranged between 64 and 74%.

The sheep flock was kept on the farm under an extensive or semi-intensive regime and comprised some 1,500 animals of the mixed breed Santa Inês, including 1,300 ewes, 200 lambs and 16 rams (five of the Doppler breed and 11 of the Santa Inês breed). In order to monitor infection by *Eimeria* parasites, fecal samples were collected on a weekly basis from each of the 27 male lambs starting at the day after birth until the age of 90 days. These lambs were raised extensively. Fecal material was prepared for analysis by mixing it in a saturated sucrose solution and using centrifugal flotation technique (MENEZES; LOPES, 1995). The samples that contained *Eimeria* species were submitted to quantitative analysis by counting the number of oocysts per gram of feces (OOPG) (UENO; GONÇALVES, 1998). After counting, the prepared samples were placed in Petri dishes containing aqueous potassium dichromate solution (2.5%, w/v) for seven days at room temperature to induce sporulation (DUSZYNSKI; WILBER, 1997). Sporulated oocysts were separated using the centrifugal flotation technique in Sheather's sugar flotation solution (DUSZYNSKI; WILBER, 1997). Oocysts were examined as proposed by McKenna (1972) at 40× and 100× magnification on an Olympus BX 40 optical microscope (Tokyo, Japan) coupled to a DP11 digital camera. *Eimeria* species were described based on morphological (size, shape, color, absence or presence of micropyle and micropylar cap, texture of the external wall, shape of sporocysts, absence or presence and characteristics of sporocyst residue, and Stieda body) and morphometric characteristics (dimensions) of oocysts and sporulating oocysts (LEVINE, 1961; VERCRUYSSSE, 1982; AMARANTE; BARBOSA, 1992; VIEIRA et al., 1999; HASSUM; MENEZES, 2005). One hundred oocysts were randomly selected and identified to determine the percentages of each *Eimeria* species present in the fecal samples and then OOPG per *Eimeria* species was counted.

Results

Oocysts were detected in the feces of 17% of lambs at week 3 after birth, and then percentage of animals excreting oocysts increased progressively and achieved 100% by week 6 when the average OOPG count was 77,501 (Figure 1). The elimination of oocysts fell dramatically by week 7 to an average OOPG count of 25,965, and then gradually fell from week 9 onwards. By week 10, some lambs either stopped eliminating oocysts or were eliminating them in very low numbers. Total OOPG counts for individual animals varied significantly during the observational period and ranged from 0 to 465,400.

Eight *Eimeria* species were identified in the fecal samples in the following frequencies: *E. crandallis* Honess, 1942 65.7%, *E. parva* Kotlan, Moscy and Vajda, 1929 54.7%, *E. granulosa* Christensen, 1938 53.7%, *E. ovinoidalis* McDougald, 1979 48.8%, *E. ahsata* Honess, 1942 43.3%, *E. ovina* Levine and Ivens, 1970 (syn. *E. bakuensis* Musaev, 1970) 43.3%, *E. faurei* Moussu and Marotel, 1902 29.0% and *E. intricata* Spiegl, 1925 3.0%. Only 32 (16.8%) of the 191 fecal samples that contained *Eimeria* oocysts were infected with a single species, while 23 (12%) were

infected with two different species and 136 (71.2%) harbored three or more species.

All animals eliminated *E. crandallis*, *E. absata*, *E. granulosa* and *E. parva* oocysts, although the prevalence of the different species varied according to age. *E. absata* and *E. granulosa* predominated in week 3 whereas *E. crandallis* was more common by week 4 and predominated until week 9 and remained present until week 12. *E. parva* predominated for a short period during week 10. In contrast, *E. granulosa* increased gradually as the lambs grew older and predominated from week 11 until the end of the experimental period (Figure 2).

The kinetics of oocyst elimination of individual *Eimeria* species were characterized by one or two peaks, although excretion peaks

of different coccidians coincided in some cases (Figure 3). Single peaks of *E. crandallis* and *E. ovinoidealisis* occurred together and overlapped with the first peak of *E. parva* by week 6 while single peaks of *E. ovina* and *E. granulosa* coincided by week 9, and that of *E. absata* overlapped with the second peak of *E. faurei* by week 8. *Eimeria intricata* had two peaks, the first one was coincident with the first peak of *E. faurei* at week 5 while the second one overlapped with the second peak of *E. parva* at week 10. In general, the second elimination peaks were associated with lower oocyst counts than those seen in the first peaks, and the counts decreased gradually and continuously thereafter until they stabilized at low values.

Discussion

This is the first study to describe the natural course of *Eimeria* infection in lambs raised in the northeastern state of Rio Grande do Norte. The finding of oocyst elimination from the third week of age onwards suggests that the lambs acquired infection during the first days of life. The primary sources of infection were most likely environmentally contaminated udders of the mothers or ingestion of contaminated soil and other materials (POUT, 1973; SILVA, 2006). It is known that oocysts are very resistant structures that can remain viable in the environment for several months under favorable conditions (FOREYT, 1990; LIMA, 2004). In general, lambs raised in pastures that have been previously used for grazing excrete more *Eimeria* oocysts than those raised on new pastures (HELLE; HILALI, 1973).

The oocyst elimination peak that occurred at week 6 is consistent with a time of infection around week 3 when the lambs

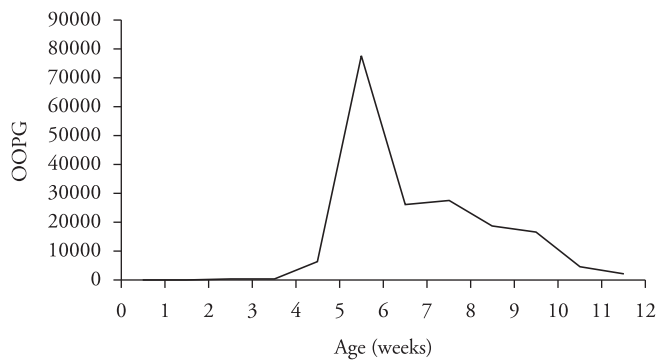


Figure 1. Average number of oocysts per gram of feces (OOPG) in samples from naturally infected lambs aged 1 to 12 weeks. The study was conducted between April and August 2008.

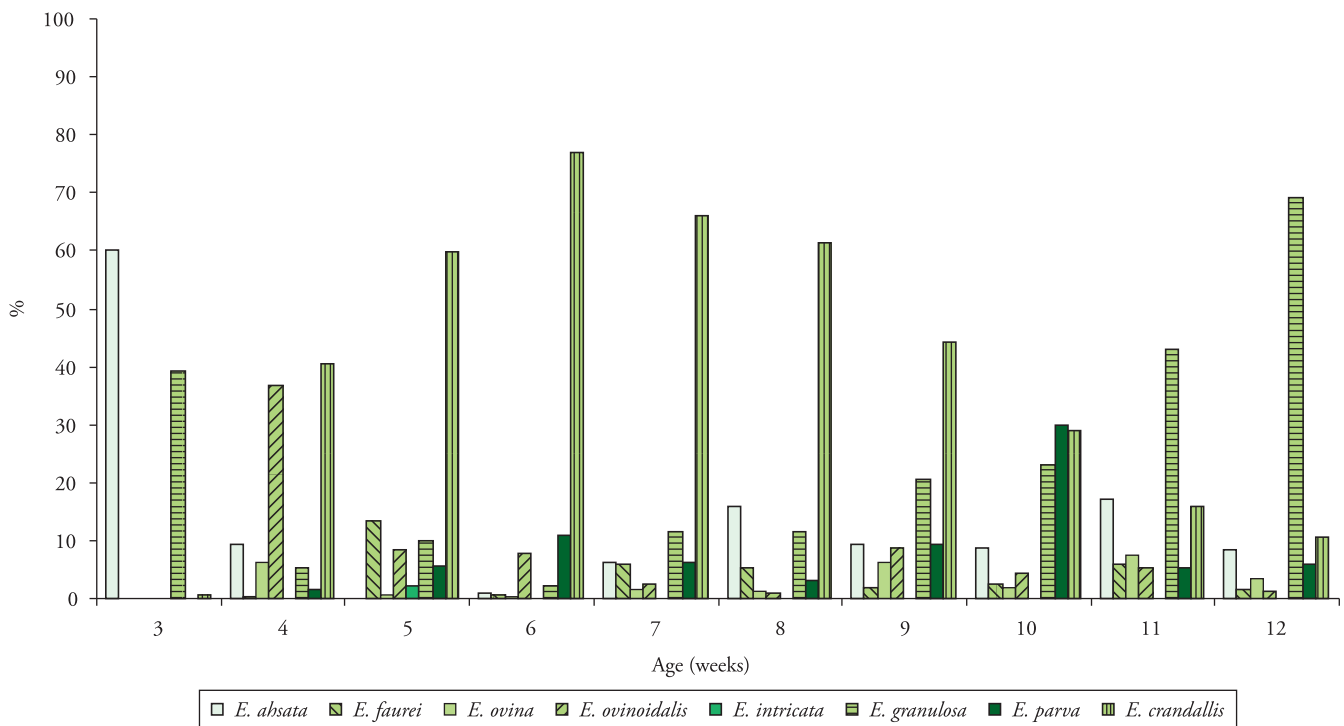


Figure 2. Percentages (%) of *Eimeria* species found in fecal samples from naturally infected lambs aged 1 to 12 weeks. The study was conducted between April and August 2008.

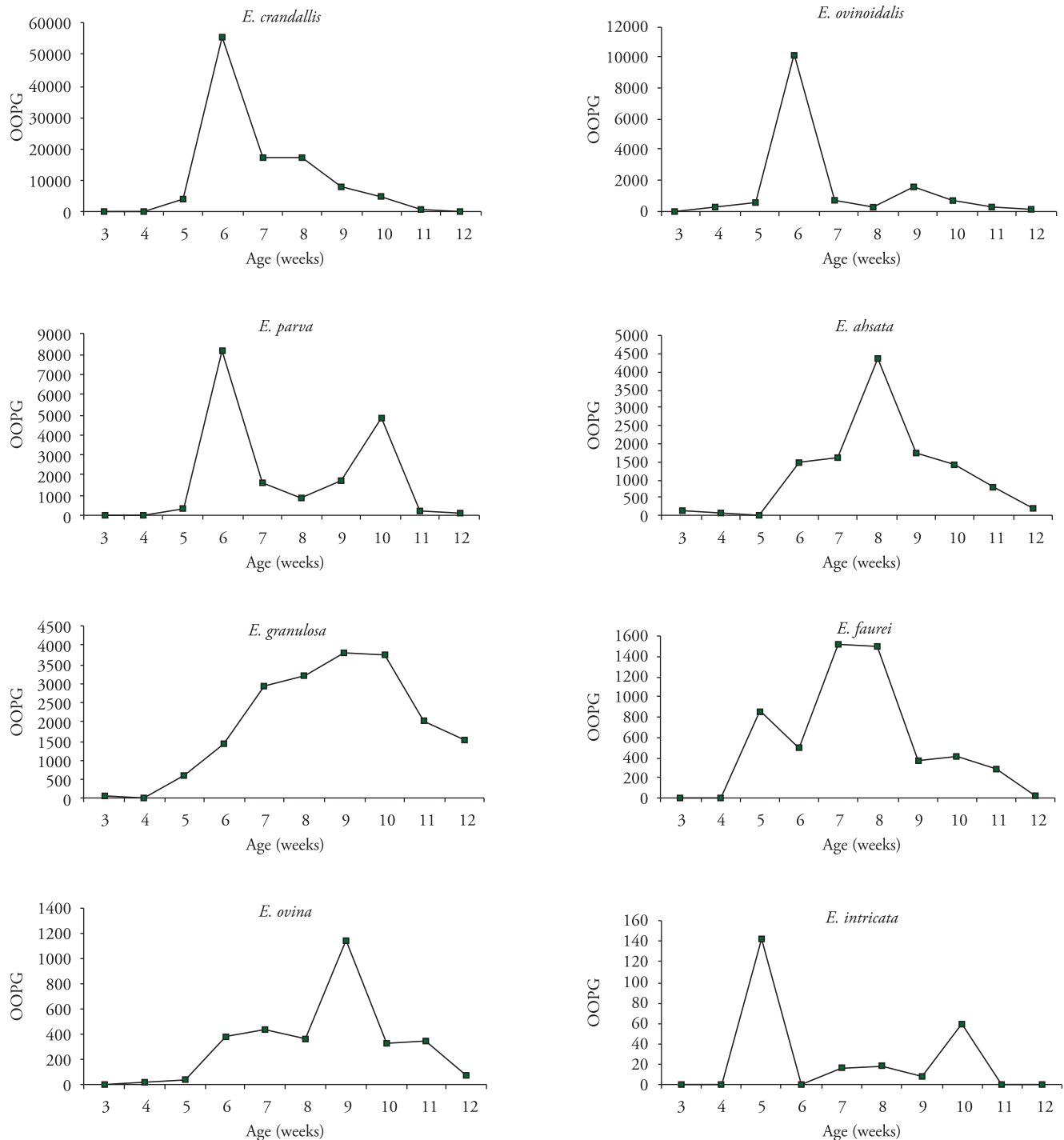


Figure 3. Total number of oocysts per gram of feces (OOPG) in samples from naturally infected lambs aged 1 to 12 weeks eliminating different *Eimeria* species. The study was conducted between April and August 2008.

started grazing on pasture (MANSON, 1977; SILVA, 2006). The progressively increasing number of oocyst-excreting lambs suggests that the animals themselves were the main source of environmental contamination. The reduction in oocyst excretion following the elimination peak was probably due to the development of resistance after initial exposure, and the consequent reduction of susceptible population (POUT et al., 1966; CHAPMAN, 1974; HELLE; HILALI, 1973).

The remarkable variation in OOPG counts among individuals within the study cohort may be attributed to a number of factors including health and nutritional status of the host, host susceptibility, infecting dose, continuous challenge imposed by the presence of oocysts, and differential ability of *Eimeria* species to form cysts in the host (POUT et al., 1966; MANSON, 1977). Other factors may be related to livestock management methods (GAULY et al., 2004) and climatic conditions (MANSON, 1977).

The majority of the eight species identified have already been described in Brazilian sheep (SANTIAGO; COSTA, 1975; SANTANA et al., 1983; AMARANTE; BARBOSA, 1992; VIEIRA et al., 1999; MENEZES et al., 2001; SILVA, 2006). Variations in the infecting species and their prevalences have been reported in different studies and may be attributed to environmental, chemical and physical factors that affect sporulation, survival and infectivity of oocysts, parasite strain and its intrinsic reproductive potential in the host, host traits (age, immunological status, race), livestock management (feeding, weaning and sanitary conditions), and concurrent diseases and stresses (GOUET et al., 1984; CATCHPOLE; HARRIS, 1989; FOREYT, 1990).

The present study found a direct correlation between increasing percentage of infected lambs and age, and this is consistent with previous findings (POUT et al., 1966; POUT, 1973). Furthermore, the study results confirm previous reports (HELLE; HILALI, 1973; VIEIRA et al., 1999; SILVA, 2006) that oocyst elimination profiles of different *Eimeria* species are characterized by one or two elimination peaks followed by an abrupt reduction in OOPG count, and that elimination peaks of some *Eimeria* spp. overlap in time. Previous studies (HELLE; HILALI, 1973; SILVA, 2006) have also reported the predominance of *E. crandallis* at peak coccidial elimination, indicating that infection was acquired around the third week of life since this species has a pre-patent period of 13 to 32 days (LEVINE, 1961; SILVA, 2006). Vieira et al. (1999) reported that *E. crandallis* was the species that produced the greatest number of oocysts in the first elimination peak, suggesting that this parasite may be associated with clinical diseases that affect lambs two to three weeks after moving them to pasture.

The oocyst elimination peak of *E. ovinoidalis*, which is one of the most pathogenic of sheep parasites (McDOUGALD, 1979; GREGORY et al., 1989), occurred at week 6 and coincided with that of *E. crandallis*. In the present study, the elimination peak of *E. intricata* occurred at week 5, which is consistent with Amarante and Barbosa findings (1992), but contrasts with Vieira et al. (1999) reports. These authors found *E. intricata* oocysts only after week 9 and suggested that this species had a preference for older animals.

The results of the present study revealed that during the observational period most lambs had multiple infections while fecal samples containing oocysts from three or more different species became more common as the lambs grew older. In fact, field-acquired infections are generally mixed as the animals are more exposed to the existing environmental biodiversity (GREGORY et al., 1980; SILVA, 2006).

In conclusion, this study has clearly demonstrated that the animals studied were affected by a mixed combination of coccidian parasites with different oocyst kinetics and pathogenicity, especially for animals grown under an extensive regime. Since subclinical coccidiosis has a significant impact on the economics of livestock production, the most cost-effective means of reducing potential losses would be to implement an ongoing prevention program by providing lambs with an environment as clean as possible by removing all waste materials, raising lamb feeding troughs above ground level to limit exposure to infective oocysts, and adopting routine practices such as appropriate drug therapy prior to parturition or during weaning.

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