

SENSITIVITY AND SPECIFICITY OF THE FAUST *ET AL.* AND WILLIS-MOLLAY METHODS IN THE DIAGNOSIS OF INTESTINAL PARASITES IN SHELTER DOGS

Sensibilidade e especificidade dos métodos Faust et al. e Willis-Mollay no diagnóstico de parasitas intestinais em cães de ONGs

Carolina Dantas Micheletti*¹, Bruna Pan dos Santos¹, Mauro Citro Lalucci², Mariana Ramos de Andrade Beraldo³

*Corresponding author: Carolina Dantas Micheletti, Via Guilherme Dibbern, nº 4015, Jardim Florença, Limeira, SP, Brazil. CEP: 13482-217.
E-mail: carolinadantas.vet@gmail.com

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Abstract

Brazil recorded approximately 8.8 million vulnerable animals between 2018 and 2020, overwhelming NGOs that face limited resources and precarious sanitary management. This scenario favors the spread of parasitic diseases, particularly those caused by pathogens such as *Ancylostoma spp.* and *Trichuris sp.* A study conducted in shelters in São João da Boa Vista (SP) and the surrounding region identified *Ancylostoma spp.* as the most prevalent parasite (50.98%). When comparing the Willis-Mollay and Faust *et al.* diagnostic methods, Kappa statistical analysis revealed poor to fair agreement between the techniques. These findings demonstrate that the combined use of diagnostic methods is essential to ensure greater accuracy in parasite detection. The results reinforce the urgency of implementing effective sanitary protocols, including periodic deworming and environmental

- 1 Undergraduate student in the Veterinary Medicine program at the Octávio Bastos Teaching Foundation University Center (Unifeob), São João da Boa Vista, SP, Brazil.
- 2 Master's student in the Statistics program at the Federal University of Lavras (UFLA), Lavras, MG, Brazil.
- 3 Faculty member in the Veterinary Medicine program at the Octávio Bastos Teaching Foundation University Center (Unifeob), São João da Boa Vista, SP, Brazil.



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management measures. The objective of this study is to compare the sensitivity and specificity of Faust *et al.* and Willis-Mollay methods and differentiate them in the diagnosis of parasites in shelter dogs.

Keywords: *Ancylostoma spp.*; dogs; endoparasites; fecal parasite examination; intestinal inflammation.

Resumo

O Brasil registrou cerca de 8,8 milhões de animais vulneráveis entre 2018 e 2020, sobrecarregando ONGs com recursos limitados e manejo sanitário precário. Esse cenário favorece a disseminação de doenças parasitárias, com destaque para patógenos como *Ancylostoma spp.* e *Trichuris sp.* Um estudo realizado em abrigos de São João da Boa Vista (SP) identificou o *Ancylostoma spp.* como o parasita mais prevalente (50,98%). Ao comparar os métodos de diagnóstico Willis-Mollay e Faust *et al.*, a análise estatística de Kappa revelou uma concordância de fraca a razoável. Isso demonstra que a utilização de técnicas combinadas é essencial para garantir maior precisão diagnóstica. Os resultados reforçam a urgência de protocolos sanitários eficazes, incluindo vermifugação periódica e controle ambiental. O objetivo deste estudo é comparar a sensibilidade e especificidade dos métodos Faust *et al.* e Willis-Mollay e diferenciá-los no diagnóstico de parasitas em cães de ONGs.

Palavras-chave: *Ancylostoma spp.*; cães; endoparasitas; exame coproparasitológico; inflamação intestinal.

Introduction

The confinement of dogs in shelters and non-governmental organizations (NGOs), often characterized by high population density and uncertain health histories (Hagiwara, 2019), creates an environment conducive to the spread of endoparasitic infections (Larson; Newbury; Schultz, 2009). The absence of strict deworming protocols and inadequate environmental management in these settings increases the prevalence of helminths and protozoa, many of which have zoonotic potential and pose risks to public health (Garcia; Calderón; Brandespim, 2019; Silva, 2023).

In this context, fecal parasitological examination (FPE) stands out as the gold standard for diagnosing these parasitic infections in dogs and cats (Antunes, 2020). This test enables the detection of helminth eggs and larvae, as well as protozoan cysts and oocysts, providing accurate analysis for monitoring intestinal parasites (Ferreira *et al.*, 2020). However, diagnostic efficacy is directly dependent on the technique employed, as different methods vary in detection sensitivity according to the density and morphology of parasitic forms (eggs, larvae, cysts, or oocysts) (Ferreira *et al.*, 2020). Although methods such as those of Faust *et al.* and Willis-Mollay are widely used, the choice between them may significantly influence the accuracy of intestinal parasite monitoring in shelter populations (Buzatti *et al.*, 2023). The comparative performance of these techniques, particularly when evaluated individually under conditions of high parasitic load, still requires further investigation to validate their diagnostic efficiency in collective screening contexts (Antunes, 2020; Buzatti *et al.*, 2023).

Thus, the objective of this study is to investigate the sensitivity and specificity of the coproparasitological methods of Faust *et al.* and Willis-Mollay in detecting endoparasitic infections in dogs housed in NGOs in the region of São João da Boa Vista (SP). The study aims to assess the comparative effectiveness of these diagnostic methods, identify the main intestinal parasites present in these animals, and determine the diagnostic limitations of each technique when used independently.

Methodology

This study was approved by the Animal Use Ethics Committee (Ceua) of the Octávio Bastos Teaching Foundation University Center (Unifeob), under protocol No. 2024.31, in accordance with Law No. 11,794/2008 (available in the Appendix). The sample comprised 51 mixed-breed dogs of both sexes, of varying ages, and without official records, originating from two animal protection shelters located in the municipalities of Aguaí (SP) and São João da Boa Vista (SP). Sampling was conducted in three stages, including 11 animals from the NGO Projeto Jurema and 40 animals from Associação Pet Feliz. Fecal samples ($n \geq 6$ g) were obtained either through spontaneous defecation or mechanical collection via digital rectal stimulation. In the latter case, the digit was inserted cranially approximately 3 cm, using procedure gloves lubricated with water-soluble gel to ensure mucosal integrity and animal welfare. The material was stored in sterile universal containers and transported under refrigeration (4 °C to 8 °C) to the Propedeutics Laboratory of the Unifeob Veterinary Center.

In the laboratory, each sample was fully processed using both the Faust *et al.* and Willis-Mollay methods. Statistical analyses were performed using R software (version 4.5.2), with the *irr* package employed to calculate the Kappa index. A significance level of 5% ($\alpha = 0.05$) was adopted for all tests. Contingency tables (2×2) were constructed based on dichotomous classifications (positive/negative) obtained from each diagnostic method and were used to perform chi-square (χ^2) tests and calculate the Kappa agreement index.

It is noteworthy that the study did not employ an independent diagnostic gold standard. Analyses were therefore conducted through direct comparison between the methods to assess agreement. Consequently, sensitivity and specificity estimates were calculated conditionally, alternately considering each method as the technical reference. These metrics were not interpreted as absolute measures of diagnostic accuracy but rather as indicators of relative performance between the compared techniques, acknowledging the intrinsic limitations associated with the absence of an absolute reference in the experimental design.

Objectives

The objective of this study was to evaluate diagnostic agreement and the relative performance of the coproparasitological methods of Faust *et al.* and Willis-Mollay in identifying parasitic structures in dogs from NGOs. To this end, 51 fecal samples ($n \geq 6$ g) were analyzed, with sensitivity and specificity estimated conditionally, as well as the detection capacity of different parasites by each technique. Additionally, the study sought to verify the applicability and comparative efficiency of both methods in routine laboratory screening for animal rescue and protection environments.

Results and Discussion

The present study aimed to evaluate the sensitivity and specificity of the coproparasitological methods of Faust *et al.* and Willis-Mollay, as well as the prevalence of endoparasitic infections in dogs housed in NGOs in the region of São João da Boa Vista (SP). Fecal samples from 51 dogs were analyzed, collected either directly or through rectal manipulation. When interpreting the sensitivity indices obtained, it is important to consider the inherent biases associated with the sampling techniques. Rectal manipulation, although ensuring individual sample origin, may yield a reduced fecal volume, which can occasionally compromise the detection of low parasitic loads. Conversely,

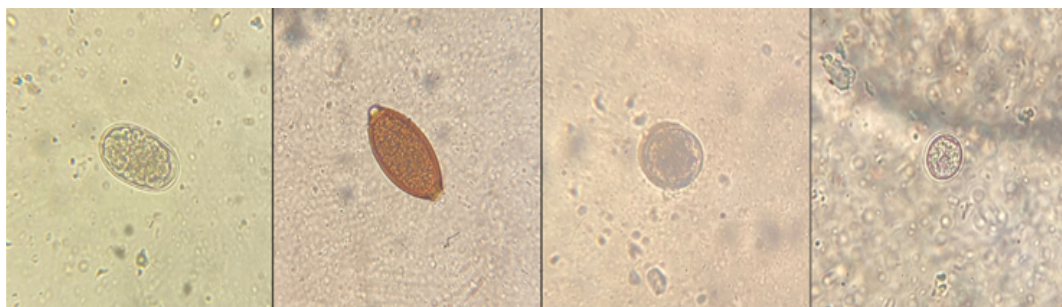
spontaneous collection, while providing a larger volume, is subject to external contamination and degradation of sensitive eggs and oocysts (Saldanha-Elias *et al.*, 2019; Monteiro, 2017). This variability in sample collection may have influenced the observed sensitivity, particularly for protozoa whose oocysts are shed intermittently.

Additionally, it is important to note that the sample size of this study (n = 51) represents a specific subset of a population under vulnerable conditions. Although the number of animals reflects the reality of regional shelters, further studies involving larger populations and serial sampling could refine the specificity parameters observed, particularly for parasites with intermittent shedding, such as *Cystoisospora spp.* and *Toxocara canis*.

The fecal samples were processed using the Willis-Mollay and Faust *et al.* laboratory methods, enabling the identification and comparison of different intestinal parasites. These coproparasitological techniques are widely used for the detection of intestinal parasites in feces. The Willis-Mollay method is based on flotation in a saturated solution and is effective in recovering helminth eggs. In contrast, the Faust *et al.* method employs centrifugation in a high-density solution, allowing greater sensitivity, including for the detection of protozoan oocysts.

The following parasites were identified: *Ancylostoma spp.*, *Toxocara canis*, *Trichuris sp.*, and *Cystoisospora canis* (Figure 1). These agents are commonly found in dogs and may cause conditions ranging from mild gastrointestinal disturbances to systemic disease, in addition to presenting zoonotic potential (Monteiro, 2017).

Figure 1 – Eggs of canine intestinal endoparasites



T Source: Micheletti *et al.* (2024).

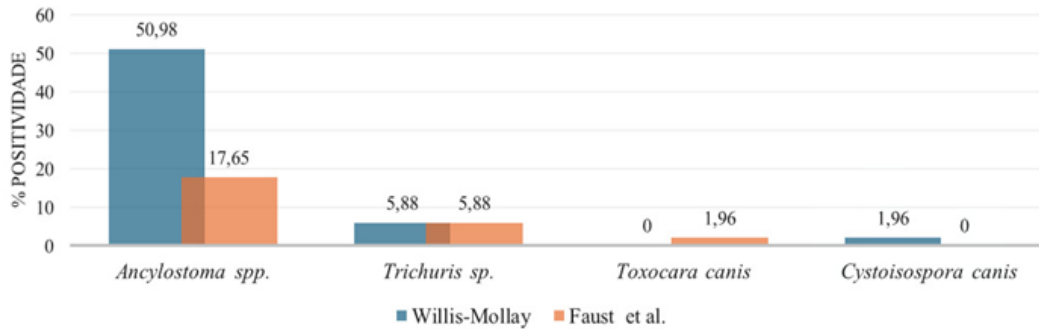
T Note: A) *Ancylostoma spp.*, B) *Trichuris sp.*, C) *Toxocara canis*, D) *Cystoisospora canis*.

In the comparison between the Faust *et al.* and Willis-Mollay methods, the Willis-Mollay method showed a higher detection rate for *Ancylostoma spp.* (50.98%) compared to Faust *et al.* (17.65%), initially suggesting greater sensitivity for this parasite (Ferreira *et al.*, 2020). This discrepancy may be attributed to the density of the saturated sodium chloride solution used in Willis-Mollay, which favors the flotation of hookworm eggs, whereas the centrifugation process in the Faust *et al.* method, although effective for cyst recovery, may sometimes distort or rupture thinner-shelled eggs (Monteiro, 2017). Furthermore, the predominance of *Ancylostoma spp.* observed in this study can also be explained by the tropical climatic conditions of the analyzed region. High temperatures and humidity favor rapid egg maturation and the survival of L3 larvae in the environment (Monteiro, 2017). According to Garcia, Calderón, and Brandespim (2019), soil in environments with high animal density, combined with tropical climate conditions, acts as a reservoir of infective forms, facilitating the occurrence of syndromes such as cutaneous and visceral larva migrans in humans who handle these environments.

For *Trichuris sp.*, both methods showed the same positivity rate (5.88%), but with low agreement between them. This phenomenon is explained by the discontinuous or intermittent

shedding characteristic of this parasite's biological cycle, reinforcing that a single negative test does not exclude infection. In the case of *Toxocara canis*, detection occurred exclusively with the Faust *et al.* method (1.96%), whereas *Cystoisospora spp.* was identified only by the Willis-Mollay method (1.96%), highlighting differences in method sensitivity across parasite types (Figure 2).

Figure 2 – Detection frequency by method



T Source: Micheletti *et al.* (2024).

T Note: Bar chart illustrating detection frequency by method.

These variations demonstrate that methodological choice is not merely a laboratory preference but a strategic decision in screening processes. As highlighted by Ferreira *et al.* (2020), the use of multiple methods is essential to avoid false negatives in at-risk populations, where underdetection of a single infected animal may result in continued environmental contamination (shedding) and failure of collective deworming protocols (Ramires; Brito; Santarém, 2019).

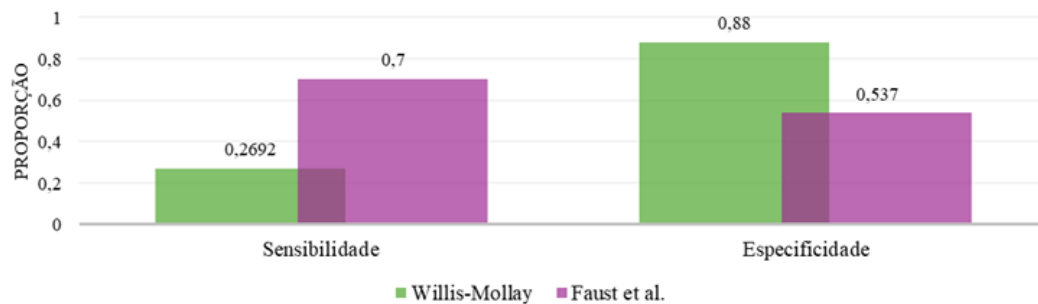
The comparison between methods also revealed important variations in terms of agreement and sensitivity. To assess the degree of agreement, the Kappa index was used, which measures the extent to which two observers (or methods) agree beyond what would be expected by chance. For *Trichuris sp.*, weak but statistically significant agreement at the 5% level was observed (Kappa = 0.292; $p = 0.0373$), indicating that both methods tend to detect this parasite similarly. For *Ancylostoma spp.*, agreement was also weak but not statistically significant (Kappa = 0.187; $p = 0.0764$), suggesting inconsistencies between the methods.

Additionally, the chi-square test was applied to assess whether there was a statistically significant difference between the proportions of positive results obtained by the different methods. This test indicated significant differences between the methods in the detection of *Toxocara canis* and *Cystoisospora spp.* ($p < 0.05$), suggesting that one method may be more sensitive or effective for these parasites.

Statistical analyses were performed using R software (version 4.5.2), with the *irr* package employed to calculate the Kappa index. A significance level of 5% ($\alpha = 0.05$) was adopted. Contingency tables (2×2) were constructed based on dichotomous classifications (positive/negative) obtained from each diagnostic method and were used for both chi-square tests and Kappa index calculation.

Furthermore, the Faust *et al.* method demonstrated higher sensitivity (70%), making it more effective in identifying intestinal parasites, whereas the Willis-Mollay method showed higher specificity (88%), implying a lower occurrence of false positives. These findings indicate that the Faust *et al.* method is more suitable for screening and initial diagnosis, while the Willis-Mollay method may be more appropriate for confirming negative results, particularly in contexts requiring high specificity (Figure 3).

Figure 3 – Sensitivity and specificity of the methods



T Source: Micheletti *et al.* (2024).

T Note: Bar chart illustrating the sensitivity and specificity of the methods.

The lower frequency of *Trichuris sp.*, along with the detection of *Toxocara canis* and *Cystoisospora canis*, highlights the importance of a comprehensive approach to sanitary management and the implementation of continuous control measures (Saldanha-Elias *et al.*, 2019). The adoption of protocols based on the 2025 guidelines of the Companion Animal Parasite Council (CAPC), combined with rigorous environmental management—such as daily removal of feces, application of moist heat to inactivate resistant oocysts and eggs, and disinfection with quaternary ammonia—is essential. Moreover, the implementation of strategic quarantine with mandatory screening using combined methods proves to be the most effective measure to interrupt the reinfestation cycle and reduce zoonotic risks within the population (Saldanha-Elias *et al.*, 2019).

Final considerations

Based on the data obtained, it can be concluded that no single method is sufficient for the accurate diagnosis of all parasitic species. Therefore, the combined application of different coproparasitological techniques is recommended in order to increase diagnostic accuracy and contribute more effectively to the control of endoparasitic infections in sheltered dog populations. Furthermore, the analyzed data highlight the urgent need for preventive strategies, such as regular deworming and proper environmental management. The literature clearly indicates that high population density in shelters contributes to parasite dissemination, and practices such as daily feces removal and appropriate disinfection are essential to reduce infestation levels. &

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Appendix A

The experimental protocol was approved by the Animal Use Ethics Committee (Ceua) of the Octávio Bastos Teaching Foundation University Center, under protocol No. 2024.31, in accordance with current legislation and institutional guidelines for the use of animals.



Comissão de Ética Comissão de Ética no Uso de Animais
 Centro Universitário da Fundação de Ensino Octávio Bastos
 CEUA UNIFEOB
 CAMPUS 2 - Mantiqueira: Av. Dr. Octávio Silva Bastos, s/nº - Jd Nova São João - Tel: (19) 3634 3205
 São João da Boa Vista-SP
 www.unifeob.edu.br
 ceua@unifeob.edu.br

São João da Boa Vista, 05 de novembro de 2024.

REF: CEUA Protocolo n. 2024.31

Título do Projeto: Análise da Prevalência de Parasitas Intestinais em Cães de ONGs de São João da Boa Vista, SP, e Região.

Responsável: Mariana Ramos Andrade Beraldo

Colaboradores: Bruna Pan dos Santos: estudante graduanda em Medicina Veterinária (19) 99808-7841; Carolina Dantas Micheletti: estudante graduanda em Medicina Veterinária (19) 99831-2257

Início: 07/11/2024

Fim: 08/05/2025

Local de realização: APEF - Associação Pet Feliz = Sítio Conceição ou Pituva, KM 4, Gleba 04 A, São João da Boa Vista, SP e Centro Veterinário Unifeob - Av. Dr. Octávio da Silva Bastos, 2439 - Jd Nova São João, São João da Boa Vista - SP.

PARECER: Os membros da Comissão de Ética no Uso de Animais em reunião extraordinária realizada dia 04 de novembro de dois mil e vinte e quatro, conforme os princípios éticos que regem a Comissão, após correções no protocolo encaminhado, deliberaram parecer FAVORÁVEL à realização do projeto encaminhado. Havendo quórum e sem mais, subscrevem os membros que constituem a Comissão de Ética no Uso de Animais - CEUA do Centro Universitário da Fundação de Ensino Octávio Bastos (UNIFEOB) assinam digitalmente o parecer os membros presentes.

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 Centro Universitário da Fundação de Ensino Octávio Bastos
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 São João da Boa Vista-SP
 www.unifeob.edu.br
 ceua@unifeob.edu.br

Suplentes:

Membro Suplente Médico Veterinário – Profa. Ms. Fernanda Leme Silva Bastos Varzim – Curso de Medicina Veterinária – Médica Veterinária.

CRedit Author Statement

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 - **Conflicts of Interest:** The authors declare that there are no conflicts of interest related to this work.
 - **Ethical Approval:** The study was conducted in accordance with ethical principles for the use of animals and was approved by the Animal Use Ethics Committee (Ceua) of the Centro Universitário Fundação de Ensino Octávio Bastos, under protocol number 2024.31.
 - **Data and Materials Availability:** The data and materials used in this study are available from the authors upon reasonable request.
 - **Author Contributions:** SANTOS, B. P. dos and MICHELETTI, C. D. were responsible for the conception, execution, and writing of the study under the supervision of Professor BERALDO, M. R. de A. LALUCCI, M. C. was responsible for the statistical analyses of the study.
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