

Analysis of the accuracy of different laboratory methods for the diagnosis of intestinal parasites from stray and domiciled cats (*Felis catus domesticus*) in Goiânia, Goiás, Brazil

Análise da acurácia de diferentes técnicas parasitológicas no diagnóstico de parasitos entéricos de gatos (*Felis catus domesticus*) errantes e domiciliados em Goiânia, Goiás, Brasil

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Abstract

Cats are carriers of zoonotic agents to humans, including intestinal parasites. The purpose of this study was to analyze the accuracy of different laboratory methods for the diagnosis of intestinal parasites. Fecal samples were processed by the Willis, Sheather, Faust and Hoffman-Janer-Pons-Lutz (HJPL) methods. Accuracy analysis was performed determining the sensitivity, specificity, positive and negative predictive value and *Kappa*. A total of 149 fecal samples were collected, 65 from stray cats and 84 from domiciled cats. The prevalence of intestinal parasites in stray cats was 60% while in domiciled cats it was 17%. In the analysis of accuracy, the techniques that showed the greatest accuracy for Ancylostomids were Willis and Faust, for *Cystoisospora* spp. Sheather with Faust or HPJL, and *Toxoplasma gondii*/ *Hammondia hammondi* the association between Willis and Faust. Therefore, for a reliable evaluation of the prevalence of intestinal parasites, at least two different techniques should be used in parasitological exams of feces.

Keywords: Cats, clinical laboratory techniques, parasitic diseases.

Resumo

Gatos são veiculadores de agentes zoonóticos para o homem, entre eles os parasitos intestinais. O objetivo foi analisar a acurácia de diferentes métodos laboratoriais para o diagnóstico de parasitos entéricos. As amostras de fezes foram processadas pelos métodos de Willis, Sheather, Faust e Hoffman-Janer-Pons-Lutz (HJPL). A análise de acurácia foi realizada determinando a sensibilidade, especificidade, valor preditivo positivo e negativo e índice *Kappa*. Foram coletadas 65 amostras de fezes de gatos errantes e 84 amostras de gatos domiciliados, total de 149 amostras. A prevalência de parasitos entéricos nos errantes foi 60% e nos domiciliados 17%. Na análise de acurácia as técnicas que associadas apresentaram maior acurácia para Ancilostomídeos foram Willis e Faust, para *Cystoisospora* spp. Sheather com Faust ou HPJL, e para *Toxoplasma gondii*/ *Hammondia hammondi* a associação entre Willis e Faust. Portanto, para uma avaliação fidedigna da prevalência de parasitos entéricos, deve-se usar no mínimo duas técnicas diferentes ao realizar os exames parasitológicos de fezes.

Palavras-chave: Gatos, técnicas de laboratório clínico, doenças parasitárias.

Introduction

Domestic and stray cats are responsible for environmental contamination and may be responsible for the transmission of zoonotic. In this respect, few studies have compared the zoonotic

potential of these two groups of animals (GENNARI et al., 1999; SERRA et al., 2003).

Cats are carriers of parasites therefore enlarging the risk of environmental contamination. Human contact with feces of infected animals is considered a public health problem due to the possibility of zoonotic transmission (TESSEROLLI et al., 2005; BRENER et al., 2005; JOFFILY et al., 2013; BEIGI et al., 2017; LIMA et al., 2017).

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The diagnosis of such parasitic infections is of utmost importance due to the possibility of transmission and environmental contamination. Therefore adequate laboratory techniques that present good sensitivity, specificity and reproducibility are necessary. The evaluation of the accuracy of different parasitological techniques allows the determination of concordance and reproducibility for application in veterinary laboratories. Thus the aim of this study was to analyze the accuracy of different laboratory methods for the diagnosis of intestinal parasite from stray and domestic cats and determine the prevalence of the detected parasites (REZENDE et al., 2015; STULL et al., 2015).

Given the possibility of transmission of intestinal parasites by cats, and their role in environmental contamination, laboratory techniques with good sensitivity, specificity and reproducibility are necessary to diagnose such parasites. The evaluation of the accuracy of different parasitological techniques allows to determine their agreement and reproducibility for application in the veterinary laboratory, being the main objective of this work, the indication of techniques to produce reliable information about the real prevalence of intestinal parasites in the study population.

Material and Methods

Study

This study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Goiás, under Protocol nos. 054/2013 and 024/2016. The animals were divided in two groups: domiciled and stray. Domiciled cats were animals living in houses or apartments with their owners, while the stray cats were captured by the Zoonosis Control Center (ZCC) in Goiânia and by a non-governmental organization (NGO) animal protection. Two groups of cats of different ages and both sexes, from Goiânia, in the state of Goiás, Brazil, were analyzed. In the group of stray cats, 33 were females and 32 males. 32 were less than one year old and 32 were over 1 year old. Of the domiciled cats, 49 were females and 35 males. 40 were less than one year old and 44 were older than 1 year.

The samples were collected on demand, totaling 149 fecal samples, 65 from stray cats and 84 from domiciled cats. The sampling period started in March 2015 and ended in May 2016. Stray cat fecal samples were collected directly from the animals' cages before they were dewormed, and the samples from the domiciled animals were collected by their owners. All the samples were stored in sterile universal containers, and care was taken not to use samples contaminated with soil.

Analysis of samples

The techniques used in this study were Sheather, Hoffman-Janer-Pons or Lutz (HPJL), Faust and Willis, used to identify protozoan cysts and oocysts, helminth eggs and larvae in the cat feces (WILLIS, 1921; SHEATHER, 1923; HOFFMAN et al., 1934; FAUST et al., 1938; COELHO et al., 2009).

Statistical analysis

An evaluation was made of the occurrence of intestinal parasites, and the frequency of positivity for each parasite and for each technique. To evaluate the diagnostic tests, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and *Kappa* (*K*) of each technique were compared each other. The χ^2 test was applied to evaluate the statistical correlation between the prevalence of parasites in the groups of stray and domiciled cats. The parasitological results were analyzed using Epi Info 3.2.1 software.

Results and Discussion

Of the total of 149 fecal samples, 65 came from stray cats and 84 from domiciled cats. Of the 65 fecal samples from stray cats, 60% were positive, while of the 84 fecal samples from domiciled cats, 17% were positive.

The test χ^2 revealed a significant difference between the groups, $p = 0.023$ ($p < 0.05$), indicating that stray cats are more likely to have parasites than domiciled cats, which may be explained by their poor and inadequate diet, lack of antiparasitic treatment, and/or exposure to infections. This corroborates the study by Serra et al. (2003) conducted in the metropolitan region of Rio de Janeiro, which evaluated the prevalence of intestinal parasites in stray and domiciled cats and also reported a high prevalence in stray cats. The present study also corroborates data reported by the study Mattos & Viol (2013) conducted in Apucarana, PR, Brazil, where the prevalence of intestinal parasites was higher in cats that had free access to the street than in cats restricted to living indoors.

The χ^2 test was also applied to sex and age between the groups of stray and domiciled cats and in each specific group, revealing no significant difference. As for types of parasites, the comparison between mono-parasitized stray and domiciled cats also revealed no significant difference.

The parasitological analysis of the cats' feces using different methods enabled the identification of intestinal parasites in 53 fecal samples. The Willis technique enabled the detection of 83% (44/53). The Faust technique enabled the detection of 74% (39/53) of the positives, followed by the HPJL technique with 46% (24/53) and the Sheather technique with 44% (23/53). An analysis was made of the frequency of positivity by each technique in the 53 positive samples from both stray cats (39 positive) and domiciled (14 positive).

The analysis of the frequency of positivity of the parasitological techniques indicated that the Willis technique presented the best performance (83%), reinforcing the use of this technique as a gold standard. This is in line with the studies of Táparo et al. (2006), who reported the good performance of the Willis technique applied to eggs, cysts and oocysts (REZENDE et al., 2015). The Faust technique obtained a frequency of positivity (74%), while the Sheather technique presented the lowest frequency of positivity (44%). None technique was able to detect 100% of the positive cases, reinforcing the need to use more than one diagnostic technique. The combined use of several methods is useful to detect intestinal infections caused by parasites, particularly helminths

and protozoa, increasing the accuracy of the laboratory diagnosis (MENEZES et al., 2013).

Rezende et al.'s study (REZENDE et al., 2015) demonstrated that the HPJL technique detected more positives, while the Faust technique presented the lowest frequency of positivity. However, their finding is discrepant with the present study, in which the Faust technique was achieved better performance. The inconsistency between the results of the present study and those reported by Rezende et al. (2015) can be explained by the fact that the study population was different, since Rezende et al. (2015) evaluated only stray cats and the present study analyzed two cat populations: stray and domiciled.

In the current study, it was found that associating the Willis with the Faust technique can increase the diagnostic efficacy. This finding diverges from that reported by Rezende et al. (2015) and Ribeiro et al. (2015), who recommend associating the Willis technique with the HPJL technique in veterinary clinical and laboratory diagnosis.

The accuracy of the HPJL, Faust, Sheather and Willis parasitological techniques were compared for the diagnosis of *Ancylostoma* sp. eggs. The association of the techniques that presented the greatest precision were Willis and Faust, with 75% sensitivity and 99.1% specificity, PPV of 96%, NPV of 93.5% and *Kappa* (0.805) (Table 1).

The study by Cerqueira et al. (2007), which compared the efficiency of the Willis technique to that of the HPJL method in the diagnosis of *Ancylostomatidae*, found that the HPJL technique was more sensitive than the Willis technique. In the present study, HPJL associated with the other techniques did not have a good performance, and the *Kappa* showed weak agreement. However, the Faust and Willis technique displayed a better performance (75% sensitivity and *Kappa*=0.805), and was considered more suitable for the diagnosis of Ancylostomids, because this parasite has light eggs. Souza-Dantas et al. (2007) demonstrated the importance of using the Faust technique, which reached 100% sensitivity in comparison to necropsy of the intestinal tract of cats. Coelho et al. (2013) also obtained a good performance using Faust compared in other techniques used in its diagnosis of *Ancylostomatidae*.

The centrifugal flotation technique with zinc sulfate (Faust's Technique) is a technique of choice in the detection of light structures. It can also be used in the detection of heavy eggs, which suggests it is a technique with good diagnostic sensitivity (FAUST et al., 1938; DUBEY, 1993; CARLETON & TOLBERT, 2004; DRYDEN et al., 2005). This finding differs from that reported by Rezende et al. (2015), who found that the Sheather technique exhibited higher sensitivity in the diagnosis of *Ancylostomatidae*.

Analyzing the precision of the diagnosis of *Cystoisospora* spp. Oocysts, Sheather and Faust presented higher precision, 100% sensitivity, 97.2% specificity, 55.6% PPV, 100% NPV and *Kappa* 0.701, demonstrating very good agreement. The Sheather and HPJL techniques showed similar accuracy, with 100% sensitivity, specificity of 98.6%, PPV of 66.7%, NPV of 100% and *K* (0.793), demonstrating a very good agreement (Table 2). The present study reinforces the use of the HPJL technique associated with Sheather technique, which corroborates the finding of Rezende et al. (2015), who stated that the HPJL technique presented higher sensitivity in the diagnosis of *Cystoisospora* spp.

Garcia (2007) recommended associating the HPJL technique with the Willis or Sheather technique for the diagnosis of *coccidia* in routine laboratory tests.

Analyzing the results of accuracy for oocysts of *T. gondii/H. hammondi*, the Faust technique associated with Willis demonstrated the highest sensitivity of 92.9%, specificity of 94.8%, PPV of 65%, NPV of 99.2% and *Kappa* (Table 3). In Rezende et al.'s study (2015), the Faust technique also exhibited higher sensitivity in the diagnosis of oocysts of *T. gondii/H. hammondi*, denoting the good performance of this technique. Flotation techniques in saturated solutions are recommended for oocysts and show good diagnostic sensitivity (SHEATHER, 1923; FAUST et al., 1938; DRYDEN et al., 2005). Therefore, for the diagnosis of oocysts of *T. gondii/H. hammondi*, we recommend using the Willis technique associated with the Faust technique, or the Willis technique associated with the Sheather method, where *Kappa*=0.735 demonstrates good agreement.

Table 1. Evaluation of the association of the Sheather, Faust and Hoffman -Pons -Janer -Lutz techniques and Willis technique for the diagnosis of *Ancylostoma* sp.

	Sensitivity	Specificity	PPV	NPV	<i>Kappa</i>
Willis + Sheather	38.9%	97.3%	82.4%	83.3%	0.411
Willis + Faust	75.0%	99.1%	96.0%	93.5%	0.805
Willis + HPJL	34.4%	100.0%	100.0%	84.8%	0.451
Sheather + Faust	86.7%	91.8%	54.2%	98.4%	0.619
Sheather + HPJL	12.5%	93.2%	18.2%	89.9%	0.060
Faust + HPJL	32%	97.6%	72.7%	87.7%	0.381

PPV: positive predictive value; NPV: negative predictive value; HPJL: Hoffman -Pons -Janer -Lutz.

Table 2. Evaluation of the association of the Sheather, Faust and Hoffman -Pons -Janer -Lutz techniques and Willis technique for the diagnosis of *Cystoisospora* spp.

	Sensitivity	Specificity	PPV	NPV	<i>Kappa</i>
Willis + Sheather	28.6%	100.0%	100.0%	93.1%	0.420
Willis + Faust	38.5%	98.5%	71.4%	94.4%	0.467
Willis + HPJL	42.9%	99.3%	85.7%	94.4%	0.542
Sheather + Faust	100.0%	97.2%	55.6%	100.0%	0.701
Sheather + HPJL	100.0%	98.6%	66.7%	100.0%	0.793
Faust + HPJL	62.5%	99.3%	83.3%	97.9%	0.700

PPV: positive predictive value; NPV: negative predictive value; HPJL: Hoffman -Pons -Janer -Lutz.

Table 3. Evaluation of the association of the Sheather, Faust and Hoffman -Pons -Janer -Lutz techniques and Willis technique for the diagnosis of *Toxoplasma gondii/Hammondia hammondi*.

	Sensitivity	Specificity	PPV	NPV	<i>Kappa</i>
Willis + Sheather	50.0%	99.2%	85.7%	95.6%	0.607
Willis + Faust	92.9%	94.8%	65.0%	99.2%	0.735
Willis + HPJL	28.6%	98.5%	66.7%	93.0%	0.364
Sheather + Faust	87.5%	92.2%	38.9%	99.2%	0.501
Sheather + HPJL	50.0%	97.9%	57.1%	97.2%	0.508
Faust + HPJL	35.3%	99.2%	85.7%	92.3%	0.464

PPV: positive predictive value; NPV: negative predictive value; HPJL: Hoffman -Pons -Janer -Lutz.

In conclusion, the association of parasitological techniques is fundamental for greater precision in the diagnosis of intestinal parasites. As expected the prevalence of intestinal parasites in stray cats was higher than that found in domiciled cats reinforcing their role as sources in the environmental contamination

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