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Eimeria leuckarti in equids from properties in the Zona da Mata of Minas Gerais

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Abstract: Brazil has a significant population of equids, which makes the equine industry an important contribution on the national Gross Domestic Product (GDP). Parasitic diseases are prevalent, particularly those affecting the gastrointestinal tract, which can cause colic, diarrhea, and, in severe cases, result in fatality. Among these diseases, the protozoan *Eimeria leuckarti*, a parasite of the small intestine in horses, mules, and zebras, predominantly affects foals. When *E. leuckarti* causes clinical disease, symptoms such as acute and chronic diarrhea, abdominal pain, colic, and urticaria may occur, although it often presents asymptomatically. The present study analyzed 247 foals from eight stud farms located in the Zona da Mata of Minas Gerais (ZMM) region, encompassing the cities of Coimbra, Guaraciaba, Ponte Nova, Ervália, São Geraldo, Ubá, Teixeiras, and Viçosa. Data were collected through questionnaires administered to owners regarding the animals' production history and characteristics, and coproparasitological examinations were conducted using the Sheather technique at the Laboratory of Parasitology and Parasitic Diseases of the Veterinary Department of the Federal University of Viçosa. The collected data were organized into Microsoft Excel spreadsheets and subjected to statistical analysis using IBM SPSS Statistics software, version 25, applying Measures of Association tests and Yates-corrected Chi-Square tests, with a significance level set at $p < 0.05$. The prevalence of *E. leuckarti* in the ZMM region was 7.3%, and silage feeding was identified as a risk factor for eimeriosis in foals ($p < 0.01$). Other factors, such as feed type, housing conditions, and fecal disposal, did not show statistically significant differences.
Keywords: Coccidiosis. Eimeriosis. Parasitic diseases.

1. Introduction

Brazil has an extensive equine herd, estimated at around 5.8 million animals, with Minas Gerais having the largest population, accounting for approximately 804,000 equids (IBGE, 2025). This sector creates around 3 million direct and indirect jobs and generates annual revenue of 30 billion reais (Lima, 2016). Historically, equids have played crucial roles in social, agricultural, sporting, leisure, and therapeutic activities (Nascimento and Nardi, 2021).

Currently, the breeding of horses for leisure, sports, and rural tourism has outpaced their traditional work roles (Tomljenovic, Boranic-Zivoder, and Corak, 2018). Horses bred for sports and leisure require more intensive care and a greater investment, mainly because of their participation in events and competitions (Lima, 2016). In this context, health management practices, sanitary care, such as deworming and vaccination, is essential to maintaining the herd's health (Guelpa, 2023).

Parasitism poses a significant challenge to equine breeding, affecting both horses kept in the field and those housed in stables, causing problems such as colic, diarrhea, and, in severe cases, death (Afonso, 2016). Although cyathostomins are the leading cause of gastrointestinal parasitosis in equines, coccidian protozoa, such as Eimeria spp., can also affect the digestive tract of these animals, especially foals (Bauer, 1988). *Eimeria leuckarti* is a cosmopolitan protozoan that parasitizes the small intestine of horses, mules, and zebras, but its detection is rare due to the high frequency of asymptomatic infections (Studzińska, Tomczuk, and Sadzikowski, 2008; Dubey and Bauer, 2018).

In the Zona da Mata of Minas Gerais (ZMM), the equine industry plays a significant role in the local economy and culture, contributing to the region's agricultural and sports activities. The health of equids directly affects their productivity and well-being. *Eimeria leuckarti* is an important etiological agent of coccidiosis in foals, potentially causing diarrhea and resulting in production losses. Understanding the prevalence of this parasite and the associated risk factors is essential for developing effective control and prevention strategies, thereby improving sanitary management practices, reducing economic losses, and enhancing animal welfare. This study aimed to determine the prevalence of *E. leuckarti* in equids in the ZMM and to identify the risk factors associated with infection. Fecal samples were collected from foals on various properties in the region and subjected to coproparasitological analysis to diagnose coccidiosis. Additionally, a questionnaire was administered to gather information on management practices and environmental conditions, and the data obtained were statistically analyzed to determine the prevalence and associated risk factors.

2. Materials and Methods

2.1. Ethics

This study was conducted with the approval of the Animal Use Ethics Committee (CEUA) of the Federal University of Viçosa (UFV), under protocol number 50554278388. The procedures were performed following the Code of Conduct for the Use of Animals in Teaching, Research, and Extension of the Department of Veterinary Medicine (DVT). A questionnaire was also administered to the owners or those responsible for the animals' maintenance to collect data on the association between risk factors and infection with the pathogen. The Human Research Ethics Committee (CEP) of the Federal University of Viçosa approved the questionnaire under protocol number 59014622.7.0000.5153.

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2.2. Animals and Sample Collection

Sample collection took place at stud farms located in the ZMM, in the micro-regions of Ubá, Viçosa, and Ponte Nova, specifically in the municipalities of Coimbra (Farm 1) (20° 50' 58" S, 42° 47' 28" W), Guaraciaba (Farm 2) (20° 33' 28" S, 43° 0' 22" W), Ponte Nova (Farm 3) (20° 24′ 57″ S, 42° 54′ 32″ W), Ervália (Farm 4) (20° 50' 25" S, 42° 39' 8" W), São Geraldo (Farm 5) (6° 40' 60" S, 44° 33' 0" W), Ubá (Farm 6) (21° 07′ 12″ S, 42° 56′ 34″ W), Teixeiras (Farm 7) (20° 39' 8" S, 42° 51' 31" W), and Viçosa (Farm 8) (20° 44′ 41″S, 42° 50′ 31″ W). Seven of the properties specialized in breeding Mangalarga Marchador horses, and one in donkeys and mules. The coproparasitological analyses were conducted at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary, Federal University of Viçosa (DVT/UFV).

Fecal samples were collected directly from the rectal ampulla of 247 animals (5 mules, 9 donkeys, and 233 equids) up to 12 months of age, from eight equid-breeding farms. The distribution of animals per property was based on their availability up to the first year of age (Table 1). To determine the sample size, the average prevalence reported by Dubey and Bauer (2018) across 77 global studies was calculated, yielding an estimate of 13.04%. The OpenEpi (https://www.openepi.com/Menu/OE_Menu.htm) was used to calculate the required sample size based on the average frequency, with a 99% confidence interval, resulting in an approximate sample size of 241 animals for the study.

Fecal samples were collected by restraining of the foals using halters or stocks (when available on the property). Using latex or silicone EVA gloves lubricated with mucilage, fecal samples were collected directly from the rectal ampulla, labeled, and stored under refrigeration. The owners or those responsible for the establishments answered a questionnaire, reporting the animal's history, housing conditions, feeding, cleaning, and health care.

Stud Farms	City	Nº of Samples
1	Coimbra	4
2	Guaraciaba	14
3	Ponte Nova	49
4	Ervália	10
5	São Geraldo	54
6	Ubá	92
7	Teixeiras	12
8	Viçosa	12

Table 1 – Distribution of the number of animal samples collected at each visited stud farm.

2.3. Coproparasitological Diagnostic

The coproparasitological diagnoses was performed at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary Medicine at the Federal University of Viçosa, using Sheather's flotation technique (Sheather, 1923) for oocyst visualization. This technique consists of centrifugal flotation procedure. In this method, the diluent solution used is a saturated sucrose solution with a density of 1.27 mg/mL (Sheather, 1923). Five grams of feces were weighed using a mini digital LCD scale, diluted in distilled water, and filtered through a 4-mesh gauze sieve. Subsequently, 10 mL of the mixture was transferred to a Falcon tube, and 10 mL of Sheather's solution, prepared in the laboratory (500g of sugar in 320ml of distilled water with 6g of phenol for preservation), was added. Centrifugation was performed at 2000 g for 5 minutes. After centrifugation, Sheather's solution was added dropwise until a meniscus was formed, where a glass slide was placed to capture the floating oocysts. The oocysts were identified using an optical light microscope (CX3) at 40x magnification.

2.4. Calculation of *E. leuckarti* prevalence

To calculate the prevalence of eimeriosis in the equids of the present study, the positive cases from each farm were divided by the sample size of the same and multiplied by 100, obtaining the percentage for each property, according to the following formula:

$$\text{Prevalence} = \frac{\text{number of positive cases on the farm}}{\text{number of animals on the farm}} \; x \; 100$$

To calculate the total prevalence data,the total number of positive cases was divided by the total number of animals collected and multiplied by 100, according to the formula:

$$\text{Total prevalence} = \frac{\text{total number of positive cases}}{\text{total number of animals in the experiment}} \; x \; 100$$

2.5. Statistics

The data were stored in Microsoft Excel 2016 and analyzed using IBM SPSS Statistics version 25. Descriptive analyses were performed, with relative and absolute frequencies presented as percentages. Measures of Association and Yates-corrected Chi-Square tests were used to assess the association between the positivity rate for *E. leuckarti* infections and potential risk factors for

infection, such as age, diarrhea, diet, fecal disposal, and housing. The strength (degree) of the association between infection prevalence and associated factors was assessed using the Risk Ratio (RR) calculated through on the OpenEpi website, in order to better understand the relationship between infection exposure and the risk factors to which the animals were exposed. A significance level of 0.05 was adopted.

3. Results

Coprological examinations identified, positive samples for *E. leuckarti* were identified (Figure 1). Based on the number of positive tests, the overall prevalence of infection with this parasite on properties within the ZMM was determined to be 7.3%. Additionally, prevalence was calculated for each property: Stud Farm 1 (Coimbra) showed a prevalence of 7.1%, Stud Farm 3 (Ponte Nova) had 14.3%, and Stud Farm 6 (Ubá) had 10.9%. No confirmed cases of *E. leuckarti* were found on the other stud farms (Table 2).

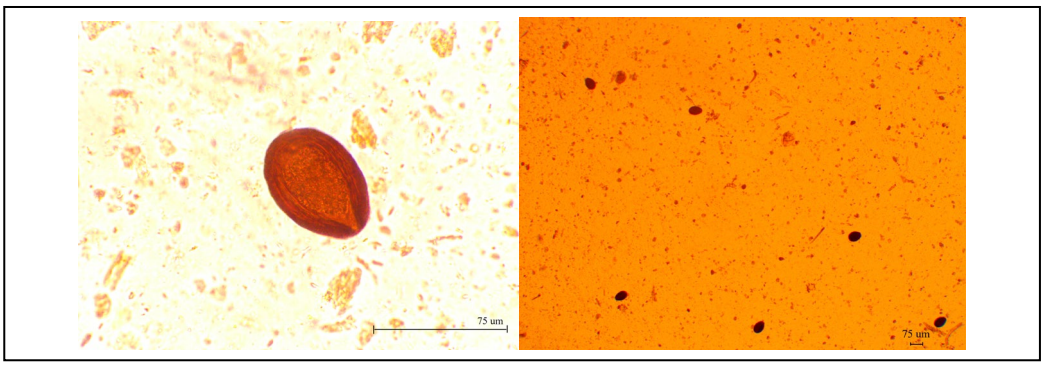

Figure 1 – *Eimeria leuckarti* diagnosed using the Sheather's method. (A) Image magnified 100x, showing the thick wall of the coccidian; (B) Image magnified 10x, displaying six oocysts of *E. leuckarti*.

An analysis was also conducted to assess the association between property risk factors and the occurrence of *E. leuckarti* infection in foals, using data from questionnaires administered to owners of the visited stud farms. Considering the fecal-oral nature of transmission, dietary characteristics, management practices, and the presence of diarrheal cases were analyzed for potential correlations (Table 3).

Stud Farms	N° of animals	Positive (%)
1	4	0 (0%)
2	14	1 (7,1%)
3	49	7 (14,3%)
4	10	0 (0%)
5	54	0 (0%)
6	92	10 (10,9%)
7	12	0 (0%)
8	12	0 (0%)
Total	247	18 (7,3%)

Table 2 – Prevalence of *E. leuckarti* in stud farms in the Zona da Mata Of Minas Gerais region.

Risk Factor	RR (IC 95%)	P-Value	EFP	EFE
Age	2,333 (0,9 - 6,0)	0,0585	38,1%	57,14%
Feeding				
Silage	12,78 (1,7 - 94,0)	0,0010**	87,05%	92,18%
Sugarcane	2,09 (0,8 - 5,2)	1,884	31,88%	52,16%
Ration	5,073 (3,1 – 8,7)	0,248	75,09%	80,29%
Grass	0,3049 (0,2 – 0,3)	0,241	52,13%	69,51%
Diarrheia	1,859 (0,7 - 4,5)	1,149	17,96%	46,2%
Destination of feces				
Pasture	0,02926 (0,009 - 0,071)	0,574	34,95%	97,07%
Hay	0,1438 (0,094 - 0,212)	0,477	38,53%	85,62%
Weeder	2,767 (2,1-3,5)	0,751	26,11%	63,86%
Composting / Other	5,88 (4,5 - 7,5)	0,751	49,39%	82,99%
Animal acesses				
Stall	41,62 (29,62 - 59,76)	0,061	82,98%	97,6%
Piquet	0,5819 (0,33 - 0,95)	0,983	4,181%	41,81%

Table 3 – Factors associated with eimeriosis in foals from properties in the Zona da Mata of Minas Gerais region. (RR - Risk Ratio; CI - Confidence Interval; EFP - Etiological Fraction in the Population; EFE - Etiological Fraction in the Exposed; P-value** p ≤0.01)

4. Discussion

The prevalence of *Eimeria leuckarti* on farms in the Zona da Mata of Minas Gerais was lower than expectations, with a rate of 7.3%, consistent with the findings of Parsani et al. (2013) in India and Canestrini-Trotti and Restani (1972) in Italy (Dubey and Bauer, 2018), which reported prevalence rates of approximately 6%. These results also align with the findings of Gundłach et al. (2004) in Poland. Despite the prevalence being lower than the estimated 13.04%, most properties did not show *E. leuckarti*, infection and where it was detected, risk factors associated with the infection were identified.

The analysis of foals indicated that, although they are more susceptible to *E. leuckarti* (Dubey and Bauer, 2018), there was no significant correlation between age and infection. Of the 247 foals, 10.5% of the younger ones and 4.5% of the older ones tested positive. This finding may be related to the observed prevalence and the study methodology, which distinguished between the nursing phase (0 to 6 months) and the weaned phase (7 to 12 months).

Dietary factors influenced the prevalence: the highest infection rates were observed in Stud Farm 3 (Ponte Nova) and 6 (Ubá), indicating a strong correlation between silage consumption and positivity ($p \leq 0.01$). Silage may affect gastrointestinal microbiota and favor the proliferation of *E. leuckarti* (Zhu et al., 2021). Out of 247 animals, 141 were fed silage, and 17 of the 18 positives were from Stud Farms 3 and 6. The intestinal microbiota plays a crucial role in immune modulation, preventing the colonization of pathogen, influencing mucosal immunity through mucus production, and activating immune cells. These processes, however, can be disrupted by abrupt dietary changes, which alter the balance of commensal microorganisms (Boucher et al., 2024).
Sugarcane, used in Stud Farms 2 (Guaraciaba) and 6 (Ubá), did not show an association with *E. leuckarti* infection. Despite being an alternative during dry periods, its high fiber content and sucrose concentration did not correlate with positivity. Neither chopped grass nor hay showed any relationship with infection.

No correlation was found between *Eimeria* infection and diarrhea, corroborating the absence of clinical signs in many cases (Gorji, Sadr, and Borji, 2023). Of the 247 animals evaluated, only 7 of the 63 with diarrhea tested positive.

Regarding fecal disposal of feces, although none of the correlations had statistically significant, pasture (RR=0.029) and hay (RR=0.14) showed risk ratios (RR) less than 1, suggesting a possible inverse association with eimeriosis. This may indicate that fecal management and environmental controls are important factors. The analysis of fecal disposal practices locations revealed that about one-third of eimeriosis cases could be attributed to pasture use, highlighting the importance of proper management.

Finally, no significant correlation was found between infection and housing conditions, such as stalls (p=0.061) and paddocks (p=0.983), since as several establishments used both types of facilities, allowing animals to come into contact with pasture.

5. Conclusion

The parasite *Eimeria leuckarti* was found in equids from the Zona da Mata region of Minas Gerais. This study found that foals fed with silage are more susceptible to *E. leuckarti* infection, possibly due to intestinal dysbiosis caused by the feed's fermentative

characteristics and resulting alterations in the microbiota. However, further studies are needed to characterize the role of silage in the equine intestinal microbiota and to understand how microbiota modulation influences the establishment of this coccidian.

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Briefing notes: CEUA Protocol: 50554278388; CEP protocol: 59014622.7.0000.5153.

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