Pascoti Bruhn, Fábio Raphael; Silva Júnior, Fidelis Antônio; de Oliveira Carvalho, André
Henrique; Ribeiro Orlando, Débora; Barcellos Magalhães da Rocha, Christiane Maria;
Marcos Guimarães, Antônio

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Ocorrência de *Eimeria* spp. e nematódeos gastrintestinais em bezerros no sul de Minas Gerais, Brasil

Fábio Raphael Pascoti Bruhn[1]; Fidelis Antônio Silva Júnior[1]; André Henrique de Oliveira Carvalho[1];
Débora Ribeiro Orlando[1]; Christiane Maria Barcellos Magalhães da Rocha[1]; Antônio Marcos Guimarães[1]*

[1]Departamento de Medicina Veterinária, Universidade Federal de Lavras – UFLA, Lavras, MG, Brasil

Abstract

The aim of this cross-sectional observational study was to determine the frequency and factors associated with infection by *Eimeria* spp. and gastrointestinal nematodes in 356 calves on 20 dairy farms located in southern Minas Gerais, Brazil. Ten species of *Eimeria* spp. were identified, of which *E. bovis* (37.6%) and *E. zuernii* (17.9%) were the most frequent. From fecal cultures, four genera of gastrointestinal nematodes were recovered, of which *Cooperia* spp. (74.6%) and *Haemonchus* (19.4%) were the most frequent. Variables relating to higher levels of technology used on dairy farms showed a significant association ($p < 0.05$) with higher OPG and EPG counts, and are discussed in this study.

Keywords: EPG, OPG, fecal cultures, risk factors.

Resumo

O objetivo deste estudo foi determinar a frequência e os fatores associados à infecção por *Eimeria* spp. e nematódeos gastrintestinais, em 356 bezerros provenientes de 20 rebanhos leiteiros, localizados no sul de Minas Gerais, Brasil. Foram identificadas dez espécies de *Eimeria* spp., sendo *E. bovis* (37,6%) e *E. zuernii* (17,9%) as mais frequentes. Nas coproculturas, foram recuperados quatro gêneros de nematódeos gastrintestinais, sendo os mais frequentes *Cooperia* spp. (74,6%) e *Haemonchus* spp. (19,4%). Variáveis relacionadas a um maior nível de tecnificação das propriedades leiteiras apresentaram associação significativa ($p < 0,05$), com maiores contagens de OoPG e OPG, e são discutidas neste estudo.

Palavras-chave: OPG, OoPG, coprocultura, fatores de risco.

Introduction

Among the factors that interfere in cattle development, gastrointestinal parasites such as *Eimeria* spp. and helminths stand out because of the economic losses that they cause in relation to low herd productivity, delayed animal development, death and significant expenses on management and medication.

Studies have shown that the intensity of *Eimeria* parasite species and gastrointestinal parasites in cattle varies between different regions of Brazil (REBOUÇAS et al., 1994; LIMA, 1998; ALMEIDA et al., 2011). This has a negative impact of varying degree on dairy production among these regions, thus indicating that there is a need for knowledge on the prevalence of these parasite species among herds. This is especially so in the south of Minas Gerais, one of the biggest dairy regions of the country, with mean annual production estimated at 1.6 billion liters of milk (IBGE, 2006). For effective control over gastrointestinal nematodes, knowledge of basic epidemiological factors is fundamental, especially regarding the regional characteristics or local specificities and the type of production system to which the ruminants are subjected (CEZAR et al., 2008).

Thus, the objectives of this study were to determine the frequencies of *Eimeria* spp. and gastrointestinal nematodes among dairy calves, and to evaluate factors inherent to zoosanitary management that are associated with occurrences of these parasites in herds in the southern region of the state of Minas Gerais. This information is relevant because it forms a preliminary stage in devising control schemes against these parasites in dairy herds.

Material and Methods

Feces samples from 356 calves on 20 dairy farms located in the municipalities of Boa Esperança, Bom Sucesso, Ijaci, Ingaí, Itumirím, Lavras and Nepomuceno were gathered between September 2008 and August 2009. A cross-sectional observational study was conducted to evaluate the frequency of *Eimeria* species

***Corresponding author:** Antônio Marcos Guimarães
Departamento de Medicina Veterinária,Universidade Federal de Lavras – UFLA,
CP 3037, CEP 37200-000, Lavras, MG, Brasil
e-mail: amg@dmv.ufla.br

and gastrointestinal nematode genera, and the risk factors associated with quantifying oocysts (OPG) and eggs per gram of feces (EPG) among calves during the pre-weaning phase (≤90 days of age) and post-weaning phase (>90 days to 12 months of age).

The 20 farms were divided equally into two groups, according to the type of milk produced: I – type B milk (BM) and II – raw refrigerated milk (RRM). The farms were visited on a single occasion, at the time of conducting interviews to gather information on the characteristics inherent to zoosanitary management of the herds. The farm properties were chosen randomly, and minimum numbers of ten fecal samples/herd and one fecal sample/animal were followed. The numbers of calves per property ranged from a minimum of 12 to a maximum of 205 animals. In all, 356 fecal samples were collected from calves between one day of age and 12 months of age. Individual fecal samples were collected directly from the rectal ampulla and were packed in plastic bags that were identified with the animal's date of birth, category (pre-weaning or post-weaning), farm name and date of sampling.

For individual quantification of eggs (EPG) and oocysts (OPG) per gram of feces, the technique of Gordon and Whitlock (1939) was used. For each BM or RRM herd, the calves were separated in two categories (pre-weaning and post-weaning), positive sample pools were formed for each of the categories, fecal cultures were performed (ROBERTS; O'SULLIVAN, 1950) and gastrointestinal nematode genera were identified (UENO; GONÇALVES, 1998). Positive sample pools for *Eimeria* spp. oocysts were placed on Petri plates with 2.5% potassium dichromate solution for seven days, in a "BOD" heating chamber (temperature of 28° and humidity >80%). After oocyst sporulation, the material was stored under refrigeration, up to the time of identifying the species, using the morphometric parameters described by Levine and Ivens (1965).

The Shapiro-Wilk normality test was applied to the quantitative variables (EPG, OPG, total area of the farm property, number of lactating cows, daily milk production and productivity). When normal distribution was absent, the median was used as a central trend measurement for the initial variables (EPG and OPG). Following this, the nonparametric Kruskal-Wallis and Mann-Whitney tests were performed on these variables with the aim of identifying differences between the pre-weaning and post-weaning phases and between the types of milk produced (BM and RRM). Evaluations on the association between positivity for *Eimeria* spp. oocysts and nematode eggs and the variables studied were made using the chi-square test. For significant variables (p < 0.05), the odds ratio (OR) was calculated with 95% confidence intervals. All analyses was performed using the SPSS 17.0 statistical software.

Results and Discussion

In the present study, there were statistical differences (p < 0.05) between the milk production systems in terms of the mean sizes of the farm properties (BM = 127.8 ha and RRM = 50.4 ha), the numbers of lactating cows (BM = 90.3 head and RRM = 33.5 head) and the total daily production of milk/farm (BM = 1568.5 l and RRM = 304.0 l). However, there was no difference in terms of the mean milk production per cow/day (BM = 15.01 l and RRM = 8.82 l) (p > 0.05).

In relation to the zootechnical and management characteristics of the herds that produced BM, 90% of cows were reared in a semi-intensive system, whereas in relation to RRM, 60% were kept in an extensive system. Milking was mechanical in 100% of the BM herds, but 90% of the cows on RRM farms were milked manually. Regarding the breeds on BM and RRM farm properties, respectively, 60 and 80% of herds were formed by purebred and half-breed animals. On BM farms, 100% of the calves were reared in individual shelters, which were located in pasture areas on 88% of the farms and on cemented floor on 12%. On 66% of BM farms, these shelters were located away from the corral, and the solar radiation and hygiene conditions were considered to be good or excellent in 100%. On RRM farm properties, 80% of calves were reared in a free-range manner, in ordinary fenced enclosures, located near to the corral in 90% of the cases, with solar radiation and hygiene conditions that were considered to be poor in 80% of the situations. On the properties that produced BM and RRM, 70 and 60%, respectively, practiced weaning of the animals at 90 and 120 days of age.

There were no significant differences (p > 0.05) in occurrences of different species of *Eimeria* and genera of gastrointestinal nematodes between calves during the pre-weaning and post-weaning phases, independent of the type of milk produced. Ten species of *Eimeria* were identified: *E. bovis* (37.6%), *E. zuernii* (17.9%), *E. ellipsoidallis* (17.3%), *E. auburnensis* (9.5%), *E. canadensis* (4.9%), *E. alabamensis* (4.5%), *E. subspherica* (3.1%), *E cylindrica* (2.3%), *E. wyomingensis* (1.9%) and *E. bukidnonensis* (1.0%). A similar result was found by Rebouças et al. (1994), in São Paulo, and Almeida et al. (2011), in Bahia. Although *E. auburnensis* and *E. wyomingensis* were not identified (respectively), they both mentioned *E. brasiliensis*. Bruhn et al. (2011) found a result similar to the present study among milking calves aged from three to seven months in the south of Minas Gerais, except for the presence of *E. pellita* and *E. brasiliensis*, thus totaling 11 species of *Eimeria* spp.

These results suggest that in Brazil, *E. bovis* is one of the most important species because it has repeatedly been found to be the most frequent species in many studies conducted in this country (REBOUÇAS et al., 1994; ALMEIDA et al., 2011; BRUHN et al., 2011). In the south of Minas Gerais, in addition to *E. bovis, E. zuernii* is also highly disseminated among calves, thus causing concern precisely because these are the species that are considered to be most pathogenic and most frequent among young cattle (TAUBERT et al., 2008; LASSEN et al., 2009).

In fecal cultures, four genera of gastrointestinal nematodes were identified: *Cooperia* spp. (74.6%), *Haemonchus* spp. (19.4%), *Strongyloides* spp. (3.5%) and *Bunostomum* spp. (2.5%). According to Bianchin et al. (1992), *Trichostrongylus* spp. is a genus that naturally competes with *Haemonchus* spp. in mixed infections, and this was a highly frequent species in the present study. Furthermore, according to Pimentel Neto and Fonseca (2002), establishment of parasites in calves is related to the animal's age, the helminth genus and the rearing location (management, regional climate, etc). Therefore, it is likely that some of these factors that were not evaluated factors in the present study may separately or in association have interfered with the results observed in the present study.

The occurrence of different species of nematodes in this study was similar to other findings in Brazil, in fecal cultures from calves on dairy farms located in the region of Campo das Vertentes, MG

(GUIMARÃES; GUEDES, 2003; ARAUJO; LIMA, 2005), in the municipality of Alegre, ES (REPOSSI JÚNIOR et al., 2006), and in the city of Paty de Alferes, RJ (ABIDU-FIGUEIREDO et al., 2011), with predominance of the genera *Cooperia* spp. and *Haemonchus* spp.

In the present study, the overall median counts for EPG (100) and OPG (300) were low, regardless of the calves rearing phase or the type of milk produced. It was also found that herds that produce BM presented higher (p < 0.05) EPG and OPG counts than found on RRM farm properties, talking calves in the pre-weaning and post-weaning phases together, and also considering only the animals in the post-weaning phase. Other than this, no difference was observed (p > 0.05) in EPG and OPG counts between calves in the pre-weaning and post-weaning phases.

The EPG counts were low, independent of the animal category (pre-weaning or post-weaning) or the type of milk produced (BM or RRM), thus characterizing subclinical nematodiasis, with values below the criteria established by Hoffmann (1987), which would justify anti-helminth treatment (≥300 EPG). However, these data must be interpreted cautiously, because of the limited correlation between EPG values and of the impact on production caused by gastrointestinal nematode infections (MOLENTO et al., 2011). During both the pre-weaning and the post-weaning phases, the calves showed low OPG counts, and this result concurs with other cross-sectional observational studies on *Eimeria* spp. in Brazil, which also did not observe cases of clinical eimeriosis among infected cattle, probably due to the low quantities of oocysts eliminated in the animals' feces (REBOUÇAS et al., 1994; ALMEIDA et al., 2011).

Table 1. Factors associated to counts for eggs (EPG) and oocysts (OPG) per gram of feces among dairy calves, during the pre-weaning and post-weaning phases, in the south of Minas Gerais, Brazil.

Quantity	Animal category	Factors	N***	p value	Odds ratio	CI (95%)
OPG	General*	Type of reproduction				
		Natural mounting	7	0.051	1	1.1-3.1
		Artificial insemination	13		1.89	
		Number of calves per hectare (ha)				
		≤15 calves/ha	13	0.046	1	1.1-106.4
		>15 calves/ha	6		11.0	
	Pre-weaning phase**	Number of calves per hectare (ha)				
		≤15 calves/ha	13	0.01	1	2.0-379.9
		>15 calves/ha	6		27.5	
		Calf shelter cleaning frequency				
		≤15 days	5	0.048	1	0.035-1.2
		>15 days	5		0.2	
		Amount of concentrate offered to the cow				
		Ad libitum	12	0.008	1	1.7-21.3
		Up to 1 kg	4		6.0	
EPG	General*	Daily milk production				
		≤500 l	9	0.005	1	1.26-6.01
		>500 l	11		2.75	
		Type of reproduction				
		Natural mounting	7	0.044	1	1.2-3.9
		Artificial insemination	13		2.17	
		Frequency of diarrhea among calves				
		Low	16	0.036	1	1.7-9.3
		Medium/high	3		4.0	
	Pre-weaning phase**	Daily milk production				
		≤500 l	9	0.014	1	1.2-4.2
		>500 l	11		2.2	
		Type of reproduction				
		Natural mounting	7	0.051	1	1.1-3.1
		Artificial insemination	13		1.9	

*Pre-weaning and post-weaning phases;**During the post-weaning phase, the variables tested did not present any significant association;***N < 20: response absence (could not be informed) by the interviewee for the variable in question.

The results from the analysis on risk factors are in Table 1, but only those with a significant association (p < 0.05) are shown. Farms that rear calves at higher densities and use artificial insemination (AI) present a higher risk of high OPG counts; and those that produce >500 L of milk/day and use AI also show a higher likelihood of higher EPG counts. Sánchez et al. (2008), in Argentina, evaluated dairy calves of up to two months of age, and also found a significant association (p < 0.05) between higher density of calf rearing and higher OPG count. The results from the present study show that, when considering calves during the pre-weaning and post-weaning phases together, and only the animals in the pre-weaning phase, the variables relating to higher rates of technification on dairy farms, such as use of AI, higher milk production and higher calf rearing densities, presented significant associations (p < 0.05) with higher OPG and EPG counts. Rehman et al. (2011) observed similar results, in finding that some variables relating to higher levels of technification, such as larger herds and confined calf rearing, constituted risk factors for *Eimeria* spp. infection. These results indicate that, on dairy farms in the south of Minas Gerais, the use of modern management practices, with the aim of increasing productivity, has not been accompanied by greater sanitary care for the herd. This situation has led to the existence of this association between factors indicative of higher degrees of technification on farms and higher levels of *Eimeria* spp. and gastrointestinal nematode infections among dairy calves.

Conclusion

Although low counts of oocysts (OPG) and eggs (EPG) per gram of feces were found, the predominant occurrence of the coccidian species *E. bovis* and *E. zuernii*, and of the nematode genus *Haemonchus* spp. (an intestinal parasite that is considered to have high pathogenic potential for young cattle), in association with a higher level of technification of the milk production system, indicates that there is a need to adopt control measures against these intestinal parasites, with the aim of minimizing the possible damage caused by subclinical infections among dairy calves in the pre-weaning and post-weaning stages.

Acknowledgements

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