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Modelling the faecal worm egg count curve during the periparturient period in Uruguayan Merino sheep

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Abstract

Disease caused by gastrointestinal nematodes (GIN) is one of the main constraints for sheep production worldwide. Although adult categories are more resistant to GIN, a temporary loss of acquired immunity is presented around parturition, which results in the so-called "periparturient rise" (PPR) in fecal excretion of GIN eggs. The objective of the present work was to study the dynamic of the PPR and to model the faecal worm egg count (FEC) curve during the periparturient period in Uruguayan Merino sheep. Faecal samples were collected from lambing ewes during 2009 and 2010 parturitions. FEC and infective larvae cultures of each sampling were recorded. A total of 2,121 records from 748 ewes, the progeny of 107 sires, were used in the statistical analysis. FEC data were normalized using two logarithmic transformations: Log FEC = Log_e (FEC + 100) and Log FEC_st (Log FEC with the variance standardized within contemporary group). Three functions were compared to shape the PPR curve. *Haemonchus* spp. was the most prevalent parasite. Log FEC_st was the selected response variable of the model, for its better adjustment to a normal distribution and a more homogeneous residual variance. The fixed regression model with Legendre polynomials was the selected one, based on the selection model criteria (Akaike & Schwarz Bayesian Information Criteria). The highest egg output was observed between two and four weeks post-lambing. In conclusion, the PPR observed in Uruguayan Merino lambing ewes had the maximum egg output matched with the milk production peak.

Additional key words: gastrointestinal nematodes; *Haemonchus contortus*; lambing ewes; periparturient rise.

Resumen

Modelación de la curva del recuento de huevos de parásitos durante el período del periparto en ovinos Merino uruguayo

La infección por nematodos gastrointestinales (GIN) es una de las principales limitantes de la producción ovina mundial. Si bien los adultos son más resistentes a los GIN, una pérdida temporal de la inmunidad adquirida se presenta en el período del periparto, resultando en el "alza de lactación" (PPR) en la eliminación de huevos de GIN (HPG). El objetivo del presente trabajo fue estudiar la dinámica del PPR y obtener la curva del recuento del HPG durante el periparto en ovejas Merino uruguayo. Se recogieron muestras de heces de ovejas en las pariciones 2009 y 2010, realizándose HPG y coprocultivo de cada muestreo. Para el análisis estadístico se utilizaron 2,121 datos de 748 ovejas, hijas de 107 padres. Se llevaron a cabo dos transformaciones logarítmicas de manera que los datos siguieran una distribución normal: Log HPG = Log_e (HPG+100) y Log HPG_st (Log HPG con la varianza normalizada dentro del grupo contemporáneo). Se compararon tres funciones para modelar la curva del PPR. *Haemonchus* spp. fue el género más frecuente. Log HPG_st fue la variable de respuesta del modelo seleccionada, por el mejor ajuste a la distribución normal y mayor homogeneidad de la varianza residual. Se seleccionó el modelo con polinomios de Legendre, en base a los criterios de selección del modelo (Akaike y Schwarz Bayesian Information Criteria). La mayor eliminación de huevos se observó entre las dos y cuatro semanas posparto. En conclusión, se observó el PPR en ovejas Merino uruguayo, coincidiendo la máxima eliminación de huevos de GIN con el pico máximo de producción de leche.

Palabras clave adicionales: alza de lactación; *Haemonchus contortus*; nematodos gastrointestinales; ovejas parturientas.

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Abbreviations used: AIC (Akaike information criteria); BIC (Schwarz bayesian information criteria); CG (contemporary group); DML (days of faecal egg count measurement respect to lambing); FEC (faecal worm egg count); FMN (Fine Merino Nucleus); GIN (gastrointestinal nematodes); INIA (Instituto Nacional de Investigación Agropecuaria, National Institute for Agricultural Research); Log FEC_st (natural logarithm of Faecal egg count with the variance standardized within contemporary group); PER (faecal egg count measurement period); PPR (periparturient rise).

Introduction

Gastrointestinal nematodes (GIN) are one of the main constraints for sheep production in Uruguay and worldwide (Castells *et al*., 1995; Perry & Randolph, 1999). Although lambs are the most susceptible category to GIN infection, lambing ewes also experience a relaxation in acquired immunity around parturition through a phenomenon called periparturient rise (PPR). The PPR was firstly documented by Taylor (1935) and it can be defined as a temporary but marked increase in nematode eggs output by lambing ewes; that begins in the last weeks of gestation and reaches the maximum peak in the first weeks post-parturition. It is an important event because it represents a pasture larval contamination source for newborn lambs (Bishop & Stear, 2001; Romero & Boero, 2001). The cause has not yet been determined, but it is generally accepted that the rise occurs after an immunity depression of the host by stressful factors such as pregnancy, parturition, lactation, climate and malnutrition (Barger, 1993).

The PPR was firstly described in Uruguay by Nari *et al*. (1977a) in the Ideal breed, where *Haemonchus* spp. represented 82% of the total of the parasite genera presented. They worked with only 71 animals which were divided in three groups: lambing ewes that were dewormed before parturition, lambing ewes without a *pre-partum* anthelmintic treatment and non-treated barren ewes (control group). These authors observed that the maximum egg output took place between six and eight weeks post-lambing, but they did not model the shape of the PPR curve.

Because it is important to know the dynamic of the PPR for the flock management and parasite control, the aim of the present study was to model the phenotypic curve of faecal worm egg count (FEC) during the periparturient period in Uruguayan Merino sheep.

Material and methods

Animals and management

Animals belong to three flocks genetically connected by reference rams: the Fine Merino Nucleus (FMN) belonging to "Glencoe", a research station of the Instituto Nacional de Investigación Agropecuaria (INIA) of Uruguay, and two Merino studs: "Talitas" and "La Gringa", belonging to the same breeder. The three flocks are located in the northern part of Uruguay, characterized by a warm and wet climate, with a mean annual temperature of 18-19°C, relative humidity of 70-72% and an average annual rainfall of 1,400-1,500 mm (Castaño *et al*., 2011). These conditions are favorable for the development and survival of larvae of GIN throughout the year, varying the presence and predominance of different parasite genus according to the season.

In FMN, ewes are managed in parturition groups according to the average expected day of lambing. A *pre-partum* strategic drenching is performed every year as a management control measure, approximately one month prior to the expected beginning of lambing. Anthelmintic used were DOVENIX® in 2009 and TRIMIX® in 2010. In "Talitas" and "La Gringa", ewes are managed in a single parturition group. In these stud flocks, ewes are not always dewormed before parturition because drenching is performed strategically; *i.e.* periodically stool samples are collected from a random sample of animals, and if FEC average is higher than 500 eggs per gram, all the parturition group is drenched immediately. For the present study, it was not necessary anthelmintic treatment in these flocks.

Ewe records

A total of 2,500 faecal samples were collected in 2009 and 2010, during lambing season. The age of ewes ranged between of 2 and 10 years-old and litter size was recorded as single or multiple (≥ two lambs). The experiment was conducted between days –50 and +68 respect to lambing (day 0). Each ewe was sampled on average three times, under natural mixed-species parasite challenge on pasture. All ewes in the same cohort were sampled on the same day. The first sample was collected in late pregnancy and the others in early and mid-lactation. The number of post-lambing measurements depended on the degree of parasitic infection; if FEC counts within a cohort reached certain levels such that animal health and welfare might be compromised, then all animals in that cohort were immediately drenched. Cohorts were defined as a parturition group in case of FMN or all the flock in case of "Talitas" and "La Gringa" studs. In FMN, ewes were sampled in the two years of the experiment (Table 1). In 2009, a total of six measurements were performed on 293 ewes divided in four parturition groups, obtaining a total of 742 records. In 2010, five measurements were performed on 345 ewes (of which 185 were also sampled

Table 1. Data collection in Fine Merino Nucleus, "Talitas" and "La Gringa"

	Cohort	Weeks respect to expecting lambing date					
Fine Merino Nucleus 2009							
Sampling date		03-sep	26-oct	13-nov	18-nov	02-dec	18-dec
Expecting lambing date							
14-sep	1	–1.6	6.0				
01-oct	2	–4.0	3.6		6.9		
18-oct	3		1.1	3.7		6.4	
05-nov	4		–1.4	1.1		3.9	6.1
Number of records		129	237	135	38	150	53
Fine Merino Nucleus 2010							
Sampling date		02-sep	20-sep	14-oct	01-nov	24-nov	
13-sep	1	–1.6		4.4	7.0		
30-sep	2	–4.0	–1.4	2.0	4.6		
15-oct	3		–3.6		2.4	5.7	
Number of records		241	173	199	219	49	
Talitas and La Gringa 2010							
Sampling date		30-aug	02-oct				
01-set		–0.3	4.4				
07-set		–1.1	3.6				
15-set		–2.3	2.4				
22-set		–3.3	1.4				
Number of records La Gringa		324	272				
Number of records Talitas		142	139				

in 2009) divided in three parturition groups, obtaining a total of 881 samples. In "La Gringa" and "Talitas" studs, only 349 and 173 females, respectively, were sampled in 2010, with two measurements performed in each flock, obtaining a total of 877 samples (596 and 281, respectively) (Table 1).

Parasitological analysis

FEC were determined using the modified McMaster technique (Whitlock, 1948), where each egg observed represented 100 eggs per gram of faeces. In addition, in 2010, faecal cultures of infective larvae were prepared to assess the species composition of nematode infection in each flock.

Statistical analysis: Exploratory analysis of ewe FEC in the periparturient period

A total of 2121 records from 748 ewes, the progeny of 107 sires, were used in the statistical analysis. FEC data were transformed prior to analysis using the following logarithmic transformations, in order to remove skewness and to normalize data:

$$\text{Log FEC} = \text{Log}_e\,(\text{FEC} + 100)$$
$$\text{Log FEC\_st} = (\text{Log}_e\,(\text{FEC} + 100) - m_{GC}) / ds_{GC}$$

where Log FEC_st is the natural logarithm of FEC records (Log FEC) with the variance standardized within contemporary group (CG); and m_{CG} and ds_{CG} are the mean and standard deviation of Log FEC of each CG, respectively.

Data were analyzed by a sire repeatability model through MIXED procedure of SAS statistical package (SAS Inst, 2004). Three functions were compared to model ewe FEC in the periparturient period ("PPR curve"): 1) fixed classes of FEC measurement period (PER); 2) days of FEC measurement respect to lambing (DML) as lineal and quadratic covariates; and 3) quadratic Legendre polynomials in function to DML. The general model common to the three functions was the following:

$$y_{ijklm} = CG_i + LS_j + EA_k + f + s_l + p_m + e_{ijklm}$$

where y is FEC transformed (Log FEC or Log FEC_st). The fixed effects are: CG (defined as sampling date by flock by parturition group, with 26 levels), LS litter size (two levels: single and multiple); EA ewe age (four levels: 2, 3, 4 and ≥ 5 years). The tested function is f,

that according to the model is: PER (4 levels: ≤–15, >–15, >15 and >30 days of FEC measurement respect to lambing), DML and DML^2 as covariates, or second-order Legendre polynomials. The random effects are: s the additive genetic effect of the sire (107 levels), p the permanent environmental effect of the animal (748 levels) and e is the residual term.

The criteria used for choosing the model with the better adjustment were the Akaike Information Criteria (AIC) and the Schwarz Bayesian Information Criteria (BIC). Once the model was selected, studentized residuals of Log FEC and Log FEC_st were calculated, in order to detect possible extreme values. Records with studentized residuals ≥ to 3.0 or ≤ to –3.0 were eliminated as outliers (Ott & Longnecker, 2001).

Descriptive statistic for FEC, Log FEC, Log FEC_st and the studentized residuals was estimated, through UNIVARIATE procedure of SAS statistical package (2004). Based on residuals analysis and descriptive statistical values of Log FEC and Log FEC_st, it was selected the response variable (y) of the model.

Results

Parasitological analysis

Results of larvae cultures are presented in Fig. 1. *Haemonchus contortus* was the most prevalent parasite species in all samples and flocks. It was followed in order of predominance, by *Trichostrongylus columbriformis* and *Teladorsagia circumcinta* spp. Other genera (*i.e. Oesophagostomum* spp., *Cooperia* spp.) were found in a very small proportion of the samples.

Statistical analysis: Exploratory analysis of ewe FEC in the periparturient period

The descriptive statistic of ewe FEC without and after logarithmic transformation is shown in Table 2. For FEC without transformation, the mean, median and mode values clearly differ among them, and data were strongly positively skewed. Due to the large difference among the measures of central tendency and the elevated values of the skewness and kurtosis, data were adjusted by logarithmic transformation, with and without variance standardization (Log FEC_st and Log FEC). Transformed variables presented closer values of measures of central tendency, and skewness and kurtosis values were close to zero; although Log FEC_st transformation had a better adjustment to a normal distribution than Log FEC.

The studentized residuals of Log FEC and Log FEC_st were also analyzed. The pattern of residuals of Log FEC, as a function of the predicted mean, showed heteroskedascity of the residual variance (Fig. 2a). The distribution of studentized residuals of Log FEC_st had a better adjustment to a normal distribution and they were more uniformly distributed when were plotted as a function of the

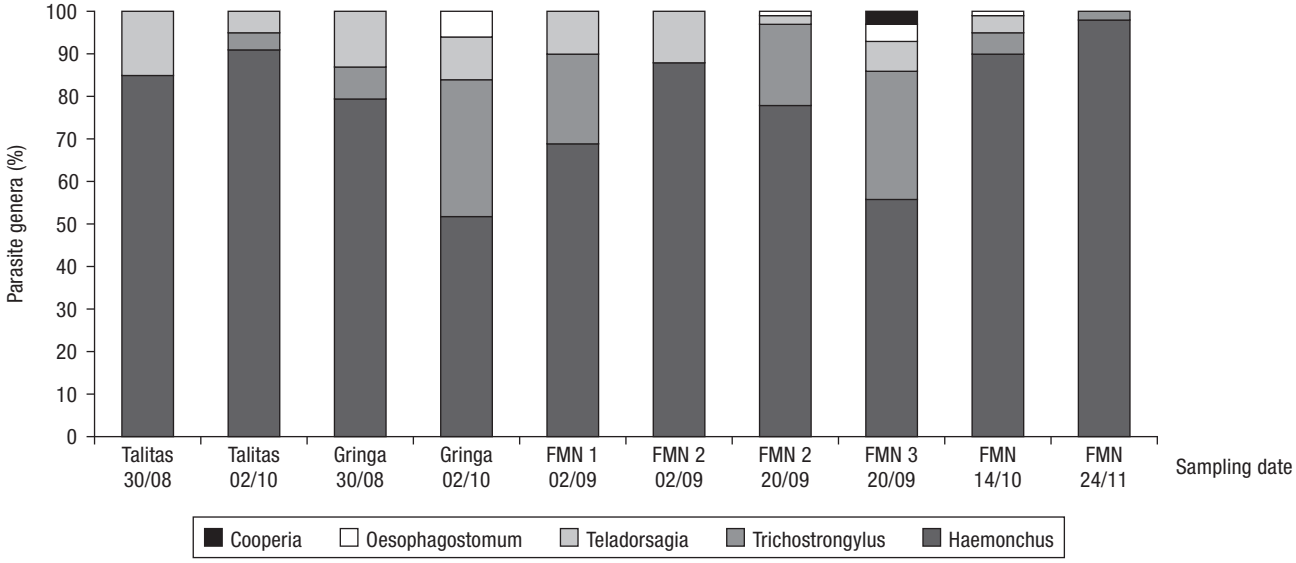

Figure 1. Parasite genera presented in the sampling performed in the three flocks. FMN 1, FMN 2, FMN 3: Fine Merino Nucleus parturition group 1, 2 and 3, respectively.

Table 2. Descriptive statistics of FEC (faecal worm egg count) and FEC after logarithmic transformation with and without variance standardization (Log FEC and Log FEC_st)

Trait	N	Mean	Median	Mode	SE	Skewness	Kurtosis
FEC	2110	765.59	300.00	0.00	1173.34	4.12	32.15
Log FEC	2110	6.07	5.99	4.61	1.21	0.19	–1.15
Log FEC_st	2110	–0.01	–0.18	–0.71	0.97	0.23	0.13

SE: standard error.

predicted mean (Fig. 2b). Thus, this transformation removed the heterogeneous residual variance pattern.

Due to a better adjustment to a normal distribution and a more homogeneous distribution of the residuals, Log FEC_st was selected as the response variable of the model.

Periparturient rise

The three functions used to shape the PPR resulted in similar curves (Fig. 3); although the model with second-order Legendre polynomials was the selected one, based on the model selection criteria (lower AIC and BIC values).

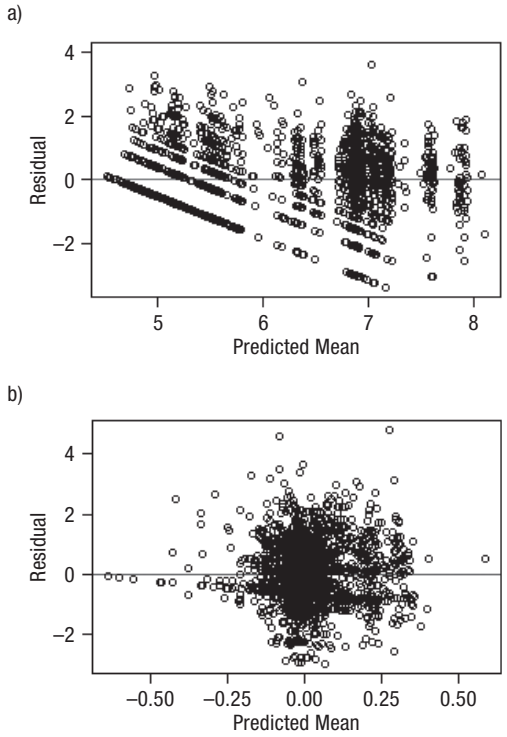

Figure 2. Distribution of studentized residuals of FEC logarithm (a) and FEC logarithm with the variance standardized within contemporary group (b) in function to the predicted mean.

The maximum nematode egg output was observed after lambing date, reaching the maximum peak in the third period of measurement (PER 3), that is, between 2 and 4 weeks post-parturition. Unfortunately, it was not possible to study the shape of PPR function beyond 68 days post-lambing, because of the high level of parasite infection in some animals began to compromise welfare and health, thus sampling end sooner than expected.

Discussion

Parasitological analysis

Results of larvae cultures are consistent with Nari *et al*. (1977b) and Castells (2009) reports, who also found that *Haemonchus* spp. and *Trichostrongylus* spp. were the two predominant parasite genera in Uruguay, both in lambs and lambing ewes.

The cause of the increase in nematode egg output during the periparturient period has not yet been determined. The PPR may derive from an increase in adult parasite populations by an increased rate of establish-

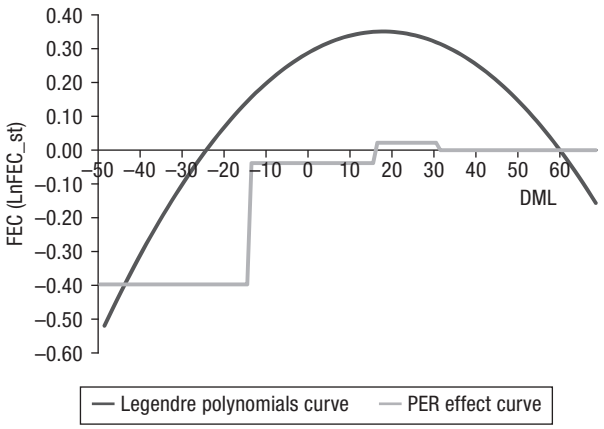

Figure 3. Phenotypic curve of ewe FEC logarithm standardized within contemporary group (Log FEC_st) during the periparturient period, in function to days of FEC measurement respect to lambing (DML).

ment of newly ingested larvae which develop to the adult stage without inhibition, as well as by the resumption of development of previously inhibited fourth-stage larvae. In addition, both newly acquired and established adult female parasites may show increased fecundity (O'Sullivan & Donald, 1970; Urquhart *et al.*, 1996). Thus, the high percentage of *H. contortus* could be explained by newly ingested larvae of this parasite and/or the maturation of hypobiotic larvae that arrested the development over winter.

Statistical analysis: Exploratory analysis of ewe FEC in the periparturient period

The results of the statistical analysis of ewe FEC were in agreement with those reported by several authors (*e.g.* Gasbarre & Miller, 2000; Stear *et al*., 2007), who described that the distribution of FEC is skewed and overdispersed; where most hosts have relatively low egg counts while a small proportion of hosts eliminate a large number of eggs.

Different authors found different transformations (*e.g.* square root, cube root, Log (FEC+25)) as the most appropriate to their data structure, being appropriate to explore and identify, which is the best transformation in each particular case (Eady, 1995; Castells, 2009). Log_e (FEC+100) transformation was also used by Watson *et al*. (1995) and Morris *et al.* (1998), as well as in the sheep genetic evaluation for resistance to GIN in Uruguay (Ciappesoni *et al.*, 2010). An alternative that has also been described is data transformation with variance standardization. Brown & Tier (2003) and Pollott & Greeff (2004) transformed FEC count through the cube root with the variance standardized within contemporary groups. Brown & Tier (2003) found a high phenotypic and genetic correlation between the variable with and without standardization of the variance (0.95 ± 0.00 and 0.95 ± 0.01, respectively).

Periparturient rise

The second reason to select the model with second-order Legendre polynomials was that orthogonal polynomials of standardized units of time, as Legendre polynomials, have been recommended as covariates in regression models because they are easy to calculate and to use, and decrease the correlation between the estimated regression coefficients (Mrode, 2005).

The observation of the present study was consistent with the reported by Herd *et al*. (1983), who also found that the PPR occurs between two and four weeks after parturition, matching the milk production peak that in Merino breed occurs approximately at three weeks post-lambing (Corbett, 1968). Salisbury & Arundel (1970) observed the maximum egg output between four and six weeks post-lambing, while Crofton (1954) and Nari *et al*. (1977a), described this event between 6 and 8 weeks post-parturition.

In conclusion, it was observed the PPR in lambing ewes belonging to three Uruguayan Merino flocks. The maximum nematode egg output was observed between two and four weeks post-lambing, matching with the maximum milk yield peak. The pathogenicity of GIN varies with species and with the number of parasites present. Thus, it is important to take into account these results for sheep flock management around parturition, when newborn lambs will be on contaminated pastures being very susceptible to parasite infection.

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