

**UNIVERSIDADE FEDERAL DE VIÇOSA**

**Species differentiation of *Parascaris* spp. by egg karyotyping: a study in equines from the zona da mata region, Minas Gerais, Brazil**

Giulia Ornellas Fuzaro Scalea  
*Magister Scientiae*

**VIÇOSA - MINAS GERAIS  
2025**

**GIULIA ORNELLAS FUZARO SCALEA**

**Species differentiation of *Parascaris* spp. by egg karyotyping: a study in equines from the zona da mata region, Minas Gerais, Brazil**

Dissertation submitted to the Veterinary Medicine Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Artur Kanadani Campos

Co-advisers: Rodrigo Alves Barros  
Mariana G. de Freitas

**VIÇOSA - MINAS GERAIS  
2025**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade  
Federal de Viçosa - Campus Viçosa**

T

S281s  
2025 Scaléa, Giulia Ornellas Fuzaro, 1997-  
Species differentiation of *Parascaris spp.* by egg  
karyotyping: a study in equines from the Zona da Mata region,  
Minas Gerais, Brazil / Giulia Ornellas Fuzaro Scaléa. – Viçosa,  
MG, 2025.

1 dissertação eletrônica (72 f.): il. (algumas color.).

Texto em inglês.

Orientador: Artur Kanadani Campos.

Dissertação (mestrado) - Universidade Federal de Viçosa,  
Departamento de Medicina Veterinária, 2025.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2025.514>

Modo de acesso: World Wide Web.

1. Cavalos - Doenças. 2. *Parascaris equorum*. 3. *Parascaris  
univalens*. 4. Cariótipo. I. Campos, Artur Kanadani, 1975-.  
II. Universidade Federal de Viçosa. Departamento de Medicina  
Veterinária. Programa de Pós-Graduação em Medicina  
Veterinária. III. Título.

CDD 22. ed. 636.10896

**GIULIA ORNELLAS FUZARO SCALEA**

**Species differentiation of *Parascaris* spp. by egg karyotyping: a study in equines from the zona da mata region, Minas Gerais, Brazil**

Dissertation submitted to the Veterinary Medicine Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

APPROVED: May 28, 2025.

Assent:

---

Giulia Ornellas Fuzaro Scalea  
Author

---

Artur Kanadani Campos  
Adviser

Essa dissertação foi assinada digitalmente pela autora em 13/08/2025 às 09:31:42 e pelo orientador em 13/08/2025 às 12:11:16. As assinaturas têm validade legal, conforme o disposto na Medida Provisória 2.200-2/2001 e na Resolução nº 37/2012 do CONARQ. Para conferir a autenticidade, acesse <https://siadoc.ufv.br/validar-documento>. No campo 'Código de registro', informe o código **IKQO.VMKJ.SAN5** e clique no botão 'Validar documento'.

To God, my greatest strength. To my Family, my foundation.

## ACKNOWLEDGMENTS

To God.

To my mother, Erleth, and my father, Luiz Augusto, for their unconditional love and encouragement at every step of my life. To my sisters, Dezire and Tamires, for being my inspirations and my certainty that they will always be by my side. To Luiz Octávio, my life partner, for supporting me, listening to me, and always finding solutions in moments of frustration. To my brothers-in-law, Paulo and Neto, who are like brothers. To my nephews and godchildren, Luiz Otávio, Vicente Luiz, and Augusto, who, even as children, brighten my days and bring hope for the future.

To my advisor, Artur Kanadani, for his patience, availability, and shared knowledge. To my co-advisors, Mariana Green and Rodrigo Barros, for their daily advice and contributions to the project. To Késsia Leite, for her dedication and willingness to embrace and support this project wholeheartedly. To my master's companion, Gabrielle Soares, for sharing the daily lab routine and academic insights. To Calzinho, the parasitology lab technician, who is always willing to help. To all the staff, master's students, residents, and colleagues who assisted in the lab and field experiments.

To Anaïs Benitez, Joice Ribeiro, and Luiza Pinheiro, who, without a doubt, have been the best friends I could have had during these four years in Viçosa, MG. To Mrs. Miltes Macedo and her entire family, and to Mrs. Vânia, who became my family at heart during this time away. To my friends from Campo Grande, MS, who, despite the distance, always remain present.

To the Federal University of Viçosa, for the excellence of its graduate programs, both in residency and in the master's program, which I had the privilege to participate in.

This work has been sponsored by the following Brazilian research agencies: Coordination for the Improvement of Higher Education Personnel (CAPES; Financing code 001), Minas Gerais State Foundation for Research Aid (FAPEMIG) and National Council of Scientific and Technological Development (CNPq).

“It is not the strongest who survives, nor the smartest, but the one who best adapts to  
change.”  
(Charles Darwin)

## ABSTRACT

SCALEA, Giulia Ornellas Fuzaro, M.Sc., Universidade Federal de Viçosa, May, 2025. **Species differentiation of *Parascaris* spp. by egg karyotyping: a study in equines from the zona da mata region, Minas Gerais, Brazil.** Adviser: Artur Kanadani Campos. Co-advisers: Rodrigo Alves Barros and Mariana Green de Freitas.

*Parascaris* spp. is a significant ascarid in foals, associated with respiratory signs such as coughing and nasal discharge, and gastrointestinal disturbances including verminous obstructions, anorexia, diarrhea, and potentially death. Given its pathogenic potential, effective parasite control in foals is essential. However, increasing reports of anthelmintic resistance to various drug classes have emerged both in Brazil and globally. Although *Parascaris equorum* was historically considered the predominant species, recent studies have demonstrated that *Parascaris univalens* is now more prevalent in several regions. Species differentiation can be achieved through karyotyping of eggs recovered from fecal samples, as *P. equorum* possesses two pairs of chromosomes, while *P. univalens* has a single pair. The present study aimed to (i) review the average prevalence of *Parascaris* spp. reported in studies conducted in Brazil, (ii) determine the prevalence of *Parascaris* spp. in the Zona da Mata region of Minas Gerais, Brazil, and (iii) establish a modified karyotyping protocol for genetic characterization of the parasite at the species level. Fecal samples were collected from horses up to two years of age in the Zona da Mata region, and egg counts per gram of feces (EPG) were performed using the Mini-FLOTAC technique. Egg karyotyping involved recovery, decortication, fixation of egg suspensions preferably during the first mitotic division, staining with Giemsa and DAPI, and chromosome visualization using an Olympus BX53 Color Photomicroscope. The systematic review identified an average prevalence of 11.8% for *Parascaris* spp. in Brazil. In the present field study, a prevalence of 11.3% was observed in the Zona da Mata region of Minas Gerais. Additionally, karyotypic analysis confirmed the presence of *Parascaris univalens* in the examined population.

Keywords: anti-helminthic; cytogenetics; chromosomes; OPG; *P. univalens*

## RESUMO

SCALEA, Giulia Ornellas Fuzaro, M.Sc., Universidade Federal de Viçosa, maio de 2025. **Diferenciação de espécie de *Parascaris* spp. por cariotipagem de ovos: um estudo em equinos da Zona da Mata - Minas Gerais, Brasil.** Orientador: Artur Kanadani Campos. Coorientadores: Rodrigo Alves Barros e Mariana Green de Freitas.

*Parascaris* spp. é um ascarídeo de grande importância em potros, associado a sinais respiratórios, como tosse e secreção nasal, além de alterações gastrointestinais, incluindo obstruções verminosas, anorexia, diarreia e, potencialmente, morte. Considerando seu potencial patogênico, o controle efetivo desses parasitas em potros é essencial. No entanto, têm sido relatados, no Brasil e em diversos países, casos crescentes de resistência anti-helmíntica a diferentes classes de fármacos. Embora *Parascaris equorum* tenha sido historicamente considerado a espécie predominante, estudos recentes demonstraram que *Parascaris univalens* é atualmente mais prevalente em várias regiões. A diferenciação entre as espécies pode ser realizada por meio da cariotipagem de ovos recuperados de amostras fecais, visto que *P. equorum* possui dois pares de cromossomos, enquanto *P. univalens* apresenta apenas um par. O presente estudo teve como objetivos: (i) revisar a prevalência média de *Parascaris* spp. relatada em estudos realizados no Brasil, (ii) determinar a prevalência de *Parascaris* spp. na região da Zona da Mata de Minas Gerais, Brasil, e (iii) estabelecer um protocolo modificado de cariotipagem para a caracterização genética do parasita em nível de espécie. Amostras fecais foram coletadas de equinos com até dois anos de idade na região da Zona da Mata, e os exames de ovos por grama de fezes (OPG) foram realizados utilizando a técnica Mini-FLOTAC. A cariotipagem dos ovos envolveu a recuperação, descorticação, fixação da suspensão de ovos preferencialmente na primeira divisão mitótica, coloração com Giemsa e DAPI, e visualização cromossômica em microscópio Olympus BX53 Color Photomicroscope. A revisão sistemática identificou uma prevalência média de 11,8% para *Parascaris* spp. no Brasil. No estudo de campo realizado, foi observada uma prevalência de 11,3% na região da Zona da Mata de Minas Gerais. Adicionalmente, a análise cariotípica confirmou a presença de *Parascaris univalens* na população examinada.

Palavras-chave: anti-helmíntico; citogenética; cromossomos; OPG; *P. univalens*

## LIST OF ILUSTRATIONS

Figure 1: Schematic representation of the life cycle of <i>Parascaris</i> spp. Source: Author's figure. ....	14
Figure 2: Flowchart of the article selection process in the systematic review .....	30
Figure 3: Map of Brazil indicating states with selected articles for systematic review on <i>Parascaris</i> spp. prevalence .....	34
Figure 4: Mini-FLOTAC technique in feces samples of animals from experiments. a. 5g of feces; b. 45 mL of saturated solution; c. Homogenizing; d. Sifting the solution; e. Fill the discs; f. Wait for 10 minutes; g. Spin the disc; h. Reading under the microscope. ....	46
Figure 5: Distribution of <i>Parascaris</i> -positive animals (based on Mini-FLOTAC results) according to age (months), from samples collected from foals in stud farms located in the Zona da Mata region of Minas Gerais. ....	49
Figure 6: Processes for recovering <i>Parascaris</i> spp. eggs. a. Recovered eggs post-wash; b. Eggs after the decortication protocol; c. Eggs of <i>Parascaris</i> spp. after 24 hours of incubation at 37 °C in Petri dishes.....	65
Figure 7: Giemsa stained metaphase of <i>Parascaris univalens</i> egg at first mitotic division showing a single pair of chromosome at anaphase in a 100x magnification	67
Figure 8: Fluorophore DAPI (4',6'-diamino-2-phenyl-indol) stained metaphase of <i>Parascaris univalens</i> egg at first mitotic division showing a single pair of chromosome at anaphase in a 100x magnification .....	68

## LIST OF TABLES

Table 1: Overview of Study Details on <i>Parascaris</i> spp. in Horses in Brazil .....	31
Table 2: Horses farms' location and faecal samples collected in each property in Zona da Mata of Minas Gerais region .....	44
Table 3: Frequency of positive and negative animals identified in the prevalence study of <i>Parascaris</i> spp. in the Zona da Mata of Minas Gerais region .....	47
Table 4: Count of positive and negative animals for <i>Parascaris</i> spp. based on their stud farm of origin, as diagnosed using the Mini-FLOTAC technique.....	47
Table 5: Age Group Distribution of Animals Positive for <i>Parascaris</i> spp. in Properties from the Zona da Mata of Minas Gerais region .....	48
Table 6: Evaluation of the association between management factors and disease risk in horses, including estimates of relative risk, statistical significance, and etiologic fractions.....	60
Table 7: Mean egg count of <i>Parascaris</i> spp. in 10 µl of the solution after the karyotyping protocol in animals from different stud farms in the Zona da Mata of Minas Gerais region .....	65

## SUMMARY

1. INTRODUCTION.....	11
2. LITERATURE REVIEW.....	12
<b>2.1 Life cycle</b> .....	12
<b>2.2 Pathogenicity</b> .....	14
<b>2.3 Diagnosis</b> .....	14
<b>2.4 <i>Parascaris equorum</i> and <i>Parascaris univalens</i></b> .....	15
<b>2.5 Karyotyping</b> .....	16
<b>2.6 Faecal Egg Count (FEC)</b> .....	17
<b>2.7 Prevalence in Brazil</b> .....	18
<b>2.8 Research rationale</b> .....	20
3. REFERENCES.....	20
CHAPTER 1 .....	26
<b>Prevalence of <i>Parascaris</i> spp. in horses in Brazil - a systematic review</b> .....	27
CHAPTER 2.....	40
Prevalence of <i>Parascaris</i> spp. in Horses from the Zona da Mata of Minas Gerais region, Minas Gerais, Brazil.....	41
CHAPTER 3 .....	58
Cytogenetic Analyses in the Species Identification of <i>Parascaris</i> spp.: Detection of <i>Parascaris univalens</i> in Zona da Mata, Minas Gerais, Brazil.....	59
<b>9. Acknowledgements</b> .....	70
<b>10. Author contribution</b> .....	70
<b>ATTACHMENT I</b> .....	72

## 1. INTRODUCTION

Brazil has one of the largest horse populations in the world, with equines playing a crucial role in various sectors, including agriculture, sports, leisure, and cultural traditions (Adelman, 2020). The production and maintenance of horses are significant not only for the economy but also for rural livelihoods and the preservation of historical practices (MAPA, 2016). However, parasitic diseases pose a major challenge to equine health and productivity. These infections, caused by a variety of parasites, can lead to poor performance, weight loss, anemia, and even death if left untreated.

Effective management and control of parasitic diseases are therefore essential to ensure the welfare of the equine population and sustain their vital contributions to Brazil's social and economic landscape. Parasitic diseases are common conditions in production animals and represent an important cause of morbidity and mortality in equines (Morales; Bello; Gómez, 2011). Despite their prevalence, they are often neglected, which exacerbates their dissemination and may lead to colic, diarrhea, or production losses (Nielsen, 2012). Infections caused by the ascarid *Parascaris* spp. are particularly common in foals (Reinemeyer, 2009), although adult horses can serve as potential reservoirs (Cirak; Hermosilla; Bauer, 1996). Clinical signs include weight loss, lethargy, nasal discharge, or no symptoms at all (Nielsen, 2016).

However, the migratory pattern of the parasite includes passage through the lungs, potentially causing alterations in this organ (Nicholls *et al.*, 1978). The most severe issue associated with this parasitosis is intestinal obstruction or rupture of the small intestine, which often requires surgical intervention and worsens the patient's prognosis (Tatz *et al.*, 2012). The control of the parasite is crucial and is typically achieved through the use of anthelmintic drugs. However, resistance of *Parascaris* spp. to various medications has been reported worldwide (Armstrong *et al.*, 2014; Lyons *et al.*, 2011; Nielsen, 2022). The commonly used method for diagnosis and control involves determining the Fecal Egg Count (FEC), particularly when performing

the Fecal Egg Count Reduction Test (FECRT) to detect anthelmintic resistance (Kaplan *et al.*, 2023).

Although it is a highly relevant parasite in equine management, *Parascaris equorum* is commonly considered the species responsible for parasitic infections. However, some studies suggest that *Parascaris univalens* may be more prevalent (Nielsen *et al.*, 2014). Differentiating between these species is challenging, as there are no morphological differences in eggs and larvae. Therefore, karyotyping is required to analyze the chromosomes, given that *P. univalens* has a single chromosome pair ( $2n=2$ ), whereas *P. equorum* possesses two pairs ( $2n=4$ ) (Samson-Himmelstjerna *et al.*, 2021).

It's essential to accurately distinguish between *Parascaris equorum* and *Parascaris univalens*, especially when considering anthelmintic resistance. While these species are morphologically indistinguishable in their egg and larval stages, they differ in their chromosomal makeup and may exhibit distinct biological and pharmacological behaviors (Nielsen *et al.*, 2014; Samson-Himmelstjerna *et al.*, 2021). Misidentification can lead to an underestimation of species-specific resistance patterns, compromising parasite control strategies. Studies have shown that *P. univalens* may be the predominant species in certain regions<sup>12</sup> previously thought to be dominated by *P. equorum* (Nielsen *et al.*, 2014). This raises concerns about treatment effectiveness and the interpretation of resistance data.

The aim of this study was to evaluate the prevalence of *Parascaris* spp. in equines from the Zona da Mata region of Minas Gerais, Brazil. Additionally, this study sought to perform, for the first time in Brazil, the karyotyping of parasite eggs to identify the species responsible for this parasitosis in the region.

## **2. LITERATURE REVIEW**

### **2.1 Life cycle**

The life cycle of *Parascaris* spp. begins when horses ingest infective eggs from the environment. These eggs, covered by a sticky protein coating, can adhere to

various surfaces, such as the horse's coat or the udder of mares, facilitating transmission, especially among foals and weanlings. After ingestion, the eggs pass through the stomach and small intestine, where sequential acidic and basic conditions remove their protective coating (Bowman, 2009). The larvae then hatch and penetrate the gut lining, entering the lymphatic or venous system. They are transported to the liver within 2 to 7 days post-infection, where they migrate through the hepatic tissue, causing inflammatory lesions and fibrotic tracts. From the liver, the larvae migrate to the lungs, where they exit the pulmonary blood vessels and enter the airways by rupturing alveolar membranes approximately two weeks post-infection. They remain in the lungs for about two weeks before migrating up the pulmonary tree or being coughed into the pharynx and swallowed (Nielsen; Reinemeyer, 2018).

Once back in the small intestine, the larvae mature into adult worms, with females beginning to shed eggs in the feces 90 to 110 days post-infection. The adults grow progressively and can persist in the gut for several months. Over time, most horses develop strong immunity against *Parascaris* spp., leading to a natural cessation of egg shedding, even without anthelmintic treatment (Taylor; Coop; Wall, 2017). This immunity is why *Parascaris* infections are typically observed in young horses, such as sucklings, weanlings, and yearlings, while being rare in horses older than 18 months. Interestingly, recent studies have identified a biphasic pattern of infection in untreated foals, with peak burdens occurring at 4–5 months and a smaller, short-lived second wave at 8–10 months. While infections in mature horses are uncommon, some adult individuals have been observed to shed eggs repeatedly after anthelmintic treatments, raising questions about immune deficiencies or possible variations in parasite response (Nielsen; Reinemeyer, 2018).

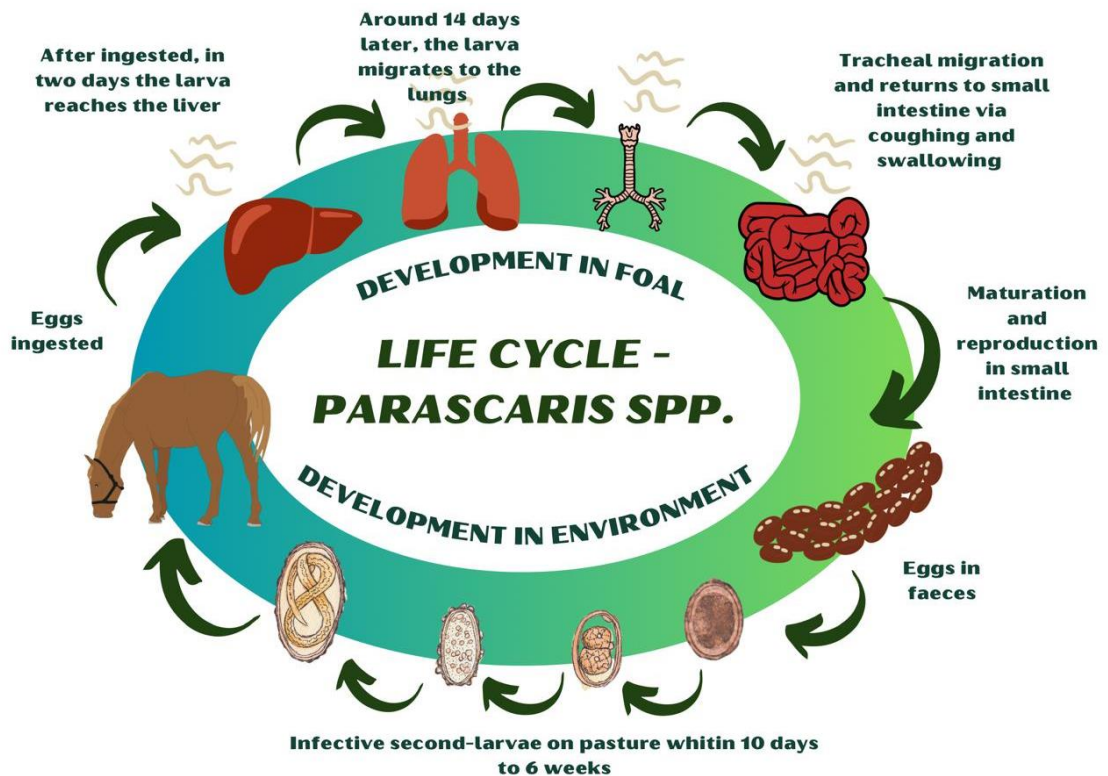


Figure 1: Schematic representation of the life cycle of *Parascaris spp.* Source: Author's figure.

## 2.2 Pathogenicity

The pathogenesis of this parasitosis in foals is primarily associated with lower body condition score, hypoproteinemia, and increased total body water, along with decreased total body solids (Clayton; Duncan, 1979). The simplest explanation for these consequences is due to the large number of adult *Parascaris spp.* in the small intestine, competing with the host for digested nutrients (Nielsen; Reinemeyer; Sellon, 2014). Another issue that can occur is small intestine impaction or rupture, and in some situations, surgical interventions are necessary (Tatz *et al.*, 2012).

## 2.3 Diagnosis

In summary, the diagnosis of *Parascaris spp.* is based on the presence of eggs in feces. These eggs are brown, approximately 100  $\mu\text{m}$  in length, and have a thick shell. They can be detected through flotation or sedimentation tests (ESCCAP, 2019), such as the Fecal Egg Count (FEC) test. A positive test result should always be

considered an indication for anthelmintic treatment (ESCCAP, 2019), given the pathogenicity of this helminth. Additionally, it is not possible to reliably correlate the intensity of intestinal worm burdens solely with the level of egg shedding in feces (Nielsen, 2014). Ultrasonographic findings for diagnosis during the pulmonary stage have been described (Clayton; Duncan, 1977). Animals dewormed less than two days ago may exhibit spontaneous elimination of this parasite in the feces (Nielsen; Reinemeyer; Sellon, 2014).

Studies indicate that coprophagy can lead to false-positive results in fecal egg count examinations (Boes et al., 1997). This phenomenon is particularly relevant in foals, as the literature confirms that this behavior is common during the first weeks of life (Crowell-Davis & Houpt, 1985; Pyles et al., 2020). Therefore, this factor should be taken into account when interpreting egg counts in the feces of young animals.

#### **2.4 *Parascaris equorum* and *Parascaris univalens***

At the end of the 1800s, two variants of *Parascaris* spp. were identified through karyotype visualization. In the field of cytogenetics, Van Beneden (1883) described the karyotyping of *Ascaris megalcephala univalens*, while Jean Baptiste Carnoy (1886) described that of *Ascaris megalcephala bivalens*. These were later recognized as distinct species by another researcher (Hertwig, 1890). Only in 1978 were these species officially named *Parascaris univalens*, which has a single pair of chromosomes, and *Parascaris equorum*, which has two pairs of chromosomes (Bullini et al., 1978). However, most researchers believed them to represent a single species and treated all infections as *P. equorum* (Lin, 1953).

As a result, it is common to refer to the equine ascarid as *P. equorum*. Both species of this ascarid exhibit no differences in their life cycles, the clinical signs they cause in the host, or explicit morphological distinctions during the various stages of their life cycle, making species-level identification challenging. Such identification is only possible through karyotype visualization (Goday; Pimpinelli, 1984), electrophoretic analyses (Bullini et al., 1978), or minor morphological differences in the termination of the spicule (Biocca et al., 1978). These methods for differentiating

*Parascaris* spp. are complex and impractical for field routine diagnostics. Consequently, few studies provide a definitive identification of the species present in practice (Samson-Himmelstjerna et al., 2021).

It is of great importance to differentiate between *Parascaris univalens* and *Parascaris equorum*. Misidentification can lead to an underestimation of species-specific resistance patterns, consequently compromising parasite control strategies. Studies have even shown that *P. univalens* may be the predominant species in certain regions previously thought to be dominated by *P. equorum* (Nielsen et al., 2014). This highlights the critical need for accurate identification to ensure effective treatment and management of these parasites.

## **2.5 Karyotyping**

Karyotyping is one of the methods used to differentiate existing species of *Parascaris* spp. (Bullini et al., 1978). It requires visualization of fixed and subsequently stained germ cells during the metaphase of the first mitotic phase of cell division. However, this approach necessitates collecting parasites with germ cells at the appropriate stage (Goday; Pimpinelli, 1984). Karyotyping can also be performed on eggs present in feces during their first mitotic division (Nielsen et al., 2014).

When conducted with adult helminths, worms are collected from the host animal, carefully dissected to locate the gonads, and the gonads are subsequently excised. The gonads are then processed for karyotyping (Goday; Pimpinelli, 1986). The modified karyotyping technique (Nielsen *et al.*, 2014) was adapted for use with eggs shed in feces. In summary, feces are collected from foals known to be shedding ascarid eggs, washed, sieved, resuspended, and subjected to multiple washes to isolate the eggs more effectively. Subsequently, the eggs are decorticated, further washed, and examined under a microscope to evaluate their developmental stage (Nielsen *et al.*, 2014).

The developmental stage of the eggs is observed microscopically, with a preference for identifying those in the first or second mitotic phase of embryonic divisions. Based on the number of chromosomes present, the species of *Parascaris* spp. can then be identified.

The first study to perform karyotyping on *Parascaris* spp. eggs present in feces was described by Nielsen et al. (2014), who adapted the technique to identify the chromosome number during the first mitotic division, allowing distinction between *P. univalens* and *P. equorum*. Later, Zhu et al. (2023), in China, applied the same methodology and confirmed its effectiveness for species differentiation, reinforcing the reliability of the technique. Overall, the adapted karyotyping method is considered useful for accurate species identification, although it requires precise timing to collect eggs at the appropriate mitotic stage and access to specialized laboratory equipment.

## **2.6 Faecal Egg Count (FEC)**

The Faecal Egg Count (FEC) is a laboratory diagnostic method used in livestock to assess parasitic load and the efficacy of anthelmintics. Treating animals with medications without prior diagnosis may contribute to the development of resistance to the active compounds used (Flanagan; Morton; Sandeman, 2013). Therefore, the American Association of Equine Practitioners (AAEP) recommends routine quantitative fecal examinations in herds (Nielsen et al., 2019).

The most widely used technique is the McMaster method, developed in Australia by Gordon and Whitlock (1939). This method uses a dual reading chamber, known as the McMaster chamber, allowing a total evaluation volume of 0.3 mL of solution. While practical, it has low sensitivity, particularly for animals with low fecal egg counts (Mes, 2003). The detection limit of this method is 50 eggs per gram of feces (FEC), meaning that low parasitic burdens often go undetected (Kaplan; Vidyashankar,

2012). It is important to note that the analytical sensitivity of fecal egg count tests may vary when sample-to-flotation-solution ratios are modified (Vadlejch *et al.*, 2011).

To address these limitations, a new technique called FLOTAC was developed and patented by Cringoli (2006). This method uses a device with a reading chamber capable of evaluating a 10 mL sample volume, significantly larger than that of McMaster. Additionally, it relies on centrifugation-based flotation, making it more sensitive than the McMaster method (Cringoli, 2006). However, FLOTAC is less practical for fieldwork due to the requirement for well-equipped laboratories, sample centrifugation, and the longer preparation time for the analysis (Cringoli *et al.*, 2017; Knopp *et al.*, 2009).

As an alternative, the Mini-FLOTAC chamber was developed. It consists of a dual reading disc that allows a total evaluation volume of 2 mL and has a detection sensitivity of 5 eggs per gram of feces (1:5) (Cringoli *et al.*, 2013, 2017).

Recent coproparasitological diagnostic techniques are based on egg flotation. These techniques include the McMaster method (Gordon; Whitlock, 1939) and the Mini-FLOTAC method (Barda *et al.*, 2013; Cringoli *et al.*, 2017). Both have been optimized to achieve improved detection limits for helminth eggs. Comparative studies between the McMaster and Mini-FLOTAC methods have shown that the former has lower accuracy and precision than the latter (Noel *et al.*, 2017). Additionally, Mini-FLOTAC recovered a higher number of parasite eggs from animal feces compared to McMaster (Johnson *et al.*, 2022). Therefore, the Mini-FLOTAC technique is highly recommended for diagnosing helminth and protozoan infections in animals (Cringoli *et al.*, 2017; de Castro *et al.*, 2017).

## **2.7 Prevalence in Brazil**

Studies reporting the prevalence of *Parascaris* spp. have been conducted in various regions of Brazil. In the southern region, a study in Curitiba, Paraná, evaluated

41 animals and found that 14.6% were positive for *Parascaris* spp. (Ferraro *et al.*, 2008). This prevalence aligns with studies conducted in Rio Grande do Sul, which reported rates of 12.21% (Martins *et al.*, 2019) and 14.40% (Martins *et al.*, 2018). However, two other studies in the same state reported differing prevalence rates, with one at 4% (Souza, 2017) and another exceeding 40% using a sample of 124 animals (Lignon *et al.*, 2020).

In the northeastern region, two studies have addressed this topic. In Paraíba, a prevalence of 31.2% was observed, with 16 positive samples detected using EPG tests (Diniz, 2013). Meanwhile, in the Sertão of Pernambuco, no significant presence of *Parascaris* spp. eggs was found in the samples, which was attributed to the age of the animals (Morais *et al.*, 2017).

In São Paulo, only one study has been conducted to assess the prevalence of this ascarid, reporting an approximate prevalence of 5% in a group of 20 animals (Pereira; Vianna, 2006). In the southeastern region, specifically in Rio de Janeiro, Martins *et al.* (2009) reported a prevalence of 7.8%, while Martins *et al.* (2001) and Martins *et al.* (2023) found rates of 20% and 22.8%, respectively.

In Minas Gerais, four studies have examined the prevalence of *Parascaris* spp. The rates identified were 9.7% in Uberlândia (Teixeira *et al.*, 2014), 20%, 1.8% in Formiga (Rosa *et al.*, 2018), and 21.2% (Rosa *et al.*, 2013).

Calculating a sample mean prevalence (Mean = Sum of prevalences / Number of studies) across these studies yields an average prevalence in Brazil of approximately 15%. However, no studies have yet been conducted in the Zona da Mata region of Minas Gerais.

## 2.8 Research rationale

The occurrence of *Parascaris* spp. in foals is widespread across various regions of the world. For this reason, the prevalence of this parasite in the Zona da Mata region of Minas Gerais must be better established. Furthermore, it is essential to determine which species of *Parascaris* spp. are present in the region, considering that many studies report only *Parascaris equorum* as the species occurring in field conditions.

This situation is further exacerbated by the lack of studies characterizing the parasite at the species level, making this the first study to perform karyotyping of *Parascaris* spp. eggs in Brazil.

The aim of this study is to provide the first description of the karyotyping protocol applied to *Parascaris* spp. eggs in Brazil, through the adaptation of the protocol described by Nielsen (2014). The execution of this experiment establishes a solid foundation for future replications of the protocol, simplifying the karyotyping process for this parasite.

## 3. REFERENCES

ADELMAN, M. Equestrian culture and heritage in Brazil: untapped potential for a tourism that favours local development - São Luiz do Paraná as a case study. **Mondes du tourisme**, [s. l.], n. 18, 2020.

ARMSTRONG, S. K. *et al.* The efficacy of ivermectin, pyrantel and fenbendazole against *Parascaris equorum* infection in foals on farms in Australia. **Veterinary Parasitology**, [s. l.], v. 205, n. 3–4, p. 575–580, 2014.

BARDA, B. D. *et al.* Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. **PLoS Neglected Tropical Diseases**, [s. l.], v. 7, n. 8, p. e2344, 2013.

BIOCCA E *et al.* Descrizione di *Parascaris univalens*, parassita degli equine, e suo differenziamento da *Parascaris equorum*. **Atti della Accademia Nazionale dei Lincei: Classe di Scienze Fisiche, Matematiche e Naturali**, [s. l.], p. 133–140, 1978.

BOES, J.; NANSEN, P.; STEPHENSON, L. S. False-positive *Ascaris suum* egg counts in pigs. **International Journal for Parasitology**, [s. l.], v. 27, n. 7, p. 833–838, 1997.

BOWMAN, D. D. **Georgis' Parasitology for Veterinarians**. 9. ed. Missouri: Elsevier Health Sciences, 2009.

BULLINI L *et al.* Ricerche cariologiche ed elettroforetiche su *Parascaris univalens* e *Parascaris equorum*. **Atti della Accademia Nazionale dei Lincei: Classe di Scienze Fisiche, Matematiche e Naturali.**, [s. l.], p. 151–156, 1978.

CARNOY, J. B. **La cellule, recueil de cytologie et d'histologie générale**. v. 3, Heft 1. La vésicule germinative et les globules polaires chez divers nématodes. La segmentation chez les nématodes. 1886.

CROWELL-DAVIS, S. L.; HOUPPT, K. A. Coprophagy by foals: effect of age and possible functions. **Equine Veterinary Journal**, [s. l.], v. 17, n. 1, p. 17–19, 1985.

CIRAK, V. Y.; HERMOSILLA, C.; BAUER, C. Study on the gastrointestinal parasite fauna of ponies in northern Germany. **Applied Parasitology**, [s. l.], v. 37, n. 4, p. 239–244, 1996.

CLAYTON, H. M.; DUNCAN, J. L. Experimental *Parascaris equorum* infection of foals. **Research in Veterinary Science**, [s. l.], v. 23, n. 1, p. 109–114, 1977.

CLAYTON, H. M.; DUNCAN, J. L. The development of immunity to *Parascaris equorum* infection in the foal. **Research in Veterinary Science**, [s. l.], v. 26, n. 3, p. 383–384, 1979.

CRINGOLI, G. *et al.* Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. **Geospatial health**, [s. l.], v. 7, n. 2, p. 399, 2013.

CRINGOLI, G. *et al.* The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. **Nature Protocols**, [s. l.], v. 12, n. 9, p. 1723–1732, 2017.

CRINGOLI, G. FLOTAC, a novel apparatus for a multivalent faecal egg count technique. **Parassitologia**, [s. l.], v. 48, n. 3, p. 381–384, 2006.

DE CASTRO, L. L. D. *et al.* Comparison of McMaster and Mini-FLOTAC fecal egg counting techniques in cattle and horses. **Veterinary Parasitology: Regional Studies and Reports**, [s. l.], v. 10, p. 132–135, 2017.

DINIZ, P. A. **Endoparasitoses em equinos (*Equus caballus*, Linnaeus, 1758) - prevalência parasitária no Município de Campina Grande e circunvizinhança**. [S. l.: s. n.], 2013.

ESCCAP (EUROPEAN SCIENTIFIC COUNSEL COMPANION ANIMAL PARASITES). **A Guide to the Treatment and Control of Equine Gastrointestinal Parasite Infections**. [S. l.]: Guideline 08 Second Edition, 2019.

FERRARO, C. C. *et al.* Prevalência parasitológica de cavalos de carroceiros em Curitiba, Paraná. **Revista Brasileira de Parasitologia Veterinária**, [s. l.], v. 17, n. 1, p. 175–177, 2008.

FLANAGAN, K.; MORTON, J.; SANDEMAN, R. Prevalence of infestation with gastrointestinal nematodes in Pony Club horses in Victoria. **Australian Veterinary Journal**, [s. l.], v. 91, n. 6, p. 241–245, 2013.

GODAY, C.; PIMPINELLI, S. Chromosome Organization and Heterochromatin Elimination in *Parascaris*. **Science**, [s. l.], v. 224, n. 4647, p. 411–413, 1984.

GODAY, C.; PIMPINELLI, S. Cytological analysis of chromosomes in the two species *Parascaris univalens* and *P. equorum*. **Chromosoma**, [s. l.], v. 94, n. 1, p. 1–10, 1986.

GORDON, H. M. C. L.; WHITLOCK, H. V. A new technique for counting nematode eggs in sheep faeces. **Journal of the Council for Scientific and Industrial Research**, [s. l.], v. 12, n. 1, p. 50–52, 1939.

HERTWIG, O. Vergleich der Ei- und Samenbildung bei Nematoden. **Archiv für Mikroskopische Anatomie**, [s. l.], v. 36, n. 1, p. 1–138, 1890.

JOHNSON, W. L. *et al.* A comparison of Mini-FLOTAC and McMaster techniques, overdispersion and prevalence of parasites in naturally infected North American bison (*Bison bison*) in the USA. **Current Research in Parasitology & Vector-Borne Diseases**, [s. l.], v. 2, p. 100103, 2022.

KAPLAN, R. M. *et al.* World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guideline for diagnosing anthelmintic resistance using the faecal egg count reduction test in ruminants, horses and swine. **Veterinary Parasitology**, [s. l.], v. 318, p. 109936, 2023.

KAPLAN, R. M.; VIDYASHANKAR, A. N. An inconvenient truth: Global worming and anthelmintic resistance. **Veterinary Parasitology**, [s. l.], v. 186, n. 1–2, p. 70–78, 2012.

KNOPP, S. *et al.* FLOTAC: A promising technique for detecting helminth eggs in human faeces. **Transactions of the Royal Society of Tropical Medicine and Hygiene**, [s. l.], v. 103, n. 12, p. 1190–1194, 2009.

LIGNON, J. S. *et al.* Infecção por *Parascaris* spp. em éguas de cria em propriedades localizadas no Sul do Rio Grande do Sul, Brasil. **Brazilian Journal of Development**, [s. l.], v. 6, n. 5, p. 26059–26066, 2020.

LIN, T. P. The chromosomal cycle in *Parascaris equorum* (*Ascaris megalcephala*): Oogenesis and diminution. **Chromosoma**, [s. l.], v. 6, n. 1, p. 175–198, 1953.

LYONS, E. T. *et al.* Further evaluation in field tests of the activity of three anthelmintics (fenbendazole, oxbendazole, and pyrantel pamoate) against the ascarid *Parascaris equorum* in horse foals on eight farms in Central Kentucky (2009–2010). **Parasitology Research**, [s. l.], v. 109, n. 4, p. 1193–1197, 2011.

MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO. **Revisão do Estudo do Complexo do Agronegócio do Cavalo**. Brasília: MAPA, 2016. Disponível em: [\[https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-setoriais/equideocultura/anos-anteriores/revisao-do-estudo-do-complexo-do-agronegocio-do-cavalo/view\]](https://www.gov.br/agricultura/pt-br/assuntos/camaras-setoriais-tematicas/documentos/camaras-setoriais/equideocultura/anos-anteriores/revisao-do-estudo-do-complexo-do-agronegocio-do-cavalo/view). Acess in: 02/12/2025

MARTINS, I. V. F.; CORREIA, T. R.; SOUZA, C. P.; FERNANDES, J. I.; SANTANNA, F. B.; SCOTT, F. B. Frequência de nematóides intestinais de eqüinos oriundos de apreensão, no estado do Rio de Janeiro. **Revista Brasileira de Parasitologia Veterinária**, v. 10, n. 1, p. 37-40, 2001.

MARTINS, I. V.; VEROCAI, G. G.; CORREIA, T. R.; MELO, R. M.; PEREIRA, M. J. S.; SCOTT, F. B.; GRISI, L. Survey on control and management practices of equine helminthes infection. **Pesquisa Veterinária Brasileira**, v. 29, p. 253-257, 2009.

MARTINS, N. *et al.* Prevalência de nematódeos intestinais em equinos da região sul do Rio Grande do Sul, Brasil. **Pubvet**, [s. l.], v. 13, n. 12, 2019.

MARTINS, N. S. *et al.* Ocorrência de helmintos com relação à idade e ao status reprodutivo de éguas da raça crioula da região sul do Rio Grande do Sul, Brasil. **Science And Animal Health**, [s. l.], v. 6, n. 3, p. 218, 2018.

MES, T. H. M. Technical variability and required sample size of helminth egg isolation procedures. **Veterinary Parasitology**, [s. l.], v. 115, n. 4, p. 311–320, 2003.

MORAIS, C. B. R. de *et al.* Parasite prevalence among equidae in the backland of the State of Pernambuco, Brazil. **Semina: Ciências Agrárias**, [s. l.], v. 38, n. 6, p. 3629, 2017.

MORALES B, A. A.; BELLO, H.; GÓMEZ R, M. S. Prevalencia de parásitos gastrointestinales en caballos pura sangre de carrera (*Equus caballus*) durante el periodo de cuarentena 2010 en el hipodromo “la rinconada” Caracas, Venezuela. **Neotropical Helminthology**, [s. l.], v. 5, n. 1, p. 85–88, 2011.

NICHOLLS, J. M. *et al.* A pathological study of the lungs of foals infected experimentally with *Parascaris equorum*. **Journal of Comparative Pathology**, [s. l.], v. 88, n. 2, p. 261–274, 1978.

NIELSEN M. K. *et al.* **AAEP Parasite Control Guidelines**. Lexington: [s. n.], 2019.

NIELSEN, M. K. Anthelmintic resistance in equine nematodes: Current status and emerging trends. **International Journal for Parasitology: Drugs and Drug Resistance**, [s. l.], v. 20, p. 76–88, 2022.

NIELSEN, M. K. *et al.* *Parascaris univalens*—a victim of large-scale misidentification?. **Parasitology Research**, [s. l.], v. 113, n. 12, p. 4485–4490, 2014.

NIELSEN, M. K. Evidence-based considerations for control of *Parascaris* spp. infections in horses. **Equine Veterinary Education**, [s. l.], v. 28, n. 4, p. 224–231, 2016.

NIELSEN, M. K. Sustainable equine parasite control: Perspectives and research needs. **Veterinary Parasitology**, [s. l.], v. 185, n. 1, p. 32–44, 2012.

NIELSEN, M. K.; REINEMEYER, C. R. **Handbook of Equine Parasite Control**. [S. l.]: Wiley, 2018.

NIELSEN, M. K.; REINEMEYER, C. R.; SELLON, D. C. **Nematodes**. *In*: Equine infectious diseases. [S. l.]: Elsevier, 2014. p. 475-489.e4.

NOEL, M. L. *et al.* Accuracy and Precision of Mini-FLOTAC and McMaster Techniques for Determining Equine Strongyle Egg Counts. **Journal of Equine Veterinary Science**, [s. l.], v. 48, p. 182-187.e1, 2017.

PEREIRA, J. R.; VIANNA, S. S. S. Gastrointestinal parasitic worms in equines in the Paraíba Valley, State of São Paulo, Brazil. **Veterinary Parasitology**, [s. l.], v. 140, n. 3–4, p. 289–295, 2006.

PYLES, M. W. *et al.* Use of fecal marker to assess coprophagic behavior in foals. **Journal of Animal Science**, [s. l.], v. 98, n. 11, p. 1–7, 2020. Disponível em: <https://doi.org/10.1093/jas/skaa352>.

REINEMEYER, C. R. Diagnosis and control of anthelmintic-resistant *Parascaris equorum*. **Parasites & Vectors**, [s. l.], v. 2, n. Suppl 2, 2009.

ROSA, M. H. F. *et al.* Factors associated with the prevalence of helminths in Mangalarga Marchador horses in southern of Minas Gerais, Brazil. **Pesquisa Veterinária Brasileira**, [s. l.], v. 38, n. 6, p. 1097–1104, 2018.

ROSA, M. H. F. *et al.* Prevalência de helmintos em equinos da raça mangalarga marchador no sul de Minas Gerais / prevalence of helminths in mangalarga marchador horses in the south of Minas Gerais. **Ars Veterinaria**, [s. l.], v. 29, n. 4, p. 81, 2013.

SOUZA, L. P. de. **Prevalência de parasitos gastrintestinais em equinos oriundos de Porto Alegre/RS**. Porto Alegre: [s. n.], 2017.

TATZ, A. J. *et al.* Surgical treatment for acute small intestinal obstruction caused by *Parascaris equorum* infection in 15 horses (2002–2011). **Equine Veterinary Journal**, [s. l.], v. 44, n. S43, p. 111–114, 2012.

TAYLOR, M. A.; COOP, R. L.; WALL, R. L. **Parasitologia Veterinária**. 4. ed. Rio de Janeiro: Guanabara Koogan, 2017.

TEIXEIRA, W. F. P. *et al.* Endoparasites of horses from the Formiga city, located in center-west region of the state of Minas Gerais, Brazil. **Revista Brasileira de Parasitologia Veterinária**, [s. l.], v. 23, n. 4, p. 534–538, 2014.

VADLEJCH, J. *et al.* Which McMaster egg counting technique is the most reliable?. **Parasitology Research**, [s. l.], v. 109, n. 5, p. 1387–1394, 2011.

VAN BENEDEN, E. **Recherches sur la maturation de l'œuf, la fécondation et la division cellulaire**. Gand; Leipzig: Clemm, 1883.

VON SAMSON-HIMMELSTJERNA, G. *et al.* Very low intraspecific sequence variation in selected nuclear and mitochondrial *Parascaris univalens* genes. **Infection, Genetics and Evolution**, [s. l.], v. 95, p. 105035, 2021.

ZHU, W. *et al.* Chromosome-level genome assembly of *Parascaris univalens* provides insights into chromatin diminution in parasitic nematodes. **Parasites & Vectors**, v. 16, n. 1, p. 1–14, 2023. DOI: 10.1186/s13071-023-05768-3.

**CHAPTER 1**

**Prevalence of *Parascaris* spp. in horses in Brazil - a systematic review**

Giulia Ornellas Fuzaro Scaléa<sup>1</sup>, Gabrielle Oliveira Soares<sup>1</sup>, Ariany Lacerda Nogueira<sup>2</sup>, Késsia Leite Souza<sup>3</sup>, Mariana Green de Freitas<sup>4</sup>, Artur Kanadani Campos<sup>1\*</sup>

<sup>1</sup>Department of Veterinary, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>2</sup>Department of Veterinary Medicine, Federal University of Juiz de Fora, Minas Gerais, Brazil

<sup>3</sup> Department of Animal Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>4</sup>Department of Veterinary Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

\*Corresponding author

**Abstract**

*Parascaris* spp. is a cosmopolitan nematode of great relevance in veterinary medicine. The objective of this study was to conduct a systematic review on the prevalence of *Parascaris* spp. in equids in Brazil. For this purpose, a search strategy was implemented and the following databases were consulted: PubMed, Scopus, and ScienceDirect, maintaining inclusion and exclusion criteria. The initial screening selected a total of 1,317 articles, among these, 134 were excluded due to duplication, remaining 1,183 titles and abstracts were analyzed. From these, only 12 were consistent with the search string, and 4 were eliminated after reading the full text, resulting in 8 final articles being used. The results highlight an average prevalence of 11.8% of *Parascaris* spp. among equids in Brazil; however, none of them performed karyotyping for species-level differentiation. This study emphasizes the gap in the differentiation of *Parascaris* spp. species, highlighting the need for new research to facilitate the implementation of the karyotyping technique. Additionally, it contributes to a broader understanding of this parasitosis in the country.

**Keywords:** Ascarid; Foals; Occurrence.

**Highlights:**

- *Parascaris* spp. is an ascarid of great importance in horse breeding;
- The prevalence of this parasite is still poorly known in most continents;
- Research about this parasite are limited.

**1. Introduction**

*Parascaris* spp. are nematodes widely distributed among equid populations worldwide (Rodostits et al., 2000). This genus of parasites represents one of the primary threats to foal health globally, causing massive intestinal blockages, developmental delays, and various other detrimental effects on overall performance. In Brazil, which stands as one of the world's most significant countries in terms of its economic and cultural importance in equid breeding, academic information regarding the parasite's prevalence remains dispersed and limited (ESCCAP, 2019; Nielsen, 2016; Reinemeyer, 2009).

Although there are studies showing the prevalence of *Parascaris* spp., few studies provide species differentiation. This is due to the difficulty of this characterization, which is done through karyotyping, identifying one or two chromosome pairs in the parasite's egg. This differentiation is crucial for understanding infection dynamics and obstacles in sanitary management (Goday and Pimpinelli, 1986; Nielsen et al., 2014).

Treating animals with anthelmintics without prior diagnosis can contribute to an increase in resistance (Flanagan et al., 2013). For this reason, the eggs per gram of feces (EPG) count is recommended to be performed routinely (Nielsen, 2019). The McMaster method for EPG is widely used due to its practicality; however, it has low sensitivity (Mes, 2003). On the other hand, the Mini-FLOTAC technique has higher sensitivity and is highly recommended for diagnosing infections caused by helminths and protozoa in animals (Cringoli et al., 2017, 2013).

Given these circumstances, this study aims to compile, analyze, and discuss available data on the frequency of *Parascaris* spp. in equids in Brazil, highlighting knowledge gaps and suggesting directions for future investigations.

## 2. Material and methods

Our study centered around a key inquiry: "What's the prevalence of *Parascaris* spp. among equids in Brazil?" As described in the methodology by Fabbri S. et al., 2016, we tapped into academic databases like PubMed, Scopus, and Science Direct for our search. Initially, we deployed an advanced search using the following search strategy: ((prevalence OR occurrence OR incident OR frequency) AND (*Parascaris*)) AND (horse OR equine OR equid OR donkey). Our inclusion criteria were strict, admitting only articles detailing *Parascaris* spp. prevalence in Brazilian equids, with studies from other countries excluded. We also excluded theses, dissertations, abstracts, proceedings, literature reviews, books, and book chapters (Fabbri et al., 2016).

Following our initial search, we cataloged all articles in the StArt Software, an innovative tool by LaPES/UFSCAR. Duplicate articles were purged, and screening commenced with a thorough examination of titles and abstracts. This task was undertaken independently by a pair of reviewers, with discrepancies resolved through author consensus. Subsequently, the remaining articles underwent full-text scrutiny to ascertain eligibility, with reasons for inclusion/exclusion duly noted. This stage involved independent assessment by a reviewer duo. Out of the 1317 initially identified articles, 1183 persisted after eliminating 134 duplicates. Upon scrutiny of titles and abstracts, 12 studies met our eligibility criteria, with 1171 discarded. Finally, after full-text review, 4 more articles were discarded, leaving us with 8 for systematic review (Figure 1).

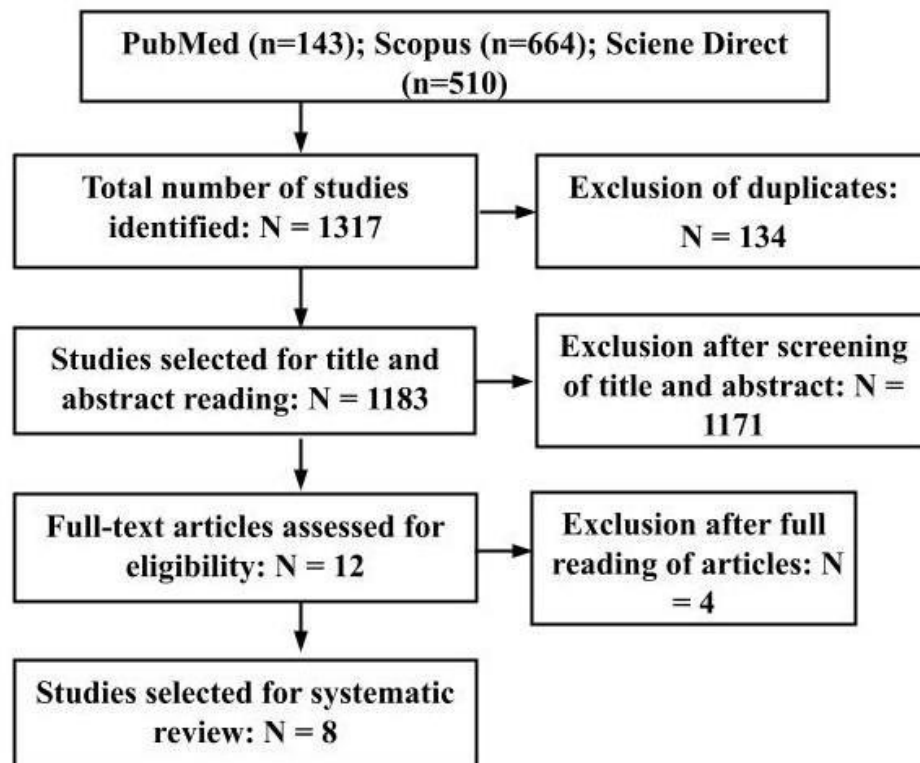


Figure 2: Flowchart of the article selection process in the systematic review

Once we finalized our article selection, data extraction ensued from the chosen eight studies (Table 1). We conducted data extraction using the StArt Software, with a pair of reviewers working independently. Key data points extracted encompassed authorship, publication year, study location, total number of animals, *Parascaris spp.* positivity rate, age, diagnostic methodology, and species-level parasite identification.

*Table 1: Overview of Study Details on Parascaris spp. in Horses in Brazil*

	Author	Year	State	Number of animals	Positive for Parascaris spp. (%)	Age	Diagnostic method	Species identification
1	Carminatti, et al.	2023	Santa Catarina	208	3,36%	Not specified	OPG - McMaster	NI
2	Martins, et al. (a)	2023	Rio de Janeiro	27	22,7%	6 months to 23 years of age	OPG - Mini-FLOTAC	NI
3	Teixeira, et al.	2014	Minas Gerais	25	20%	Beyond 36 months	Identification after necropsy	NI
4	Martins, et. al. (b)	2023	Rio de Janeiro	520	22,8%	Average age of 3,7 years	OPG - Mini-FLOTAC	NI
5	Rodrigues Morais, de	2017	Pernambuco	185	1,62%	Not specified	OPG - McMaster	NI

6	Rosa, et al.	2018	Minas Gerais	602	1,8%	6 months to 23 years of age	OPG - McMaster	NI
7	Pereira, et al.	2006	São Paulo	20	5%	7 months to 30 years of age	Identification after necropsy	NI
8	Ferraro, et al.	2008	Paraná	41	14,6%	Not specified	OPG - McMaster	NI

---

NI – Unidentified.

### 3. Results and discussion

The analysis of our findings unveils the existence of 8 published articles detailing the prevalence of *Parascaris spp.* in Brazil, adhering to the inclusion and exclusion criteria outlined earlier. The mean prevalence stands at 11.8%, mirroring findings from previous studies conducted in Finland, where it was detected in 11.5% of assessed samples (Aromaa et al., 2018). This consistency extends globally, as evidenced by studies such as that of (Tavassoli et al., 2016), reporting 15.51% prevalence in Iran, and Rehbein et al. (2013), detecting 11.3% in Germany.

In Lyons and Tolliver (2004) study, 773 foals aged between 10 and 223 days were utilized, justifying the heightened presence of *Parascaris spp.* owing to the age range covered, a phenomenon echoed in earlier research indicating lactating foal's elevated susceptibility compared to older counterparts (Relf et al., 2013). Notably, the reviewed articles lack strict criteria concerning animal age, resulting in significant age range variation, compounded by three studies omitting this detail (studies 1, 5, and 8). Consequently, observed prevalence rates fall short of Lyons and Tolliver (2004) findings, which exclusively focused on foals.

Article 4 reveals a higher average prevalence of *Parascaris spp.*, concomitant with reaffirmation that infections taper off after 7 months of age (Taylor et al., 2017). This study categorizes animals into two groups: those under 3 years old and those over 3 years old. The former exhibits a markedly higher prevalence compared to the latter, across both *Parascaris spp.* and Strongyles. Conversely, study 5 boasts the lowest average prevalence. Despite lacking age-specific parameters, the author notes a predominantly adult population, also attributing reduced parasite incidence to concurrent rearing of other domestic species like cattle, goats, and sheep (Morais et al., 2017).

Selected studies span various regions across Brazil, encompassing Minas Gerais, Rio de Janeiro, Pernambuco, São Paulo, Paraná, and Santa Catarina (Figure 3). Notably, there are no articles from the North and Midwest regions, and just one

study from Pernambuco representing the Northeast. In the South region, Paraná and Santa Catarina contribute with one study each, while the Southeast region presents two articles from Minas Gerais, two from Rio de Janeiro, and one from São Paulo. This geographical distribution underscores the limited breadth of research on the subject nationwide.



*Figure 3: Map of Brazil indicating states with selected articles for systematic review on *Parascaris* spp. prevalence*

Besides other studies, the studies by Martins et al. (2023) (numbers two and four) utilized the Eggs per Gram of Feces (EPG) diagnostic method in the MINI-Flotac

chamber, a disc with a dual reading field that allows for a total volume of 2 mL to be evaluated, reaching a detection sensitivity of 5 eggs per gram of feces (1:5) (Cringoli et al., 2017, 2013). Opting for this chamber is advantageous, as it boasts superior accuracy and precision compared to the McMaster chamber (Noel et al., 2017). Conversely, studies do Carminatti et al. (2023) 1, 5, 6, and 8 utilized the McMaster chamber for EPG, a widely disseminated technique pioneered in Australia by Gordon and Whitlock (1939), with the dual reading field allowing for a total volume of 0.3 mL to be evaluated. Despite its practicality, it's considered less sensitive, especially in animals with low fecal egg counts (Mes, 2003). Endorsing comparative studies indicating the Mini-FLOTAC method outperforming the McMaster method in diagnostic results, both in pig feces (Class et al., 2023), and in samples from horses and cattle (de Castro et al., 2017; Noel et al., 2017).

Studies 3 and 7, unlike others, were conducted by counting helminths found during animal parasitological necropsy. In study 3, 25 animals were used, with only 5 presenting *Parascaris spp.* Additionally, the mean intensity of parasitism by this helminth in each individual animal was determined, yielding an average of 5.48 helminths per necropsied equine. Once again, the article underscores that the most plausible reason for the reduced quantity of adult *Parascaris spp.* in each animal is the decreased susceptibility to infection in foals over one year old (Bowman, 2009).

Karyotyping is one way to differentiate *Parascaris spp.* species. This method visualizes fixed and subsequently stained germ cells during the metaphase of their first mitotic phase of cell division but requires the collection of parasites with germ cells at the appropriate stage (Goday and Pimpinelli, 1984). The egg development phase will be observed under a microscope to visualize, preferably, the first or second mitotic phase of embryonic divisions. Subsequently, the *Parascaris spp.* species will be identified according to the number of chromosomes. *Parascaris equorum* has two pairs of chromosomes, while *Parascaris univalens* has only one pair of chromosomes (Goday and Pimpinelli, 1984). None of the articles selected in this study performed this

characterization; thus, according to Nielsen et al. (2014), it is suggested that the term *Parascaris* spp. should be used to refer to these ascarids.

It's natural that few studies mention the species identification process, as this differentiation is complex and challenging since both species of this ascarid lack differences in their life cycle, clinical signs caused in the host, and explicit differences in the morphology of the various phases of their life cycle (Goday and Pimpinelli, 1984).

#### **4. Conclusion**

In light of the presented evidence, we've identified a total of 8 studies meeting our predefined inclusion and exclusion criteria, all addressing the prevalence of *Parascaris* spp. in Brazil. The collective findings from these studies yield an average prevalence rate of 11.8% for this infection. It's worth noting, however, that none of the studies included in our analysis conducted species-level characterization of this parasite. This underscores a notable gap in research on *Parascaris* spp. prevalence in Brazil, indicating a clear need for further investigation in this area. Moving forward, it's imperative that future research endeavors adopt standardized methodologies and explore crucial aspects such as species characterization, thereby advancing our understanding of the prevalence and geographical distribution of this parasite among Brazilian equids.

#### **5. Acknowledgements**

The authors are grateful to the support provided by Federal University of Viçosa and the Laboratory of Veterinary Parasitology.

#### **6. Author contribution**

Giulia Ornellas Fuzaro Scaléa: Writing – original draft; Data curation; Formal analysis; Methodology.

Gabrielle Oliveira Soares: Writing – original draft; Data curation; Formal analysis; Methodology.

Ariany Lacerda Nogueira: Writing – original draft; Data curation.

Késsia Leite Souza: Writing – original draft; Data curation.

Mariana Green de Freitas: Writing – review and editing; Project administration; Methodology.

Artur Kanadani Campos: Writing – review and editing; Project administration; Methodology.

## 7. References

Aromaa, M., Hautala, K., Oksanen, A., Sukura, A., Näreaho, A., 2018. Parasite infections and their risk factors in foals and young horses in Finland. *Vet. Parasitol. Reg. Stud. Rep.* 12, 35–38. <https://doi.org/10.1016/j.vprsr.2018.01.006>.

Bowman, D.D., 2009. *Georgis' Parasitology for Veterinarians*, ninthth ed. Elsevier Health Sciences, Missouri.

Carminatti, A., Chitolina, M.B., Ribeiro, A.B., Forest, M., Collet, S.G., Prestes, A.M., Camillo, G., 2023. Occurrence and risk factors associated with gastrointestinal parasitism in horses reared in different systems. *Vet. Parasitol. Reg. Stud. Rep.* 42, 100890. <https://doi.org/10.1016/j.vprsr.2023.100890>.

Class, C.S.C., Fialho, P.A., Alves, L.C., Silveira, R.L., Amendoeira, M.R.R., Knackfuss, F.B., Barbosa, A. da S., 2023. Comparison of McMaster and Mini-FLOTAC techniques for the diagnosis of internal parasites in pigs. *Rev. Bras. de Parasitol. Vet.* 32. <https://doi.org/10.1590/s1984-29612023013>.

Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruyssen, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nat. Protoc.* 12, 1723–1732. <https://doi.org/10.1038/nprot.2017.067>.

Cringoli, G., Rinaldi, L., Albonico, M., Bergquist, R., Utzinger, J., 2013. Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. *Geospat. Health* 7, 399. <https://doi.org/10.4081/gh.2013.97>.

de Castro, L.L.D., Abrahão, C.L.H., Buzatti, A., Molento, M.B., Bastianetto, E., Rodrigues, D.S., Lopes, L.B., Silva, M.X., de Freitas, M.G., Conde, M.H., Borges, F. de A., 2017. Comparison of McMaster and Mini-FLOTAC fecal egg counting techniques in cattle and horses. *Vet. Parasitol. Reg. Stud. Rep.* 10, 132–135. <https://doi.org/10.1016/j.vprsr.2017.10.003>.

ESCCAP (European Scientific Counsel Companion Animal Parasites), 2019. A Guide to the Treatment and Control of Equine Gastrointestinal Parasite Infections.

Fabbri, S., Silva, C., Hernandez, E., Octaviano, F., Di Thommazo, A., Belgamo, A., 2016. Improvements in the StArt tool to better support the systematic review process, in: Proceedings of the 20th International Conference on Evaluation and Assessment in Software Engineering. ACM, New York, NY, USA, pp. 1–5. <https://doi.org/10.1145/2915970.2916013>.

Flanagan, K., Morton, J., Sandeman, R., 2013. Prevalence of infestation with gastrointestinal nematodes in Pony Club horses in Victoria. *Aust. Vet. J.* 91, 241–245. <https://doi.org/10.1111/avj.12052>.

Goday, C., Pimpinelli, S., 1986. Cytological analysis of chromosomes in the two species *Parascaris univalens* and *P. equorum*. *Chromosoma* 94, 1–10. <https://doi.org/10.1007/BF00293524>.

Goday, C., Pimpinelli, S., 1984. Chromosome Organization and Heterochromatin Elimination in *Parascaris*. *Science* (1979) 224, 411–413. <https://doi.org/10.1126/science.224.4647.411>.

Gordon, H.M.C.L., Whitlock, H. V., 1939. A new technique for counting nematode eggs in sheep faeces. *J. Counc. Sci. Ind. Res.* 12, 50–52.

Lyons, E.T., Tolliver, S.C., 2004. Prevalence of parasite eggs (*Strongyloides westeri*, *Parascaris equorum*, and strongyles) and oocysts (*Eimeria leuckarti*) in the feces of Thoroughbred foals on 14 farms in central Kentucky in 2003. *Parasitol. Res.* 92, 400–404. <https://doi.org/10.1007/s00436-003-1068-2>.

Martins, A. V., Corrêa, L.L., Ribeiro, M.S., Lobão, L.F., Dib, L. V., Palmer, J.P.S., de Moura, L.C., Knackfuss, F.B., Uchôa, C.M.A., Molento, M.B., Barbosa, A. da S., 2023. Prevalence, Risk Factors and Diagnosis of Helminths in Thoroughbred Horses Kept at Training Centers in Rio de Janeiro, Brazil. *J. Equine Vet. Sci.* 127, 104536. <https://doi.org/10.1016/j.jevs.2023.104536>.

Mes, T.H.M., 2003. Technical variability and required sample size of helminth egg isolation procedures. *Vet. Parasitol.* 115, 311–320. [https://doi.org/10.1016/S0304-4017\(03\)00219-X](https://doi.org/10.1016/S0304-4017(03)00219-X).

Morais, C.B.R. de, Santiago, J.M., Lima, M.M. de, Lucena, J.E.C., 2017. Parasite prevalence among equidae in the backland of the State of Pernambuco, Brazil. *Semin. Cienc. Agrar.* 38, 3629. <https://doi.org/10.5433/1679-0359.2017v38n6p3629>.

Nielsen M. K., Mittel L., Grice A., Erskine M., Grave E., Vaala W., Tully R.C., French D.D., Bowman R., Kaplan R.M., 2019. AAEP Parasite Control Guidelines. Lexington.

Nielsen, M.K., 2016. Evidence-based considerations for control of *Parascaris* spp. infections in horses. *Equine Vet. Educ.* 28, 224–231. <https://doi.org/10.1111/eve.12536>.

- Nielsen, M.K., Wang, J., Davis, R., Bellaw, J.L., Lyons, E.T., Lear, T.L., Goday, C., 2014. *Parascaris univalens*—a victim of large-scale misidentification? *Parasitol. Res.* 113, 4485–4490. <https://doi.org/10.1007/s00436-014-4135-y>.
- Noel, M.L., Scare, J.A., Bellaw, J.L., Nielsen, M.K., 2017. Accuracy and Precision of Mini-FLOTAC and McMaster Techniques for Determining Equine Strongyle Egg Counts. *J. Equine Vet. Sci.* 48, 182-187.e1. <https://doi.org/10.1016/j.jevs.2016.09.006>.
- Rehbein, S., Visser, M., Winter, R., 2013. Prevalence, intensity and seasonality of gastrointestinal parasites in abattoir horses in Germany. *Parasitol. Res.* 112, 407–413. <https://doi.org/10.1007/s00436-012-3150-0>.
- Reinemeyer, C.R., 2009. Diagnosis and control of anthelmintic-resistant *Parascaris equorum*. *Parasit. Vectors* 2. <https://doi.org/10.1186/1756-3305-2-S2-S8>.
- Relf, V.E., Morgan, E.R., Hodgkinson, J.E., Matthews, J.B., 2013. Helminth egg excretion with regard to age, gender and management practices on UK Thoroughbred studs. *Parasitology* 140, 641–652. <https://doi.org/10.1017/S0031182012001941>.
- Rodostits, O.M., Gay, C.C., Blood, D.C., Hinchcliff, K.W., 2000. *Clínica veterinária: um tratado de doenças dos bovinos, ovinos, suínos, caprinos e equinos.*, ninth ed. Guanabara Koogan, Rio de Janeiro.
- Tavassoli, M., Arjmand Yamchi, J., Hajipour, N., 2016. A survey on the prevalence of strongyles species in working donkeys in North-West of Iran. *J. Parasit. Dis.* 40, 1210–1212. <https://doi.org/10.1007/s12639-015-0651-7>
- Taylor, M.A., Coop, R.L., Wall, R.L., 2017. *Parasitologia Veterinária*, fourth ed. Guanabara Koogan, Rio de Janeiro.

## CHAPTER 2

## Prevalence of *Parascaris* spp. in Horses from the Zona da Mata of Minas Gerais region, Minas Gerais, Brazil

Giulia Ornellas Fuzaro Scaléa<sup>1</sup>, Gabrielle Oliveira Soares<sup>1</sup>, Ariany Lacerda Nogueira<sup>2</sup>, Késsia Leite Souza<sup>3</sup>, Mariana Green de Freitas<sup>4</sup>, Artur Kanadani Campos<sup>1\*</sup>

<sup>1</sup>Department of Veterinary, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>2</sup>Department of Veterinary Medicine, Federal University of Juiz de Fora, Minas Gerais, Brazil

<sup>3</sup>Department of Animal Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>4</sup>Department of Veterinary Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

\*Corresponding author

### Abstract

This study investigates the prevalence of *Parascaris* spp. in foals up to two years old on stud farms in the Zona da Mata region of Minas Gerais, Brazil, a significant equideoculture hub with a substantial equine population. *Parascaris* spp. is a crucial pathogen in young horses, causing gastrointestinal and respiratory symptoms that can lead to severe health and economic impacts. Despite its importance, data on its prevalence in Brazil, particularly in this region, remain scarce. Fecal samples were collected from 450 foals across 15 studs farms, and the Mini-FLOTAC technique was utilized for parasitological diagnosis. Additionally, a questionnaire about the animals' history was completed by their owners. The prevalence of *Parascaris* spp. was found to be 11.3%, with significant variation among the farms. 7 and 12 Stud Farms exhibited the highest rates of infection, while others, such as 1, 4, and 10, showed no positive cases. Additionally, age group analysis revealed that foals aged 7 to 12 months had

the highest infection rate. Statistical analyses were performed using IBM SPSS Statistics version 25.0, considering a significance level of  $p < 0.01$ . The findings align with reports from other Brazilian regions, demonstrating variability in prevalence, possibly due to differences in management practices, climatic conditions, and diagnostic methods. This study provides valuable epidemiological data, highlighting the need for effective control strategies tailored to local conditions to minimize the health and economic impacts of *Parascaris* spp. infections in Brazilian equideoculture.

**Keywords:** Ascarid; Faecal Egg Count; Mini-FLOTAC

**Highlights:**

- *Parascaris* spp. is an ascarid of great importance in veterinary parasitology due to this importance in horse breeding;
- The prevalence of this parasite is poorly known in Brazil;
- Knowledge about the prevalence of the parasite in Brazil is essential for developing effective control measures.

## 1. Introduction

Equideoculture represents a significant economic potential within Brazilian agribusiness, ranking as the fourth largest in the world, with a population of 5.834 million animals. The state with the largest equine herd in Brazil is Minas Gerais, with approximately 811,000 equines (IBGE, 2021). Given the magnitude of this population, it is crucial to address helminth infections, which constitute one of the primary health challenges in horses.

*Parascaris* spp. is a major pathogen affecting foals, leading to gastrointestinal disturbances, verminous obstructions, inappetence, diarrhea, and respiratory symptoms such as coughing and nasal discharge, which can result in fatal outcomes (Nielsen, 2016; Reinemeyer, 2009). In addition to its impact on animal welfare, the disease can cause significant economic losses due to the costs of medication,

veterinary treatment, decreased animal performance, and potential mortality (Cain and Nielsen, 2022).

Considering the scarcity of studies on *Parascaris* spp. infections in foals in the Zona da Mata region of Minas Gerais, this study aimed to assess the prevalence of this ascarid in foals up to two years old from stud farms located in the Zona da Mata of Minas Gerais region, Brazil.

## **2. Materials and methods**

### **2.1. Ethics**

The study was conducted on stud farms in the Zona da Mata, region of Minas Gerais, Brazil. The animal work was approved by University of Viçosas's Institutional Care and Use Committee (CEUA) under protocol number 16/2024.

### **2.2. Statistical analysis**

For prevalence and mean of *Parascaris* spp. intensities were calculated according, providing 95% confidence intervals (C.I. 99,9%). According to OpenEpi version 3, the calculated population size for this study was 396 animals. However, a total of 450 samples were collected from foals.

The statistical analyses were performed using IBM SPSS Statistics version 25.0, considering  $p < 0.01$  as a significance threshold.

### **2.3. Study Location**

The study was conducted on horse farms located in the Zona da Mata, region of Minas Gerais state, Brazil, described in Table 1. The diagnostic tests were conducted at the Laboratory of Parasitology and Parasitic Diseases of the Department of Veterinary Medicine.

*Table 2: Horses farms' location and faecal samples collected in each property in Zona da Mata of Minas Gerais region*

<b>Stud farms' name</b>	<b>City</b>	<b>Nº of samples</b>
Stud farm 1	Viçosa - MG	4
Stud farm 2	Ubá - MG	92
Stud farm 3	Urucânia - MG	8
Stud farm 4	Viçosa - MG	12
Stud farm 5	Viçosa - MG	2
Stud farm 6	Silveirânia - MG	11
Stud farm 7	Muriaé - MG	91
Stud farm 8	Ervália - MG	14
Stud farm 9	Rodeiro - MG	16
Stud farm 10	Viçosa - MG	10
Stud farm 11	Teixeiras - MG	17
Stud farm 12	São Geraldo - MG	102
Stud farm 13	Guaraciaba - MG	14
Stud farm 14	Ponte Nova - MG	49
Stud farm 15	Coimbra - MG	8
Total: 15 Studs farms	12 cities	450 samples

MG - Minas Gerais state; Nº - Number

## **2.4 Questionnaire**

A questionnaire (Attachment 1) was administered to the owners or managers of the studied stud farms to later correlate potential risk factors associated with the disease.

## **2.5 Faecal samples**

Faecal samples from 450 foals under two years old were collected from different properties, as described in Table 1. All samples were obtained from naturally infected animals and collected directly from the rectum. Samples were transported in

a refrigerated styrofoam box to the parasitology laboratory of veterinary medicine of UFV and the samples were analyzed immediately upon arrival.

### **2.6 *Parascaris* spp. detection**

The Mini-FLOTAC was conducted according to Barda et al. (2013), Noel et al. (2017) and Cringoli et al. (2017) with adaptations. The technique was performed on 5g faeces from each horse, mixed with a volume of 45 mL of saturated sodium nitrate solution (NaNO<sub>3</sub>) (solution density (SD) =1.2), The mixture was homogenized by stirring and subsequently passed through a double layer of cheesecloth. The filtrate was then extracted through a spout located on the lid, simultaneously being filtered through an integrated filter within the lid. The resulting liquid was transferred into the two 1-mL chambers of the Mini-FLOTAC slides. After a 10-minute incubation period, the top section of the reading disc was rotated, enabling the flotation of eggs for counting under magnification (Figure 1).

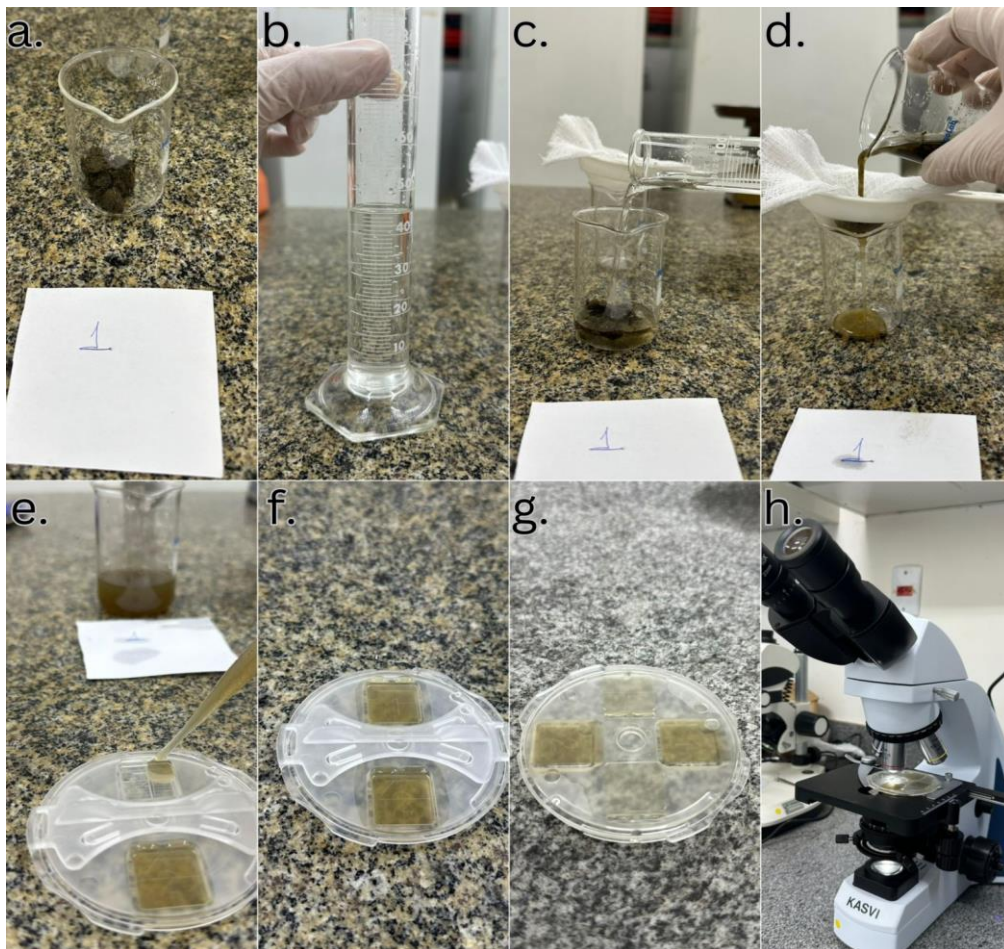


Figure 4: Mini-FLOTAC technique in feces samples of animals from experimental farms. a. 5g of feces; b. 45 mL of saturated solution; c. Homogenizing; d. Sifting the solution; e. Fill the discs; f. Wait for 10 minutes; g. Spin the disc; h. Reading under the microscope.

### 3. Results

The analysis of the collected samples revealed that, out of the total 450 samples examined, 88.7% showed no presence of *Parascaris* spp., while 11.3% tested positive for the parasite in feces (Table 2). Stud farm 7 and 12 had the highest number of positive animals, with 17 and 10 foals, respectively (Table 3). The 1, 4, and 10 Stud Farm presented only negative results.

*Table 3: Frequency of positive and negative animals identified in the prevalence study of *Parascaris* spp. in the Zona da Mata of Minas Gerais region*

	Frequency	Percentage (%)
<b>Negative</b>	399	88,7
<b>Positive</b>	51	11,3
<b>Total</b>	450	100,0

*Table 4: Count of positive and negative animals for *Parascaris* spp. based on their stud farm of origin, as diagnosed using the Mini-FLOTAC technique*

Property	Negative	Positive	% positive animals in each property
Stud farm 1	4	0	0
Stud farm 2	87	5	5,43
Stud farm 3	5	3	60
Stud farm 4	12	0	0
Stud farm 5	0	2	100
Stud farm 6	10	1	9,09
Stud farm 7	74	17	18,68
Stud farm 8	12	2	14,28
Stud farm 9	14	2	12,5
Stud farm 10	10	0	0
Stud farm 11	14	3	17,65
Stud farm 12	92	10	9,8
Stud farm 13	12	2	14,28
Stud farm 14	46	3	6,12
Stud farm 15	7	1	12,5

For the age group analysis, the animals were categorized into four distinct groups based on their age in months (Table 4). The first category included animals aged 0 to 6 months, represented by the value 0. The second group comprised animals aged 7 to 12 months, represented by the value 1. The third category consisted of

animals aged 13 to 18 months, classified under the value 2, while the fourth group included animals aged 19 to 24 months, represented by the value 3.

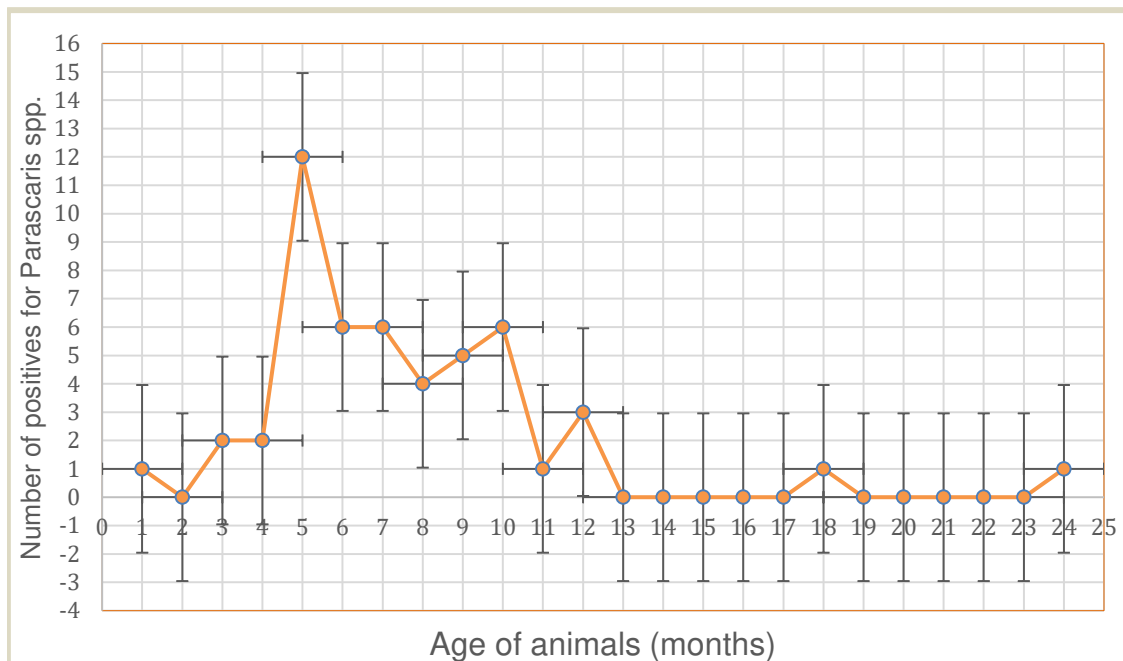
*Table 5: Age Group Distribution of Animals Positive for *Parascaris* spp. in Properties from the Zona da Mata of Minas Gerais region*

<b>Age</b>	<b>Frequency</b>	<b>Percentage (%)</b>
<b>0</b>	196	43,6
<b>1</b>	213	47,3
<b>2</b>	14	3,1
<b>3</b>	27	6,0
<b>Total</b>	450	100,0

The distribution of *Parascaris*-positive animals by age (in months) is shown in figure 2. The highest occurrence was observed at 5 months of age, with 12 positive cases, followed by 6, 7, and 10 months, each with 6 positive cases. Subsequently, 9-month-old animals had 5 cases, while those at 8 months had 4. Only 3 positive cases were recorded at 12 months, and 2 at 3 and 4 months. Animals at 1, 11, 18, and 24

months each had a single positive case, whereas no positive cases were observed at 2, 13, 14, 15, 16, 17, 19, 20, 21, 22, or 23 months.

*Figure 5: Distribution of Parascaris-positive animals (based on Mini-FLOTAC results) according to age (months), from samples collected from foals in stud farms located in the Zona da Mata region of Minas Gerais.*



The analysis of relative risk (RR), p-value, population attributable fraction (PAF), and attributable fraction among the exposed (AFE) for the variables studied regarding their association with disease occurrence are presents on Table 5. For the variable "age group," animals up to one year of age presented a relative risk (RR) of 0.9222 (95% CI: 0.3882–2.191), with a p-value of 0.8553.

Regarding the treatment decision method, the comparison between random decision and veterinarian guidance resulted in an RR of 1.234 (95% CI: 0.6554–2.322), with a p-value of 0.5120.

For the feces disposal method, the comparison between composting and disposal in the forage field showed an RR of 1.665 (95% CI: 0.9642–2.874),  $p = 0.0646$ ; composting versus feces remaining on pasture showed an RR of 0.6645 (95% CI: 0.26–1.698),  $p = 0.4069$ ; and feces remaining on pasture versus disposal in the forage

field resulted in an RR of 2.505 (95% CI: 0.9531–6.585),  $p = 0.0688$ .

With regard to feeding, animals fed exclusively with forage, compared to those receiving concentrate, presented an RR of 0.2527 (95% CI: 0.03605–1.772), with a  $p$ -value of 0.1185. The comparison between lactating and non-lactating animals resulted in an RR of 1.055 (95% CI: 0.6257–1.777), with a  $p$ -value of 0.8420.

*Table 6: Evaluation of the association between management factors and disease risk in horses, including estimates of relative risk, statistical significance, and etiologic fractions*

<b>Variable</b>	<b>RR (95% IC)</b>	<b>P-value</b>	<b>PAF</b>	<b>AFE</b>
<b>Age</b>				
Up to one year	0.9222 (0.3882 – 2.191)	0.8553	7067%	7775%
<b>Treatment decision method</b>				
Random vs. veterinarian's advice	1.234 (0.6554 - 2.322 <sup>1</sup> )	0.5120	14.86%	18.85%
<b>Feces disposal method</b>				
Composting vs. forage field	1.665 (0.9642, 2.874 <sup>1</sup> )	0.0646 0	22.94%	39,93%
Composting vs. remains on pasture	0.6645 (0.26, 1.698 <sup>1</sup> )	0.4069	30.54%	33.55%
Remains on pasture vs. forage field	2.505 (0.9531, 6.585 <sup>1</sup> )	0.0687 7	10.01%	60.08%
<b>Feeding</b>				
Forage only vs. with concentrate	0.2527 (0.03605, 1.772 <sup>1</sup> )	0.1185	5.48%	0,75%
Lactating vs. non-lactating	1.055 (0.6257, 1.777 <sup>1</sup> )	0.8420	2.941%	51,72%

Legend: RR – Relative Risk; CI – Confidence Interval; P-value – Probability value; PAF – Population Attributable Fraction; AFE – Attributable Fraction among the Exposed.

#### **4. Discussion**

Previous studies on the prevalence of *Parascaris* spp. in equines from different regions of Brazil indicate variability in the occurrence rates of this parasite. In the southern region, Ferraro et al. (2008) reported an incidence of 14.6% in the municipality of Curitiba, Paraná state. In Rio Grande do Sul, Piccoli et al. (2015) identified a prevalence of 4.2% in leisure horses and 7.4% in working horses, while Martins et al. (2019) documented a prevalence of 12.21% in the same region. In

Pelotas, also in Rio Grande do Sul, Lignon et al. (2020b) observed a prevalence of 13.4%, whereas in the southeastern part of the state, Lignon et al. (2020a) reported a rate of 40.3%. In Santa Catarina state, Carminatti et al. (2023) described a prevalence of 3.36% in an experimental study.

In the southeastern region, specifically in the state of Rio de Janeiro, Pereira and Vianna (2006) reported a prevalence of 5% for *Parascaris* spp. in equines, while Martins et al. (2021) reported 14.8%, and Martins et al. (2023a) observed 38.7%, both in the municipality of Teresópolis. In Minas Gerais state, Teixeira et al. (2014) recorded an occurrence of 20% in the municipality of Formiga. In São Paulo state, Pereira and Vianna (2006) reported a prevalence of 5% in the Vale do Paraíba region.

In a retrospective study, Molento et al. (2024) identified a prevalence of 5.8% for *Parascaris* spp., analyzing studies conducted in states with the largest equine populations, including Paraná, Rio de Janeiro, São Paulo, Minas Gerais, Rio Grande do Sul, Mato Grosso, and Mato Grosso do Sul. These findings differ from those of other authors, who reported lower (Carminatti et al., 2023; Pereira and Vianna, 2006; Rosa et al., 2013) or higher (Martins et al., 2023b; Teixeira et al., 2014) prevalence rates in different Brazilian regions.

The prevalence of *Parascaris* spp. in Brazil shows significant variability, likely due to the scarcity of studies on this subject. The rates recorded in the Zona da Mata region of Minas Gerais are comparable to those reported in studies conducted in the southern region (Ferraro et al., 2008; Lignon et al., 2020b; Martins et al., 2019) and southeastern region (Martins et al., 2021).

According to the graph showing the distribution of animals testing positive for *Parascaris* spp. by age group, the highest concentration of cases occurs at 5 months of age, followed by 6 and 7 months. This pattern suggests that foals gradually develop immunological resistance to the parasite as they grow older. Furthermore, the low detection rates in younger animals may be attributed to the long prepatent period of *Parascaris* spp., which prevents early identification of the parasite in the first weeks of

life (Reinemeyer, 2012). Studies indicate that coprophagy can lead to false-positive results in fecal egg count examinations (Boes et al., 1997). This phenomenon is particularly relevant in foals, as the literature confirms that this behavior is common during the first weeks of life (Crowell-Davis & Houpt, 1985; Pyles et al., 2020). Therefore, this fact may contribute to the appearance of positive animals in the first months, before the prepatent period.

Regarding age, no statistically significant association was observed between animals up to one year old and the presence of the disease (RR = 0.9222; 95% CI: 0.3882–2.191;  $p = 0.8553$ ). The estimates for population attributable fraction (PAF = 7.067%) and attributable fraction among the exposed (AFE = 7.775%) were also low, indicating that age, in this case, did not contribute significantly to the occurrence of the disease. This may be explained by the fact that the study included only animals up to two years of age, a range considered susceptible to *Parascaris* spp. — with infections becoming increasingly rare after 18 months of age (Nielsen & Reinemeyer, 2018). It is likely that studies including foals and animals older than two years would show a different correlation.

In relation to the choice of antiparasitic treatment, no significant associations were observed. Although the PAF and AFE were 14.86% and 18.85%, respectively, these values suggest that management practices such as veterinary guidance in the selection of antiparasitic drugs may reduce the risk of *Parascaris* spp. infection (Alonso et al., 2015; Pires, 2024). Along with veterinary guidance, the disposal of feces is crucial in sanitary management. Composting presented the lowest relative risk of *Parascaris* spp. infection compared to grass paddocks and continuous grazing, a result consistent with previous studies that highlight the role of environmental management in the dynamics of parasitic infections such as *Parascaris* spp (O'Meara & Mulcahy, 2002) (Matthews, 2014).

Although the association did not reach statistical significance, previous studies suggest that a balanced diet may influence the immune response, particularly in the

gastrointestinal system of foals, promoting greater resistance to parasitic infections. (Williams et al., 2020). Moreover, adequate nutrition is essential for the development of an effective immune response, which may directly impact the elimination of parasites, as observed by Laroche et al. (2024) in infections caused by *Strongylus* spp.

In the comparison between suckling and weaned foals, no significant associations were observed. This finding may be explained by the fact that, although colostrum provides passive immunity, its effectiveness against intestinal parasites and no studies correlates that. Furthermore, the high environmental resistance of parasite eggs and the contamination of foaling areas contribute to the early exposure of young animals (Ripley et al., 2023). Additionally, *Parascaris* eggs possess a proteinaceous and mucopolysaccharide-rich surface coat that confers adhesive properties, enabling them to stick to a variety of surfaces, including the udder of the mare, thus facilitating indirect transmission to foals (Kleine et al., 2016).

## **5. Conclusion**

In this study, based on the diagnosis of *Parascaris* spp., it was possible to determine the frequency of parasitized foals in the Zona da Mata region of Minas Gerais. The results indicated a prevalence of 11.3% in stud farms within the region, with a higher occurrence between 5 and 7 months of age, a significant finding given the absence of prior records or studies on the prevalence of this parasite in this locality.

Although no statistically significant associations were found, a tendency toward lower infection risk was observed with composting of feces and the use of antiparasitic treatments guided by veterinary recommendations. Neither the suckling phase nor an exclusive forage-based diet influenced infection rates, highlighting the importance of environmental and sanitary management. Further studies are recommended, incorporating immunological factors and integrated management practices.

## 6. Acknowledgements

The authors are grateful to the support provided by Federal University of Viçosa and the Laboratory of Veterinary Parasitology.

## 7. Author contribution

Giulia Ornellas Fuzaro Scaléa: Writing – original draft; Data curation; Formal analysis; Methodology.

Gabrielle Oliveira Soares: Writing – original draft; Data curation; Formal analysis; Methodology.

Ariany Lacerda Nogueira: Writing – original draft; Data curation.

Késsia Leite Souza: Writing – original draft; Data curation.

Mariana Green de Freitas: Writing – review and editing; Project administration; Methodology.

Artur Kanadani Campos: Writing – review and editing; Project administration; Methodology.

## 8. References

Alonso, J.M., Kauer, D.P., Alves, A.L.G., Watanabe, M.J., Rodrigues, C.A., Geraldini, C.M., Hussni, C.A., 2015. Efeito da orientação sobre o controle parasitológico dos equídeos de tração do município de Botucatu/SP. *Anais do 8º Congresso de Extensão Universitária da UNESP*.

Barda, B.D., Rinaldi, L., Ianniello, D., Zepherine, H., Salvo, F., Sadutshang, T., Cringoli, G., Clementi, M., Albonico, M., 2013. Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field. *PLoS Negl. Trop. Dis.* 7, e2344. <https://doi.org/10.1371/journal.pntd.0002344>.

Cain, J.L., Nielsen, M.K., 2022. The equine ascarids: resuscitating historic model organisms for modern purposes. *Parasitol. Res.* 121, 2775–2791. <https://doi.org/10.1007/s00436-022-07627-z>.

Carminatti, A., Chitolina, M.B., Ribeiro, A.B., Forest, M., Collet, S.G., Prestes, A.M., Camillo, G., 2023. Occurrence and risk factors associated with gastrointestinal

parasitism in horses reared in different systems. *Vet. Parasitol. Reg. Stud. Rep.* 42, 100890. <https://doi.org/10.1016/j.vprsr.2023.100890>.

Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nat. Protoc.* 12, 1723–1732. <https://doi.org/10.1038/nprot.2017.067>.

Ferraro, C.C., Kloss, A.B., Souza, D.F. De, Deconto, I., Biondo, A.W., Molento, M.B., 2008. Prevalência parasitológica de cavalos de carroceiros em Curitiba, Paraná. *Rev. Bras. Parasitol. Vet.* 17, 175–177.

IBGE (Instituto Brasileiro de Geografia e Estatística), 2021. Rebanho de Cavalos no Brasil [<https://www.ibge.gov.br/explica/producao-agropecuaria/equinos/br> (accessed 2.12.25)].

Kleine, L., Hasselmann, M., Greif, G., Hollensteiner, J., Strube, C., 2016. Adhesive properties of ascarid egg surface coats – composition and persistence in the environment. *Vet. Parasitol.* 227, 58–66. <https://doi.org/10.1016/j.vetpar.2016.07.012>

Laroche, N., Grimm, P., Julliand, S., Sorci, G., 2024. Diet modulates strongyle infection and microbiota in the large intestine of horses. *PLoS One* 19, e0301920. <https://doi.org/10.1371/journal.pone.0301920>.

Lignon, J.S., Martins, N.S., Gonçalves, N.F., Leão, M.S., Camassola, J.L.T., Cardoso, T.A.E.M., Antunes, T. de A., Pappen, F.G., Pinto, D.M., 2020a. Infecção por *Parascaris* spp. em éguas de cria em propriedades localizadas no Sul do Rio Grande do Sul, Brasil. *Braz. J. Dev.* 6, 26059–26066. <https://doi.org/10.34117/bjdv6n5-166>.

Lignon, J.S., Soares Martins, N., Mueller, A., Siegert, F., Seibert De Leão, M., Luis, J., Camassola, T., Gomes Pellegrin, T., De, T., Antunes, A., Pappen, F.G., Pinto, D.M., 2020b. Prevalência de nematódeos intestinais em equinos de tração na cidade de Pelotas/RS, Brasil.

Martins, A. V., Corrêa, L.L., Ribeiro, M.S., Lobão, L.F., Dib, L. V., Palmer, J.P.S., de Moura, L.C., Knackfuss, F.B., Uchôa, C.M.A., Molento, M.B., Barbosa, A. da S., 2023a. Prevalence, Risk Factors and Diagnosis of Helminths in Thoroughbred Horses Kept at Training Centers in Rio de Janeiro, Brazil. *J. Equine Vet. Sci.* 127, 104536. <https://doi.org/10.1016/j.jevs.2023.104536>.

Martins, A. V., Corrêa, L.L., Ribeiro, M.S., Lobão, L.F., Dib, L. V., Palmer, J.P.S., de Moura, L.C., Knackfuss, F.B., Uchôa, C.M.A., Molento, M.B., Barbosa, A. da S., 2023b. Prevalence, Risk Factors and Diagnosis of Helminths in Thoroughbred Horses Kept at Training Centers in Rio de Janeiro, Brazil. *J. Equine Vet. Sci.* 127, 104536. <https://doi.org/10.1016/j.jevs.2023.104536>.

Martins, A.V., Cavalcante De Moura<sup>2</sup>, L., Luiz, P., Mota, M., Ferreira Bastos<sup>1</sup>, B., Da, A., Barbosa<sup>3</sup>, S., 2021. Research for gastrointestinal parasites in horses with a focus on thoroughbred maintained on properties in Teresópolis, Rio de Janeiro, Brazil- preliminary results.

Martins, N., Moscarelli Pinto, D., Cozza dos Santos, T., De Ávila Antunes, T., Evaristo, T.A.E., Janczak Torres, A., Kutscher Ripoll, P., Quintana Nizoli, L., 2019. Prevalência de nematódeos intestinais em equinos da região sul do Rio Grande do Sul, Brasil. *Pubvet* 13. <https://doi.org/10.31533/pubvet.v13n12a464.1-5>.

Matthews, J.B., 2014. Anthelmintic resistance in equine nematodes. *Int. J. Parasitol. Drugs Drug Resist.* 4, 310–315. <https://doi.org/10.1016/j.ijpddr.2014.10.003>.

Molento M. B., Pires L. S. A., Dall'Anese J., Yoshitani U. Y., Almeida T. Prevalence and risk factors of gastrointestinal helminths infection in Brazilian horses: A retrospective study of a 12-year (2008-2019) diagnostic data. *Res. Vet. Sci.*, 2024 doi: 10.1016/j.rvsc.2024.105272.

Nielsen, M.K., 2016. Evidence-based considerations for control of *Parascaris* spp. infections in horses. *Equine Vet. Educ.* 28, 224–231. <https://doi.org/10.1111/eve.12536>.

Nielsen, M.K.; Reinemeyer, C.R. *Handbook of Equine Parasite Control*. Hoboken [S. l.]: Wiley, 2018.

Noel, M.L., Scare, J.A., Bellaw, J.L., Nielsen, M.K., 2017. Accuracy and Precision of Mini-FLOTAC and McMaster Techniques for Determining Equine Strongyle Egg Counts. *J. Equine Vet. Sci.* 48, 182-187.e1. <https://doi.org/10.1016/j.jevs.2016.09.006>.

O'Meara, B., Mulcahy, G., 2002. A survey of helminth control practices in equine establishments in Ireland. *Vet. Parasitol.* 109, 101–110. [https://doi.org/10.1016/S0304-4017\(02\)00249-2](https://doi.org/10.1016/S0304-4017(02)00249-2).

Pereira, J.R., Vianna, S.S.S., 2006. Gastrointestinal parasitic worms in equines in the Paraíba Valley, State of São Paulo, Brazil. *Vet. Parasitol.* 140, 289–295. <https://doi.org/10.1016/j.vetpar.2006.03.036>.

Piccoli, C., Marques, S.M.T., Appel, G., Da Silveira, E., Siqueira, G.B., Loos, D.E., Mattos, M.J.T., 2015. Helmintos intestinais em cavalos de trabalho e de lazer de Porto Alegre/RS. *Sci. And Anim. Health* 3, 56. <https://doi.org/10.15210/sah.v3i1.4227>.

Pires, L.S.A., 2024. Parasitismo gastrintestinal em equinos no Brasil: avaliação, riscos e práticas de controle. PhD thesis, Universidade Federal do Paraná, Curitiba, Brazil, 267 pp.

Reinemeyer, C.R., 2009. Diagnosis and control of anthelmintic-resistant *Parascaris equorum*. *Parasit. Vectors* 2. <https://doi.org/10.1186/1756-3305-2-S2-S8>.

Reinemeyer, C.R., 2012. Anthelmintic resistance in non-strongylid parasites of horses. *Vet. Parasitol.* 185, 9–15. <https://doi.org/10.1016/j.vetpar.2011.10.009>.

Ripley, N.E., Gravatte, H.S., Britton, L.N., Davis, S.M., Perrin, G.M., Warner, S., Rexroat, E.K., Vetter, A.L., Maron, E.E.S., Finnerty, C.A., Stanton, V., Nielsen, M.K., 2023. *Parascaris* spp. eggs shedding patterns in juvenile horses. *Vet. Parasitol.* 322. <https://doi.org/10.1016/j.vetpar.2023.110029>.

Rosa, M.H.F., ROCHA, C.M.B.M., GARCIA, A.M., LIMA, I.G., BIIHRER, D.A., FÉLIX, M.B., 2013. Prevalência de helmintos em equinos da raça mangalarga marchador no sul de Minas Gerais / prevalence of helminths in mangalarga marchador horses in the south of Minas Gerais. *Ars Vet,erinaria* 29, 81. <https://doi.org/10.15361/2175-0106.2013v29n4p81>.

Teixeira, W.F.P., Felippelli, G., Cruz, B.C., Maciel, W.G., Fávero, F.C., Gomes, L.V.C., Buzzulini, C., Prando, L., Bichuette, M.A., Lopes, W.D.Z., Oliveira, G.P. de, Costa, A.J. da, 2014. Endoparasites of horses from the Formiga city, located in center-west region of the state of Minas Gerais, Brazil. *Rev. Bras. Parasitol. Vet.* 23, 534–538. <https://doi.org/10.1590/s1984-29612014072>.

Williams, A.R., Andersen-Civil, A.I.S., Zhu, L., Blanchard, A., 2020. Dietary phytonutrients and animal health: regulation of immune function during gastrointestinal infections. *J. Anim. Sci.* 98, skaa030. <https://doi.org/10.1093/jas/skaa030>.

**CHAPTER 3**

## **Cytogenetic Analyses in the Species Identification of *Parascaris* spp.: Detection of *Parascaris univalens* in Zona da Mata, Minas Gerais, Brazil**

Giulia Ornellas Fuzaro Scaléa<sup>1</sup>, Gabrielle Oliveira Soares<sup>1</sup>, Ariany Lacerda Nogueira<sup>2</sup>, Késsia Leite Souza<sup>3</sup>, Mariana Green de Freitas<sup>4</sup>, Artur Kanadani Campos<sup>1\*</sup>

<sup>1</sup>Department of Veterinary, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>2</sup>Department of Veterinary Medicine, Federal University of Juiz de Fora, Minas Gerais, Brazil

<sup>3</sup>Department of Animal Biology, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil

<sup>4</sup>Department of Veterinary Medicine, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil

\*Corresponding author

### **Abstract:**

*Parascaris* spp. is a common ascarid in foals and weanlings, historically attributed to *Parascaris equorum*, but recent findings suggest that *Parascaris univalens* may also be involved. Differentiating between these species requires chromosome counting, as *P. univalens* has one chromosome pair, whereas *P. equorum* has two. This study aimed to differentiate *Parascaris* spp. species in foals from the Zona da Mata region of Minas Gerais, Brazil, using a karyotyping technique adapted for eggs. Fecal samples were collected from 450 foals, and the Mini-FLOTAC technique was used for parasite detection. Positive samples were pooled, and eggs were recovered using flotation and centrifugation techniques. The eggs underwent a decortication process to remove the outer layers, facilitating chromosome visualization. To optimize chromosome analysis, colchicine was used to halt mitosis, and the eggs were stained with Giemsa and DAPI for microscopic examination. Only eggs from Stud farm 12 were suitable for karyotyping, revealing a single pair of chromosomes consistent with *P. univalens*, confirming this species as the sole ascarid present in the study area. This finding aligns with previous studies in Europe and North America, challenging the long-

held belief that *P. equorum* is the predominant species. This study marks the first application of karyotyping for *Parascaris spp.* eggs in Brazil, providing a valuable epidemiological tool for species identification and aiding in the development of targeted control measures. The protocol's adaptation and successful implementation emphasize its relevance in veterinary parasitology.

**Keywords:** Ascarid; Chromosome; Mini-FLOTAC; Precision.

**Highlights:**

- *Parascaris spp.* is an ascarid of great importance in equine veterinary parasitology, especially in foals;
- The karyotyping technique is effective for determining the infecting species of the parasite;
- This is the first time the technique has been reproduced in Brazil, following a protocol for egg recovery from feces.

## 1. Introduction

In horses, *Parascaris spp.* is a ascarid common in foals and weanlings (Clayton and Duncan, 1979). Often, this parasitosis is associated with *Parascaris equorum* species, however, some authors mention that is not the unique considered pathogenic, *Parascaris univalens* can be other species related to the disease. For species differentiation, it is necessary to count the chromosome present in the parasite. As the name indicates, *P. univalens* has a pair of chromosomes and *P. equorum* has two pairs (Biocca et al., 1978).

Two techniques are used for differentiating the species of *Parascaris spp.*, karyotyping (Goday and Pimpinelli, 1984) or some molecular approach, as protein electrophoresis (Bullini et al., 1978). The karyotyping technique consist in fix the mitotic metaphase chromosomes from the early embryos or gonadal germ cells in their mitotic cell division and verified the presence of one, or two, chromosome under microscopy (Goday and Pimpinelli, 1984).

The first study to perform karyotyping on *Parascaris spp.* eggs present in feces was described by Nielsen et al. (2014), who adapted the technique to identify the chromosome number during the first mitotic division, allowing distinction between *P.*

*univalens* and *P. equorum*. Later, Zhu et al. (2023), in China, applied the same methodology and confirmed its effectiveness for species differentiation, reinforcing the reliability of the technique. Overall, the adapted karyotyping method is considered useful for accurate species identification, although it requires precise timing to collect eggs at the appropriate mitotic stage and access to specialized laboratory equipment.

The objective of this study was to use the karyotyping technique performed by Nielsen et al. (2014), adapted for karyotyping in eggs of this worm, in order to differentiate the *Parascaris* spp. species found in the Zona da Mata of Minas Gerais region in Brazil.

## **2. Materials and Methods**

### **2.1 Faecal samples**

The animal experiment was approved by the University of Viçosa's Institutional Animal Care and Use Committee (CEUA), under protocol number 16/2024.

Faecal samples were collected directly from the rectum of 450 foals under 24 months old, sourced from various properties within the ZMM region. The samples were packed in oxygen free bags and transported in refrigerated thermal boxes to the parasitology laboratory of UFV. Upon arrival, the samples were immediately analyzed and the Faecal Eggs Count (FEC) were carried out for each horse using MiniFLOTAC technique, with a theoretical sensitivity of 5 Eggs Per Gram (EPG). Ascarid eggs in faecal samples (5 g) were floated using 45mL of the saturated Sodium Nitrate ( $\text{NaNO}_3$ ) (SG = 1.2) (Cringoli et al., 2017, 2013).

### **2.2 Recovery of eggs**

A pool of positive samples from each property was prepared. Each pool consisted of approximately 200 g of feces, which were washed under running water and sieved (Bertel Indústria Metalúrgica Ltda ®). The initial sieving was performed using a 40-mesh screen (425  $\mu\text{m}$ ), followed by a second sieving with a 270-mesh screen (53  $\mu\text{m}$ ). The suspension was collected in 10 mL tubes and centrifuged at 2,000 g for 5 minutes, after which the supernatant was discarded. The tube contents were resuspended, and 10 mL of  $\text{NaNO}_3$  solution was carefully added to the bottom of the tube. The tube was then centrifuged again at 2,000 g for 5 minutes. Subsequently, 2

mL of the flotation suspension from the upper layer of each tube was carefully removed and transferred to a new container. The new tubes were filled with cold distilled water and centrifuged at 2,000 g for 5 minutes. The supernatant was discarded, and the sediment containing the eggs was resuspended; this washing procedure was repeated twice. During the final wash, the eggs were resuspended and were examined under a trinocular optical microscope (10x) (Kasvi® model K223), performing three egg counts in 10 µl of the suspension. After the three counts, the average egg quantification was calculated and the tubes were stored under refrigeration.

### **2.3 Karyotyping technique adapted to *Parascaris* spp. eggs identification**

For decortication, 6-8 mL of 0.5 M Sodium Hydroxide (NaOH) was added to each tube containing the egg suspension and maintained at room temperature for 60 minutes, with agitation two to three times during this period. Subsequently, the eggs were washed three times with cold distilled water and centrifuged at 2,000 g for 5 minutes.

Following this, 6-8 mL of 0.4 M Potassium Hydroxide (KOH) was added to the suspension and maintained at room temperature for 30 minutes, with gentle agitation twice during this period. Subsequently, the eggs were again washed three times with cold distilled water and centrifuged at 2,000 g for 5 minutes.

The solution was then treated with 6% Hypochlorite and 0.4 M KOH (17:83%) for 60 minutes at room temperature, with periodic resuspension of the solution two to three times. Finally, after this last treatment, the eggs were washed three times and centrifuged under the same previous conditions. The eggs were subsequently resuspended in 0.7% saline solution (NaCl).

The egg solution was examined under trinocular optical microscopy (10x) (Kasvi® K223), performing three egg counts in 10 µL of the suspension. After the three counts, the mean egg quantification was calculated, and the suspension was incubated at 37°C in Petri dishes.

The eggs were incubated for approximately 24 hours, with observations after 12 hours to verify the stage of embryonic mitotic development. Before initiating egg fixation, 1000 µL of colchicine (0.0125%) was added for 60 minutes, as the acquisition

of chromosomes in mitosis is generally achieved by treating cells with tubulin inhibitors, such as colchicine (Bickmore, 2001).

To compare the methodology adapted by our laboratory with that developed by Nielsen et al. (2014), a slide was also prepared without the use of colchicine. The application of colchicine, although not employed in Nielsen's original methodology, was adjusted to optimize the acquisition of chromosomes suitable for karyotyping.

Subsequently, the eggs were fixed to stabilize them in the metaphase of mitosis by adding 1000  $\mu$ L of fixative - a mixture of methanol and acetic acid (3:1) (Carnoy's Solution) - and maintained for one hour at room temperature. The tubes were then centrifuged at 14,000 g for 10 minutes, after which the supernatant was carefully removed to avoid discarding the pellet, and the tubes were refilled with fixative followed by another centrifugation. This step was repeated once more, discarding the supernatant. Subsequently, the pellet was resuspended in 1.5 mL of fixative, transferred to a microtube, and maintained under refrigeration for future analyses.

For slide preparation, a drop of the suspension containing the parasite eggs was added to the surface of a glass slide and covered with a coverslip. Manual pressure was then applied to the coverslip on the slide for one minute. Following this process, the slide was immersed in liquid nitrogen for one minute using forceps. It was then left to rest at room temperature for approximately one minute until the removal of the upper coverslip; these processes aim to rupture the *Parascaris* spp. egg shells. The slides were then stained with 10% Giemsa solution (Giemsa stock solution diluted in phosphate buffer pH 6.8) at a 1:9 ratio, leaving the slides submerged for five minutes and subsequently washing under running water, thus enabling visualization under optical microscopy using a photomicroscope (Olympus BX53®) with camera (Olympus DP73®). For fluorescence microscopy, the slide was stained with the fluorescent marker DAPI (4',6-diamidino-2-phenylindole) and observed under a fluorescence photomicroscope (Olympus BX53®) with camera (Olympus XM10®), using a coverslip over the slide. Fluorescence was observed for only a few seconds.

## 2.5 Species identification

The prepared and stained slides were examined under a Fluorescence photomicroscope (Olympus BX53®) with camera (Olympus XM10®) to identify the early mitotic phases of embryonic divisions, as metaphase is the most suitable for

counting the number of chromosomes. All slides were carefully examined to clearly and unmistakably identify the chromosomes. Only with a detailed analysis was it possible to find viable *Parascaris* spp. eggs with visible chromosomes. Ten viable *Parascaris* spp. eggs were counted and used for species differentiation.

### **3. Results**

After recovery, it was observed that the *Parascaris* spp. eggs still had their outer layers intact. Additionally, the samples contained approximately 17 to 45 eggs per 10  $\mu$ l, although four stud farms showed no eggs during recovery (Table 1). Despite the presence of *Parascaris* spp. eggs in the Fecal Egg Count (FEC) test from the samples of 6, 7, 13, and 15 Stud Farms, it was not possible to recover the eggs due to the collection technique and recovery protocol not being fully standardized among those involved, in addition to the extended refrigeration time of the samples, which exceeded that of the other samples used in the study. After decortication, the outer layers of the eggs were removed, facilitating the visualization of the genetic material. Incubating the suspension at 37°C in Petri dishes allowed the embryo to enter mitosis (Figure 5).

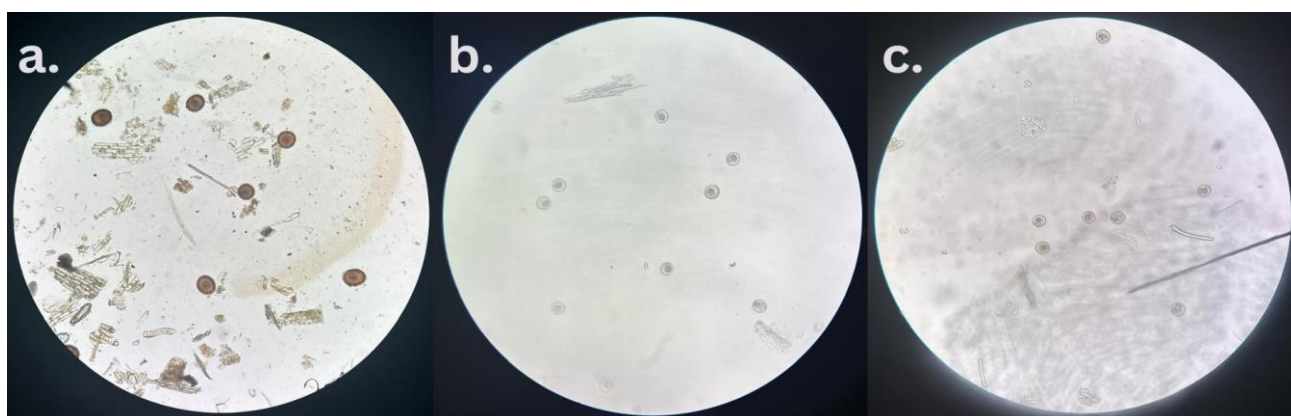


Figure 6: Processes for recovering *Parascaris* spp. eggs. a. Recovered eggs post-wash; b. Eggs after the decortication protocol; c. Eggs of *Parascaris* spp. after 24 hours of incubation at 37 °C in Petri dishes

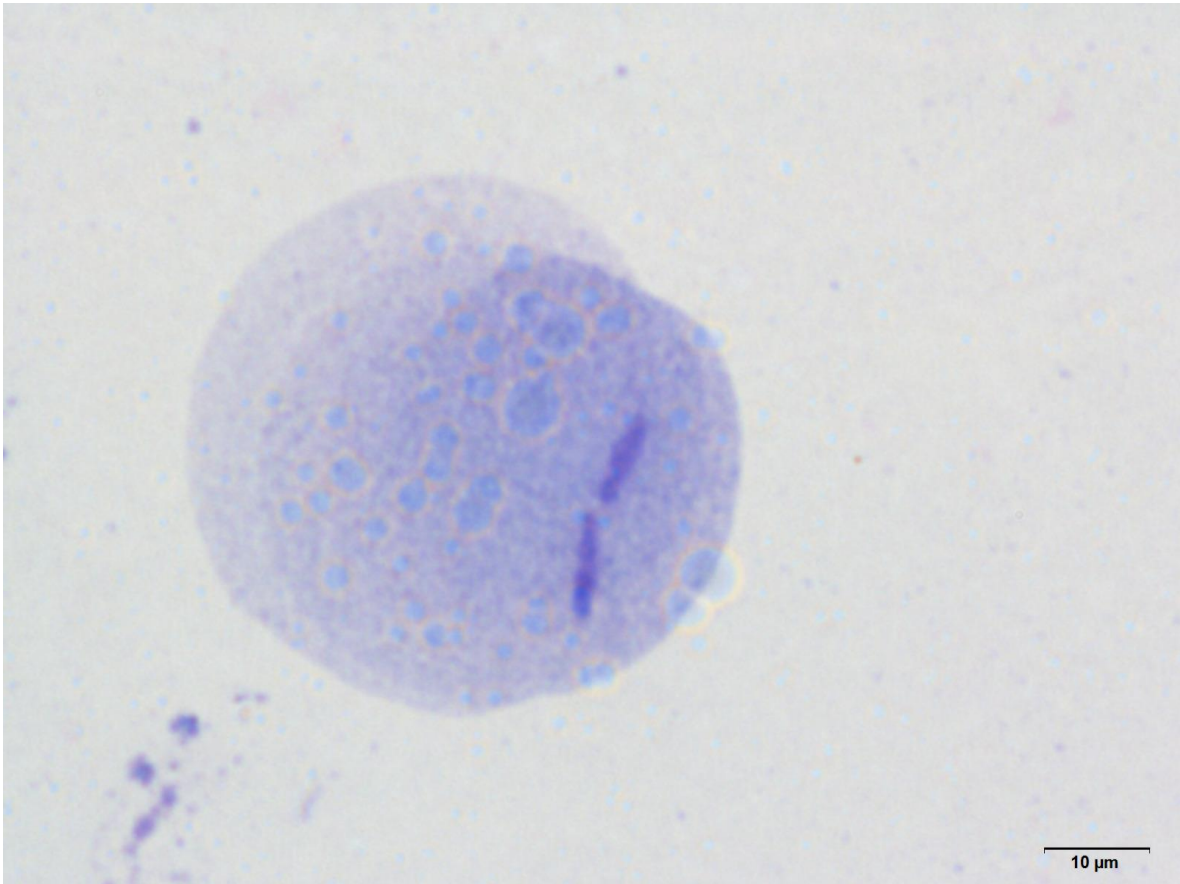
Table 7: Mean egg count of *Parascaris* spp. in 10  $\mu$ l of the solution after the karyotyping protocol in animals from different stud farms in the Zona da Mata of Minas Gerais region

Property names	Mean egg count in 10 $\mu$ l of the recovery solution	Mean egg count in 10 $\mu$ l of the decortication solution
Stud farm 1	Negative	-
Stud farm 2	37	16
Stud farm 3	24	9
Stud farm 4	Negative	-
Stud farm 5	17	11
Stud farm 6	Insufficient	-
Stud farm 7	Insufficient	-
Stud farm 8	42	16
Stud farm 9	27	11
Stud farm 10	Negative	-
Stud farm 11	27	18
Stud farm 12	45	27
Stud farm 13	Insufficient	-
Stud farm 14	42	20
Stud farm 15	Insufficient	-

In addition to decortication, other techniques to weaken the shell of *Parascaris* spp. eggs were useful in allowing access to the genetic material. These included manual pressure and freezing with liquid nitrogen (-196°C), which causes a rapid contraction of the inner and outer layers of the egg, facilitating the penetration of the dye into the interior of the egg. All viable eggs for chromosomal evaluation were obtained from slides prepared with colchicine. Colchicine is used to block mitosis, promoting the accumulation of cells in the metaphase stage, when the chromosomes are more condensed and visible, thereby facilitating their analysis.

After coproparasitological examinations, the egg was identified in infected animals from different stud farms, as described in Table 1. However, only at Stud farm 12 were ten viable eggs found for karyotyping, as they exhibited visible chromosomes after the decortication, fixation, and staining processes described in the protocol. In the other stud farms, all steps of the procedure were performed, but despite the efforts, satisfactory results for chromosome observation could not be achieved.

All the eggs examined in microscopy were identified as *P. univalens* (10 eggs), the karyotyping protocol was successful and generated useful images of mitotic chromosomes from early embryonic cell division. Figure 2 shows a single pair of chromosomes in the mitotic anaphase stage, and represents the only species present in the Zona da Mata of Minas Gerais region stained with Giemsa. Figure 3 also shows *Parascaris univalens*, due to the presence of only one pair of chromosomes, following the methodology of Nielsen et al. (2014), stained with the fluorophore DAPI.



*Figure 7: Giemsa stained metaphase of Parascaris univalens egg at first mitotic division showing a single pair of chromosome at anaphase in a 100x magnification*

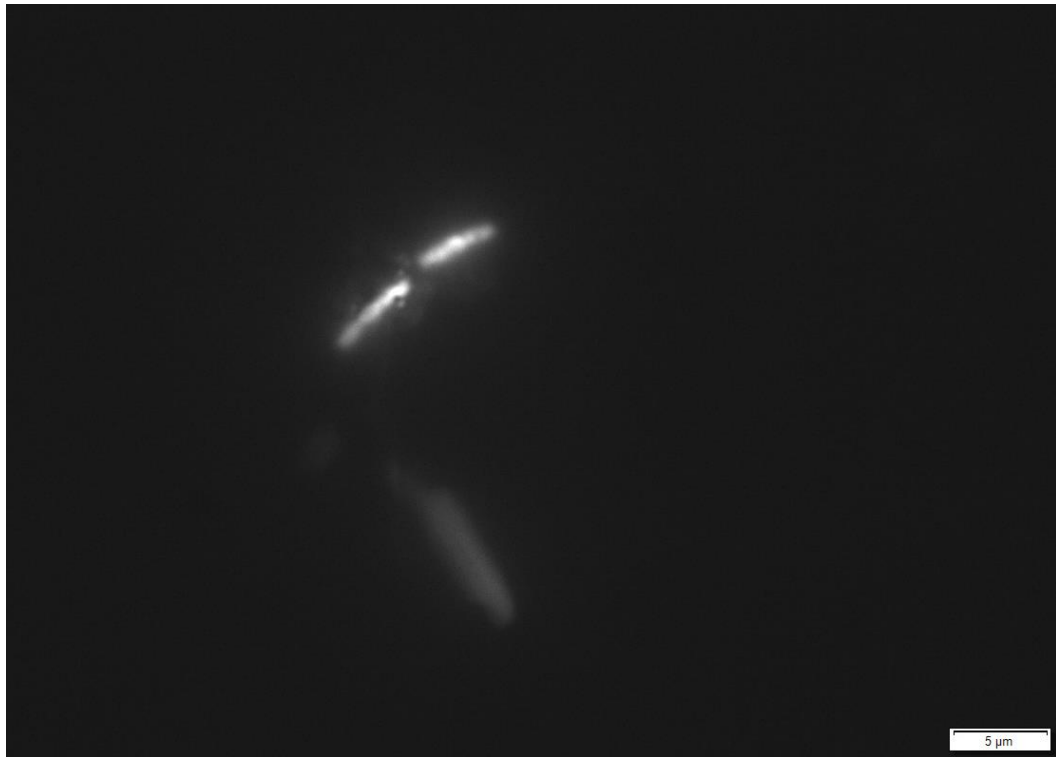


Figure 8: Fluorophore DAPI (4',6'-diamino-2-fenil-indol) stained metaphase of *Parascaris univalens* egg at first mitotic division showing a single pair of chromosome at anaphase in a 100x magnification

#### 4. Discussion

In this study, we present insights into this parasitosis in horses, with results indicating that *P. equorum* is not the predominant ascarid species. Instead, the primary species identified was *P. univalens*. This finding aligns with the results reported by Nielsen et al. (2014). Similarly, in Italy, *P. univalens* was identified as the main ascarid species within herds (Bullini et al., 1978).

In addition to species-level identification, it was possible to carry out the karyotyping protocol on *Parascaris* spp. eggs. What can be observed is that, although the protocol by Nielsen et al. (2014) demonstrates the use of DAPI, it was also possible to use Giemsa (Ford and Hamerton, 1956; Pimpinelli and Goday, 1989; Zhou et al., 2023), which is widely used in cytogenetic assays for chromosomal evaluation. The protocol standardized with Giemsa becomes more accessible due to the lower cost of the dye and its availability in laboratories.

Furthermore, the previously provided description outlines the process for breaking the walls of the parasite's eggs, emphasizing the need for efficient decortication, manual pressure of the coverslip against the glass slide, followed by

rapid freezing in liquid nitrogen. This demonstrates the thickness and rigidity of the outer layers of the ascarid egg (Nielsen et al., 2014).

Another important step in this process is the use of colchicine to inhibit the formation of the mitotic spindle, which prevents the separation of chromosomes during mitosis, causing the chromosomes to remain predominantly in metaphase, thereby facilitating their visualization and counting. Nielsen et al. (2014) do not describe colchicine as responsible for this process, but in cytogenetic analyses, this substance is commonly used (Ford and Hamerton, 1956; Guerra and Souza, 2002). In this study, we used colchicine, and this substance proved effective in halting mitosis in *Parascaris* spp. eggs, allowing for chromosomal identification and counting. Colchicine played a critical role in the quality of the karyotypic analyses.

Moreover, cytogenetic studies are important tools for the identification of *Parascaris* spp. species, and can assist in epidemiological monitoring and in the detection of genetic patterns related to anthelmintic resistance (Zhou et al., 2023).

This study is the first to perform karyotyping of *Parascaris* spp. eggs in Brazil. The importance of this work lies in the replication of the karyotyping protocol previously described by Nielsen et al. (2014), with adaptations. Considering the recent repetition of this protocol in China (Zhou et al., 2023) and now in Brazil, the relevance and applicability of this methodology in parasitology are highlighted.

The standardization of the protocol is crucial for enabling the antemortem identification of species and assessing their prevalence, the presence or absence of co-infections, variations in pathogenic impact, and resistance profiles to anthelmintics (Reinemeyer, 2009).

## **5. Conclusion**

Karyotyping is an excellent tool for differentiating *Parascaris* spp. species in eggs obtained from fecal samples of parasitized animals. This information facilitates the characterization of species occurrence in the studied region and enables the identification of potential associations with pathogenicity, drug resistance, and the determination of co-infections in equine herds.

## **6. Funding**

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de

Minas Gerais (FAPEMIG) and Conselho Nacional de Desenvolvimento Tecnológico (CNPq).

### **9. Acknowledgements**

The authors are grateful to the support provided by Federal University of Viçosa and the Laboratory of Veterinary Parasitology.

### **10. Author contribution**

Giulia Ornellas Fuzaro Scaléa: Writing – original draft; Data curation; Formal analysis; Methodology.

Gabrielle Oliveira Soares: Writing – original draft; Data curation; Formal analysis; Methodology.

Ariany Lacerda Nogueira: Writing – original draft; Data curation.

Késsia Leite Souza: Writing – original draft; Data curation.

Mariana Green de Freitas: Writing – review and editing; Project administration; Methodology.

Artur Kanadani Campos: Writing – review and editing; Project administration; Methodology.

### **7. Declaration of interest**

The authors declare no conflict of interest.

### **8. References**

Bickmore, W.A., 2001. Karyotype Analysis and Chromosome Banding, in: Encyclopedia of Life Sciences. Wiley. <https://doi.org/10.1038/npg.els.0001160>.

Biocca E, Nascetti G, Iori A, Constantini R, Bullini L, 1978. Descrizione di *Parascaris univalens*, parassita degli equine, e suo differenziamento da *Parascaris equorum*. Atti Accad. Naz. Lincei: Cl Sci. Fis., Mat. Nat. 65(3–4)133–140.

Bullini L, Nascetti G, Carrè S, Rumore F, Biocca E, 1978. Ricerche cariologiche ed elettroforetiche su *Parascaris univalens* e *Parascaris equorum*. Atti Accad. Naz. Lincei: Cl. Sci. Fis., Mat. Nat. Serie 8 151–156.

Clayton, H.M., Duncan, J.L., 1979. The development of immunity to *Parascaris equorum* infection in the foal. Res. Vet. Sci. 26, 383–384. [https://doi.org/10.1016/S0034-5288\(18\)32899-6](https://doi.org/10.1016/S0034-5288(18)32899-6).

Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruyse, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12, 1723–1732. <https://doi.org/10.1038/nprot.2017.067>.

Cringoli, G., Rinaldi, L., Albonico, M., Bergquist, R., Utzinger, J., 2013. Geospatial (s)tools: integration of advanced epidemiological sampling and novel diagnostics. Geospat. Health 7, 399. <https://doi.org/10.4081/gh.2013.97>.

Ford, C.E., Hamerton, J.L., 1956. A Colchicine, Hypotonic Citrate, Squash Sequence for Mammalian Chromosomes. Stain. Technol. 31, 247–251. <https://doi.org/10.3109/10520295609113814>.

Goday, C., Pimpinelli, S., 1984. Chromosome Organization and Heterochromatin Elimination in *Parascaris*. Science (1979) 224, 411–413. <https://doi.org/10.1126/science.224.4647.411>.

Guerra, M., Souza, M.J. de, 2002. Como observar cromossomos: Um Guia de Técnicas em Citogenética Vegetal, Animal e Humana. Ribeirão Preto.

Nielsen, M.K., Wang, J., Davis, R., Bellaw, J.L., Lyons, E.T., Lear, T.L., Goday, C., 2014. *Parascaris univalens*—a victim of large-scale misidentification? Parasitol. Res. 113, 4485–4490. <https://doi.org/10.1007/s00436-014-4135-y>.

Pimpinelli, S., Goday, C., 1989. Unusual kinetochores and chromatin diminution in *Parascaris*. Trends in Genetics 5, 310–315. [https://doi.org/10.1016/0168-9525\(89\)90114-5](https://doi.org/10.1016/0168-9525(89)90114-5).

Reinemeyer, C.R., 2009. Diagnosis and control of anthelmintic-resistant *Parascaris equorum*. Parasit. Vectors 2. <https://doi.org/10.1186/1756-3305-2-S2-S8>.

Zhou, M., Lu, Y., Han, L., Lu, M., Guan, C., Yu, J., Liu, H., Chen, D., Li, H., Yang, Y., Zhang, L., Tian, L., Liu, Q., Hou, Z., 2023. Exploration of *Parascaris* species in three different Equus populations in China. Parasit. Vectors 16, 202. <https://doi.org/10.1186/s13071-023-05768-3>.

## **ATTACHMENT I**

### **History and Questionnaire**

The animals' history and all necessary information regarding the property of origin were obtained from data collected through a questionnaire registered in Plataforma Brasil, under protocol number 5.532.592 of the Research Ethics Committee (REC). The questionnaire contains the following questions:

1. Animal's name/identification marking;
2. Age;
3. Breed;
4. Sex;
5. What type of establishment?;
6. How many animals are there on the property?;
7. What is the purpose/sport of these animals?;
8. Where do the animals live?;
9. What is the stocking density of this location?;
10. What is these animals' meals?;
11. Which is the water source provided?;
12. How and how frequently are the stalls cleaned and manure managed?;
13. Which anthelmintics have been used in the last five years?;
14. What are the criteria for selecting anthelmintics?;
15. What is the interval between treatments?;
16. How is the treatment administered?;
17. How is the dosage determined, are the animals weighed, and if so, how?;
18. Has any drug efficacy testing been performed?;
19. Do the animals show any signs of helminth infection, and if so, which ones?