PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 43:e07192, 2023
DOI: 10.1590/1678-5150-PVB-7192

Original Article
Wildlife Medicine

Evaluation of five parasitological techniques for diagnosing phylum Ciliophora cysts in fecal samples from free-living wild artiodactyls[1]

Jessica L. Pinheiro[2], Sávio F. Bruno[2], Laís V. Dib[2], Claudijane R. dos Santos[2], Camila S.C. Class[2], Marcelo S. Lima[2], Paulo Rogério A. Motoyama[2], and Alynne S. Barbosa[2]*

ABSTRACT.- Pinheiro J.L., Bruno S.F., Dib L.V., dos Santos C.R., Class C.S.C., Lima M.S., Motoyoma P.R.A. & Barbosa A.S. 2023. **Evaluation of five parasitological techniques for diagnosing phylum Ciliophora cysts in fecal samples from free-living wild artiodactyls**. *Pesquisa Veterinária Brasileira 43:e07192, 2023*. Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Prof. Hernani Pires de Melo 101, São Domingos, Niterói, RJ 24210-130, Brazil. E-mail: alynnedsb@gmail.com

A variety of laboratory techniques are used in parasitological diagnosis. However, studies that analyze their laboratory efficiency are very scarce, especially with regard to biological samples from wild animals that are little known, with little popular attachment, such as artiodactyls. These can be infected by different parasites, including protozoa of the phylum Ciliophora, which includes the parasites *Balantioides coli* and *Buxtonella sulcata*. In this light, the aim of this study was to compare the efficiency of five coproparasitological techniques for diagnosing protozoan cysts of the phylum Ciliophora in the feces of free-living artiodactyls. To this end, 101 fecal samples were collected from trails in Pedra Selada State Park, Rio de Janeiro state, from 2020 to 2021. All the samples were analyzed using the qualitative techniques of modified Sheather floatation, modified Ritchie sedimentation and Lutz, as well as the quantitative techniques of Mini-FLOTAC and McMaster. Cyst recovery was best achieved using the modified Ritchie technique, in which 62.5% positivity was detected, followed by Lutz (47.5%), modified Sheather (37.5%) and the quantitative techniques of Mini-FLOTAC (30%) and McMaster (17.5%). In most of the comparisons between the techniques, reasonable agreement regarding the diagnosis was observed (Kappa 0.21 to 0.40), which was statistically significant ($p \leq 0.05$). McMaster showed higher mean and standard deviation values for counts of cysts per gram of feces than Mini-FLOTAC. However, there was no significant difference in the estimates for cyst counts (Wilcoxon $p > 0.05$). Sedimentation qualitative techniques were more indicated for diagnosing cysts of protozoa of the phylum Ciliophora in the feces of free-living wild artiodactyls. These techniques can therefore be used as laboratory tools for environmental parasite monitoring. In addition, between the two quantitative techniques, Mini-FLOTAC presented better performance, thus showing its potential as a tool for estimating the abundance of cystic forms of the phylum Ciliophora in environmental samples.

INDEX TERMS: Parasitological diagnosis, cyst, phylum Ciliophora, wild animals.

RESUMO.- [**Avaliação de cinco técnicas parasitológicas para o diagnóstico de cistos do filo Ciliophora em amostras fecais de artiodáctilos silvestres em vida livre.**] Diversas são as técnicas laboratoriais utilizadas no diagnóstico parasitológico. No entanto, estudos que analisam a eficiência laboratorial das mesmas são muito escassos, principalmente quando se trata de amostras biológicas de animais silvestres, que possuem pouca divulgação e apego popular, como os artiodáctilos. Estes podem se infectar por diferentes parasitos, incluindo os protozoários do Filo Ciliophora, no qual se inclui *Balantioides coli*, bem como *Buxtonella sulcata*. Mediante o exposto, este estudo objetivou comparar a eficiência entre cinco técnicas

[1] Received on July 17, 2022.
Accepted for publication on May 9, 2023.
[2] Departamento de Microbiologia e Parasitologia, Instituto Biomédico, Universidade Federal Fluminense (UFF), Rua Prof. Hernani Pires de Melo 101, São Domingos, Niterói, RJ 24210-130, Brazil. *Corresponding author: alynnedsb@gmail.com

coproparasitológicas para o diagnóstico de cistos de protozoário do Filo Ciliophora em fezes de artiodáctilos em vida livre. Entre 2020 e 2021 foram coletadas 101 amostras fecais em trilhas do Parque Estadual da Pedra Selada localizado no estado do Rio de Janeiro. Todas as amostras foram analisadas pelas técnicas qualitativas de flutuação de Sheather modificada, sedimentação de Ritchie modificada e Lutz, bem como por meio das técnicas quantitativas de Mini-FLOTAC e McMaster. Pode-se verificar uma superioridade na recuperação dos cistos por meio da técnica de Ritchie modificada, no qual foi detectado 62,5% de positividade, seguida pelo Lutz (47,5%), Sheather modificada (37,5%) e pelas técnicas quantitativas de Mini-FLOTAC (30%) e McMaster (17,5%). Na maioria das comparações entre as técnicas foi possível verificar uma concordância razoável (Kappa 0,21 a 0,40) em relação ao diagnóstico entre as mesmas, sendo este estatisticamente significativo ($p \leq 0,05$). McMaster em comparação com o Mini-FLOTAC foi a que apresentou os maiores valores médio e de desvio padrão na contagem dos cistos por grama de fezes. No entanto, não foi evidenciado diferença significativa na estimativa de contagem dos cistos (Wilcoxon=$p>0,05$). Pode-se verificar que as técnicas qualitativas de sedimentação foram consideradas as mais indicadas para o diagnóstico de cistos do protozoário do Filo Ciliophora em fezes de artiodáctilos silvestres em vida livre, podendo ser utilizadas como ferramentas laboratoriais de monitoramento parasitário ambiental. Além disso, dentre as técnicas quantitativas, o Mini-FLOTAC foi a que apresentou a melhor performance, denotando o seu potencial como uma ferramenta para se estimar a abundância de formas císticas do Filo Ciliophora em amostra ambiental.

TERMOS DE INDEXAÇÃO: Diagnóstico parasitológico, cisto, filo Ciliophora, animais silvestres.

INTRODUCTION

The order Artiodactyla is a very varied group of animals that includes ungulate mammals inhabiting all world biomes. Most species are terrestrial, with walking and running abilities. Some are herbivores, and others are omnivores. In Brazil, four families of artiodactyls are present: Suidae, Tayassuidae, Cervidae and Bovidae (Tiepolo & Tomas 2006). In addition, artiodactyls play an important role in energy transfer within an ecosystem since they usually form the prey for animals at the top of the food chain, such as carnivores (Marchini et al. 2011). In Rio de Janeiro state, where the Atlantic Forest biome predominates, the wild artiodactyls present are *Pecari tajacu*, *Tayassu pecari*, *Mazama gouazoubira* and the bioinvader *Sus scrofa scrofa* (Desbiez et al. 2012, Duarte et al. 2012, Keuroghlian et al. 2013, Ministério do Meio Ambiente 2017).

Many gastrointestinal parasites can infect these animals and harm them. These include protozoa of the phylum Ciliophora, especially *Balantioides coli*, a parasite with zoonotic potential. Evolutionary forms compatible with this protozoon have been reported in other studies on artiodactyls in Brazil, including in *P. tajacu*, in captivity in Rio de Janeiro Zoo; in *Sus scrofa scrofa*, free-living in the Itatitaia National Park, which straddles the border between Minas Gerais and Rio de Janeiro states; and in *T. pecari*, in captivity in Rio Grande do Sul state (Farret et al. 2010, Barbosa et al. 2018, Dib et al. 2020). In general, *B. coli* cysts are morphologically similar to other protozoa of the phylum Ciliophora, especially concerning *Buxtonella* sp. This ciliated protozoon has already been detected in the feces of other herbivore animals, including cattle and buffaloes, and in non-human primates (Honer & Neitz 1981, Pomajbíková et al. 2013, Omeragić & Crnkić 2015). Although ciliated protozoa can infect several species of animals, the clinical changes determined by these agents are little known (Barbosa et al. 2018). As well as for humans, in non-human primates, especially in gorillas, infections with *B. coli* can manifest as chronic diarrhea with mucus or dysentery with mucus and blood (Lee et al. 1990). This protozoan has also been identified as a gastrointestinal parasite capable of altering the milk fat index in lactating female rhesus macaques (Hinde 2007).

The diagnosis of protozoa of the phylum Ciliophora can be made through the detection of trophozoites and cysts: the first is detected mainly in diarrheal feces and the second in solid and semi-solid feces (Schuster & Ramirez-Avila 2008). Although cysts can be recovered through microscopic coproparasitological techniques with different bases, there is little information on the efficiency of these techniques for diagnosing these structures. Especially with regard to material collected directly from the environment, wild animals in free-living of elusive nature, and wild animals under human care such as requests in Zoos.

Therefore, this study aimed to compare the efficiency of recovery of cysts of the phylum Ciliophora among five coproparasitological techniques based on floatation and sedimentation, including in this analysis some more modern laboratory tests such as Mini-FLOTAC, using fecal samples from free-living wild artiodactyls.

MATERIALS AND METHODS

Ethical considerations. This study was approved by the "Instituto Estadual do Ambiente" (State Institute of the Environment- INEA), Rio de Janeiro, under no. 070002/002752/2020, given that the place of sample collection is a state park. The fecal samples were collected from the ground without any direct contact with the animals, then the Animal Ethics Committee of the "Universidade Federal Fluminense" (UFF) stressed that no approval was needed from that committee.

Place of collection of fecal samples. A total of 101 fecal samples from free-living wild artiodactyls were collected from trails in the "Parque Estadual da Pedra Selada" from July 2020 to August 2021. This park is located in Visconde de Mauá, Rio de Janeiro state, with an approximate area of 8,036 hectares. It cuts into the municipalities of Resende and Itatiaia, in the middle of Paraíba Valley. The Serra da Mantiqueira is in the southern region of Rio de Janeiro state (Fig.1). This state conservation unit lies within the Atlantic Forest biome at altitudes ranging from 600 to 2,100 meters. The climate in the region is classified as high-altitude subtropical, with minimum temperature that can reach 11ºC. This park's area has several endemic and endangered animal species, including birds, amphibians and mammals. In the latter group, non-human primates, artiodactyls and felids can be highlighted (Parques Estaduais 2017).

Collection of fecal samples. The Fecal samples were collected at the same point on the trails where the fauna had previously been monitored by park guards, mainly through trap cameras. Six areas were covered; these presented a very high difficulty level because they included hills and valleys with closed forests. During the fieldwork, to be collected, fecal samples needed to be intact and to have morphology compatible with that of artiodactyls, following the characteristics described by Chame (2003) and Borges & Tomás (2004), i.e., feces with a cylindrical to rounded pelota shape,

with one of the extremities pointed and the other concave. The samples were stored individually in plastic bags without chemical preservatives. They were properly enumerated and sent to the parasitology laboratories of the Biomedical Institute, Fluminense Federal University, in thermal boxes.

Parasitological techniques. As soon as it arrived at the laboratory, not exceeding one week of storage in the fridge, parts of each fecal sample were subjected to five coproparasitological techniques: three qualitative and two quantitative. For the qualitative techniques, part of the fecal sample was homogenized in distilled water and filtered in pots containing gauze, and the filtrate was deposited in a conical-bottomed 250mL beaker. This filtrate was aliquoted into conical-bottomed 15mL centrifuge tubes to perform centrifugal sedimentation, as described by Ritchie (1948) and modified by Young et al. (1979), and centrifugal floatation, as described by Sheather (1923) and modified by Huber et al. (2003). The remaining filtrate was left to settle in a conical-bottomed beaker for 24 hours to perform the Lutz technique (Lutz 1919). In addition to these qualitative techniques, feces were also subjected to the quantitative techniques of Mini-FLOTAC, as described by Cringoli et al. (2017); and McMaster, adapted from Gordon & Whitlock (1939). The Fill-FLOTAC® type 5 fecal collector, which was designed for fecal samples from production animals, was used in both techniques. Through this device, a standardized amount of 5 grams of fecal material that is then homogenized with sodium chloride solution at 1,200g/mL can be analyzed. At the end of the cyst count, the result was multiplied by a correction factor: 5 and 33 for Mini-FLOTAC and McMaster, respectively. The microscopy slides obtained through the qualitative techniques and from the Mini-FLOTAC and McMaster chambers were read using an Olympus BX 41 binocular optical microscope, firstly at a magnification of approximately 100X and then at 400X for confirmation, when possible. This microscope was coupled to a Samsung SDC415 digital camera equipped with Honestech TVR capture software to produce photomicrographs. The same microscopist was in charge of reading all the samples.

Analysis of results. The results regarding the frequency of cysts of the phylum Ciliophora obtained through qualitative and quantitative techniques were tabulated. They were presented descriptively and together, highlighting their intersections through Venn diagrams.

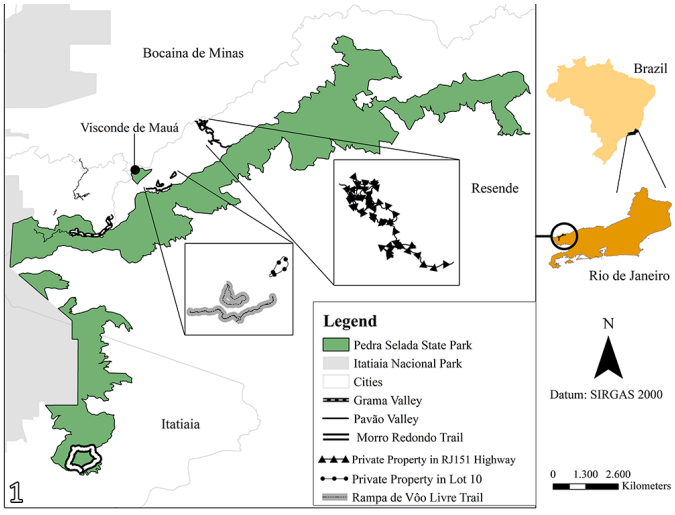

Fig.1. Location map of "Parque Estadual da Pedra Selada", in Rio de Janeiro, Brazil. Produced by the authors and with the ArcGIS software version 10.8.

The McNemar test, Kappa concordance statistics (k) and Fisher's exact statistical test were used to compare the recovery of cysts of protozoa of the phylum Ciliophora from the fecal material of the wild artiodactyls among five laboratory coproparasitological techniques. These statistical analyses were performed using the online version of the GraphPad Prism software (2014), with a significance level of 5%. The Kappa index was interpreted using the classification of Landis & Koch (1977). The McNemar test was interpreted based on the null hypothesis, i.e., assuming that the coproparasitological techniques should agree in the investigations of protozoan cysts and that the *p*-value would be greater than 0.05 in such cases. The difference in quantitative capacity between the McMaster and Mini-FLOTAC techniques was analyzed by means of Wilcoxon's nonparametric statistical test, i.e., for paired samples that did not follow the normal distribution, using a significance level of 5%, utilizing the Jamovi® version 2.2 software.

RESULTS

Among the 101 fecal samples collected, cysts of the phylum Ciliophora were detected in 40 (39.6%) by combining the results obtained from all the coproparasitological techniques. The largest number of positive samples was obtained by using the modified Ritchie centrifugal sedimentation technique, from which cysts of these protozoa were diagnosed in 25 samples (62.5%), followed by the Lutz spontaneous sedimentation technique (19, 47.5%), the modified Sheather centrifugal technique (15; 37.5%) and the quantitative techniques of Mini-FLOTAC (12, 30%) and McMaster (7, 17.5%) (Table 1) (Fig.2-7).

Analysis of the results separately showed that the modified Ritchie technique yielded the highest frequency of diagnoses, followed by Lutz, modified Sheather and Mini-FLOTAC. All the samples that were positive for cysts through the McMaster technique were also positive through another technique, and no sample was diagnosed positive only through the McMaster technique. The cysts of these protozoa were detected in only three fecal samples using all the laboratory techniques performed. Concordant diagnosis through performing four techniques was found in three fecal samples, using the modified Ritchie, Lutz, modified Sheather and Mini-FLOTAC techniques, and in one other sample in which cysts were detected using the modified Ritchie, modified Sheather, Mini-FLOTAC and McMaster techniques (Fig.8).

From comparisons between the techniques, it could be seen that they were not fully concordant in their results regarding the diagnosis of cysts. In general, a reasonable agreement was shown, reaching kappa 0.21 to 0.40 for most of the comparations that presented statistically significant differences in Fisher's exact

Table 1. Frequency of detection of cysts of the phylum Ciliophora in fecal samples from free-living wild artiodactyls through different coproparasitological techniques

Technique	Number of positive samples
Modified Ritchie	25 (62.5%)
Lutz	19 (47.5%)
Modified Sheather	15 (37.5%)
Mini-FLOTAC	12 (30%)
McMaster	7 (17.5%)
Total number of positive samples	40

test. This low agreement was confirmed by the McNemar test when it presented p≤0.05. These situations were observed with regard to comparisons between Ritchie modified and Sheather modified; between Ritchie modified and Mini-FLOTAC; between Ritchie modified and McMaster; between Lutz and McMaster; and between Sheather modified and McMaster (Table 2).

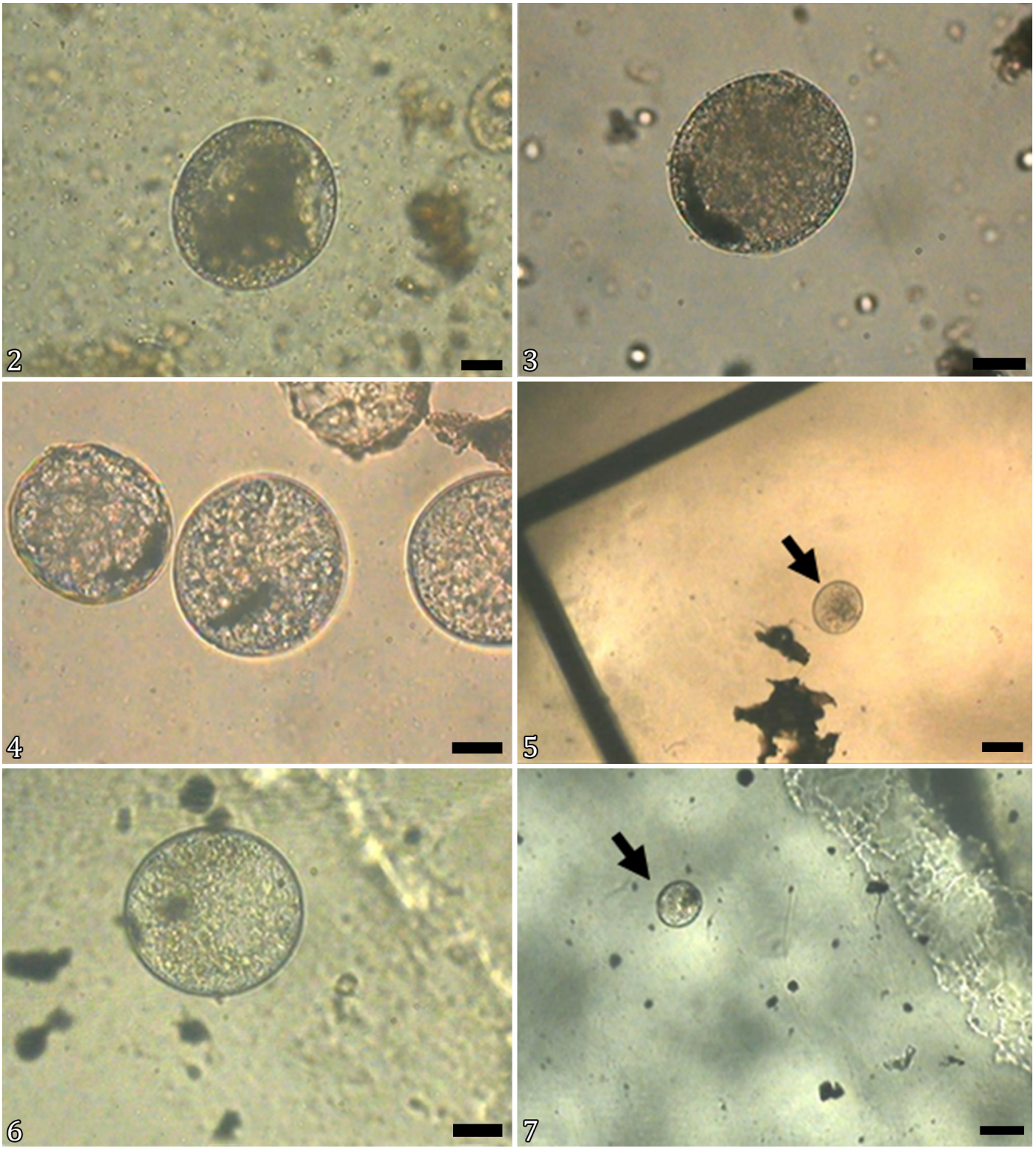

Fig.2-7. Photos of cysts of protozoa of the phylum Ciliophora detected in fecal samples collected in the "Parque Estadual da Pedra Selada", RJ. (**2**) Cyst recovered using the Lutz technique. (**3**) Cyst recovered using the modified Ritchie technique. (**4**) Cysts recovered using the modified Sheather technique. (**5**) Cyst viewed in McMaster chamber. (**6**) Cyst viewed in Mini-FLOTAC chamber. (**7**) The same cyst viewed in a Mini-FLOTAC chamber, as shown in Figure 6 but at lower magnification. (**2**-**3** and **6**) Bar = 40µm. (**5** and **7**) Bar= 25µm.

The McMaster technique presented higher mean and standard deviation values than the Mini-FLOTAC technique regarding the counts of cysts of the phylum Ciliophora. However, there was no overall significant difference in the estimated counts of cysts recovered from fecal samples between the two quantitative techniques (p>0.05) (Table 3).

DISCUSSION

In this study, we chose to use coproparasitological techniques with different bases for detecting cysts of protozoa of the phylum Ciliophora, given that the efficiency of these techniques for recovery of these structures from the feces of free-living wild artiodactyls remains unknown. This is especially so when the fecal material of these animals is obtained directly from the environment and may be in varying stages of conservation.

In our study, cysts of protozoa of the phylum Ciliophora were mainly recovered through sedimentation techniques, especially using the Ritchie modified by Young et al. (1979) technique. A similar result was previously reported by Barbosa et al. (2016), who detected cysts of the phylum Ciliophora in feces from pigs and captive non-human primates mainly through the Lutz and modified Ritchie sedimentation techniques when comparing the recovery of *Balantioides coli*-like cysts using different qualitative coproparasitological techniques. As well as in the present study, sedimentation techniques have presented the best results regarding the recovery of different parasitic structures in feces from wild animals in other places in Brazil (Carvalho et al. 2017, Dib 2019).

Cysts detected in the stool had large dimensions: on average, 105.3µm (±48.9) for the largest diameter and 101.4µm (±26.6) for the smallest diameter. This was compatible with phylum Ciliophora structures specifically relating to cysts of *B. coli* and *Buxtonella sulcata*. The large dimensions of the cysts and possibly their high density, as already pointed out by Barbosa et al. (2016), seems to have favored the recovery of these structures by means of sedimentation techniques. In addition, it is important to highlight that the use of ethyl acetate, a solvent solution together with a drop of neutral detergent, in the modified Ritchie technique, seems to have favored cleaning of feces, as this biological material contained a lot of debris and vegetable fibers. Although the cysts showed similar morphometry to *B. coli*, the fact that they belonged to free-living protozoan cysts cannot be ruled out since the samples were collected directly from the environment due to sample contamination.

The techniques based on floatation presented lower frequencies of diagnoses of cyst-positive fecal samples, including the modified Sheather and the quantitative Mini-FLOTAC and McMaster techniques. Although salt or sucrose-based solutions with different densities were used in the present study, these could not supplant the results obtained through coproparasitological sedimentation techniques. These solutions used in the flotation techniques separate the parasitic structures from the fecal material using a solution with specific density, being indicated in the recovery of protozoan cysts and oocysts (Dryden et al. 2005, Ballweber 2006). However, in a small number of fecal samples, cysts of the phylum Ciliophora were detected alone only when the modified Sheather or Mini-FLOTAC techniques were used. The diagnosis of isolated cysts in these techniques may have occurred due to an uneven distribution of these parasitic forms in the fecal material.

The low agreement of the diagnostic results obtained between the coproparasitological techniques may have been related to the fact that fecal samples are formed by a variety of types of food remnants, given that some artiodactyls, such as wild pigs and others, are omnivorous animals, and samples may also vary because of age-related differences. It is also worth noting that although the length of exposure of these fecal samples to environmental weather conditions was unknown, obtaining them noninvasively, directly from the

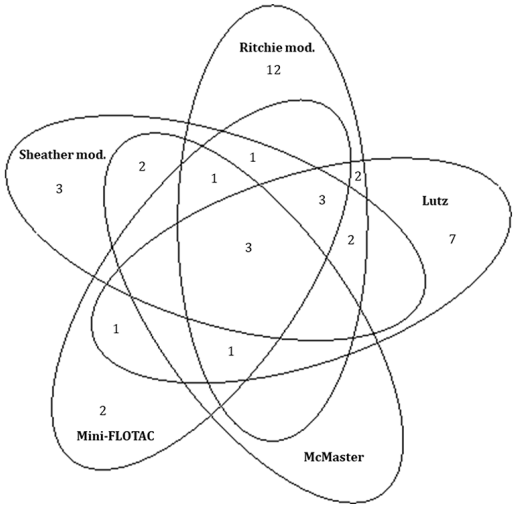

Fig.8. Diagram showing fecal samples that were positive for cysts of the phylum Ciliophora according to each coproparasitological technique. Produced by the authors and with the PowerPoint version Microsoft Office 365 and Adobe Photoshop version CS6 software.

Table 2. Comparison between five coproparasitological techniques regarding the efficacy of detection of cysts of the phylum Ciliophora present in feces from wild artiodactyls

Techniques	Kappa	McNemar	p-value
			Fisher's Exact test
Ritchie mod. vs. Lutz	0.364	0.2864	0.0006*
Ritchie mod. vs. Sheather mod.	0.386	0.0442*	0.0002*
Ritchie mod. vs. Mini-FLOTAC	0.388	0.0059*	0.0001*
Ritchie mod. vs. McMaster	0.229	0.0003*	0.0096*
Lutz vs. Sheather mod.	0.365	0.4795	0.0011*
Lutz vs. Mini-FLOTAC	0.434	0.1213	0.0001*
Lutz vs. McMaster	0.23	0.0095*	0.0223*
Sheather mod. vs. Mini-FLOTAC	0.531	0.5465	0.0001*
Sheather mod. vs. McMaster	0.498	0.0269*	0.0001*
McMaster vs. Mini-FLOTAC	0.481	0.1824	0.0002*

*p-value ≤0.05.

Table 3. Mean cyst counts per gram of feces obtained using the quantitative McMaster and Mini-FLOTAC techniques in fecal samples from wild artiodactyls

Quantitative technique	Mean ± SD	Maximum
McMaster (n=7)	79.8 (157.2)	594
Mini-FLOTAC (n=12)	50 ± 75.7	265
Wilcoxon test (p-value)	0.572	

p-value ≤0.05.

environment, was a prerequisite for this survey. It is important to highlight that it was prioritized to avoid collecting unshaped fecal samples, with the presence of arthropods and trampled on. Thus, fecal samples in different conservation states may have been included in this survey. This situation may have influenced the recovery of cystic forms of protozoa differently among the laboratory techniques used. Each technique uses different solutions and chemical reagents that may or may not have favored the recovery of parasitic forms, especially in more dry and deteriorated feces.

Even so, it should stand out that noninvasive sampling is a method that allows studies of free-ranging animals without the need to capture, manipulate, or even observe them. In this context, analyzing noninvasive samples becomes an alternative with great cost-benefit for monitoring free-living animals (Dib 2019).

In comparing the results obtained using quantitative coproparasitological techniques, it was seen that Mini-FLOTAC presented a better performance for diagnosing protozoan cysts of the phylum Ciliophora than the McMaster technique. The superiority of Mini-FLOTAC over McMaster has been widely reported with regard to diagnosing the presence of helminths in the feces of other hosts and different studies (Barda et al. 2014, Castro et al. 2017, Noel et al. 2017, Alowanou et al. 2021). The higher efficiency of Mini-FLOTAC, compared with McMaster, for diagnosing phylum Ciliophora cysts in feces obtained from the environment in the present study was already expected. Since the counting chamber of Mini-FLOTAC contains a greater volume of fecal suspension (Cringoli et al. 2017). In addition, it is important to emphasize that viewing and recognizing the parasitic structure was found to be easier in the Mini-FLOTAC technique because, with the Mini-FLOTAC chamber, it was possible to view the cysts at a magnification of 100X (Fig.7) and then confirm this at 400X (Fig.6), which cannot be done with the McMaster chamber.

In this study, the Fill-FLOTAC device was used to perform both quantitative techniques. This device eliminates the need for other equipment to perform the techniques, including the scale for weighing the material, which is not always available in all laboratories, especially those far from large urban centers. In addition, because Fill-FLOTAC is a closed system, it minimizes the operator's exposure to infectious agents (Barda et al. 2014, Lima et al. 2015, Capasso et al. 2019). These infectious agents are often unknown, but they may be zoonotic and harmful to the operator, especially in analyzing biological material from free-living wild animals.

Although quantitative techniques do not present parasite load estimates for protozoa in the way that they do for helminths and especially for nematodes, quantifications of cysts through McMaster and Mini-FLOTAC were performed to estimate the number of parasitic structures that were present in the fecal material and which could, to a greater or lesser extent, contaminate the environment. Despite the higher correction factor applied to McMaster counts of parasitic structures, this adjustment could not statistically outdo the superior performance of Mini-FLOTAC regarding the frequency of diagnoses. In addition, Mini-FLOTAC presented a lower mean standard deviation, thus denoting better efficiency in the cyst count between the samples. These observations, therefore, demonstrate that concerning quantitative techniques, Mini-FLOTAC presented better performance as a diagnostic tool for parasites and as a means for estimating the abundance of cystic forms of the phylum Ciliophora in feces from free-living wild animals that may be contaminating the environment. However, this technique presented lower diagnostic efficiency for cysts than the sedimentation techniques in the present study.

The performance of the Ritchie modified, and Lutz techniques stood out in the present study. This highlights the need for further analysis and comparisons, including with other floatation solutions and other quantitative techniques with a different basis, such as Kato-Katz. The latter was used to detect *B. coli* cysts in the feces of schoolchildren in Bolivia (Esteban et al. 1998). From further comparisons, it will be possible to verify and validate the performance of qualitative and quantitative coproparasitological techniques as appropriate laboratory tools for diagnosing and monitoring the abundance of parasitic structures such as cysts of the phylum Ciliophora. These include the zoonotic protozoon *B. coli* which may contaminate the environment and consequently non-habitual hosts such as felids, canids and humans and species with unknown zoonotic potential, such as *Buxtonella* sp.

CONCLUSION

In general, sedimentation techniques such as modified Ritchie followed by Lutz demonstrated the highest frequency of recovery of phylum Ciliophora cysts when compared with the parasitological techniques of modified Sheatther flotation, McMaster and Mini-FLOTAC. Then, sedimentation techniques were more indicated for diagnosing cysts of protozoa of the phylum Ciliophora in the feces of free-living wild artiodactyls. In addition, between the two quantitative techniques, Mini-FLOTAC presented better performance, thus showing its potential as a tool for estimating the abundance of cystic forms of the phylum Ciliophora in environmental samples.

Acknowledgments.- We thank the managers of Pedra Selada State and Itatiaia National Parks for their collaboration in carrying out this study.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Alowanou G.G., Adenilé A.D., Akouèdegni G.C., Bossou A.C., Zinsou F.T., Akakpo G.-C.A., Kifouly H.A., Rinaldi L., von Samson-Himmelstjerma G., Cringoli G. & Hounzangbé-Adoté S. 2021. A comparison of Mini-FLOTAC and McMaster techniques in detecting gastrointestinal parasites in West Africa Dwarf sheep and goats and crossbreed rabbits. J. Appl. Anim. Res. 49(1):30-38. <https://dx.doi.org/10.1080/09712119.2021.1876703>

Ballweber L.R. 2006. Diagnostic methods for parasitic infections in Livestock. Vet. Clin. N. Am., Food Anim. Pract. 22(3):695-705. <https://dx.doi.org/10.1016/j.cvfa.2006.06.001>

Barbosa A.S., Bastos O.M.P., Uchôa C.M.A., Dib L.V. & Amendoeira M.R.R. 2016. Avaliação da frequência de *Balantidium coli* em suínos, tratadores de suínos e primatas não humanos no estado do Rio de Janeiro. Revta Patol. Trop. 45(3):285-293. <https://dx.doi.org/10.5216/rpt.v45i3.43505>

Barbosa A.S., Dib L.V. & Uchôa C.M.A. 2018. *Balantidium coli*, p.531-540. In: Liu D. (Ed.), Handbook of Foodborne Diseases. 1st ed. CRC Press, Boca Raton. <https://dx.doi.org/10.1201/b22030>

Barda B., Cajal P., Villagran E., Cimino R., Juarez M., Krolewiecki A., Rinaldi L., Cringoli G., Burioni R. & Albonico M. 2014. Mini-FLOTAC, Kato-Katz and McMaster: three methods, one goal; highlights from north Argentina. Parasites Vectors 7:271. <https://dx.doi.org/10.1186/1756-3305-7-271> <PMid:24929554>

Borges P.A.L. & Tomás W.M. 2004. Guia de rastros e outros vestígios de mamíferos do Pantanal. 1ª ed. Embrapa Pantanal, Corumbá. 148p.

Capasso M., Maurelli M.P., Ianniello D., Alves L.C., Amadesi A., Laricchiuta P., Silvestre P., Campolo M., Cringoli G. & Rinaldi L. 2019. Use of Mini-FLOTAC and Fill-FLOTAC for rapidly diagnosing parasitic infections in zoo mammals. Braz. J. Vet. Parasitol. 28(1):168-171. <https://dx.doi.org/10.1590/S1984-296120180087> <PMid:30785552>

Carvalho A.F., Barnabe A.S., Federsoni I.P., Ferraz R.R.N., De Marco R.M. & Garcia I.P. 2017. Efficacy of parasitological diagnosis methods in wild animals kept in captivity. Arq. Inst. Biol. 84:e0502016. <https://dx.doi.org/10.1590/1808-1657000502016>

Castro L.L.D., Abrahão C.L.H., Buzatti A., Molento M.B., Bastianetto E., Rodrigues D.S., Lopes L.B., Silva M.X., Freitas M.G., Conde M.H. & Borges F.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://dx.doi.org/10.1016/j.vprsr.2017.10.003> <PMid:31014585>

Chame M. 2003. Terrestrial Mammal Feces: a morphometric summary and description. Mem. Inst. Oswaldo Cruz 98(Supl.1):71-94. <https://dx.doi.org/10.1590/S0074-02762003000900014> <PMid:12687767>

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(9):1723-1732. <https://dx.doi.org/10.1038/nprot.2017.067> <PMid:28771238>

Desbiez A.L.J., Keuroghlian A., Beisiegel B.M., Medici E.P., Gatti A., Pontes A.R.M., Campos C.B., Tófoli C.F., Moraes Júnior E.A., Azevedo F.C., Pinho G.M., Cordeiro J.L.P., Santos Júnior T.S., Morais A.A., Mangini P.R., Flesher K., Rodrigues L.F. & Almeida L.B. 2012. Avaliação do risco de extinção do cateto *Pecari tajacu* Linnaeus, 1758, no Brasil. Biodivers. Bras. 2(3):74-83.

Dib L.V. 2019. Helmintos e protozoários gastrintestinais em material fecal de mamíferos carnívoros e artiodáctilos do parque nacional de Itatiaia, Brasil. Dissertação de Mestrado, Universidade Federal Fluminense, Niterói. 289p.

Dib L.V., Palmer J.P.S., Class C.S.C., Pinheiro J.L., Ramos R.C.F., Santos C.R., Fonseca A.B.M., Rodríguez-Castro K.G., Gonçalves C.F., Galetti Jr. P.M., Uchôa C.M.A., Corrêa L.L., Bastos A.C.M.P., Amendoeira M.R.R. & Barbosa A.S. 2020. Noninvasive sampling in Itatiaia National Park, Brazil: wild mammal parasite detection. BMC Vet. Res. 16:295. <https://dx.doi.org/10.1186/s12917-020-02490-5> <PMid:32807197>

Dryden M.W., Payne P.A., Ridley R. & Smith V. 2005. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet. Ther. 6(1):15-28. <PMid:15906267>

Duarte J.M.B., Vogliotti A., Zanetti E.S., Oliveira M.L., Tiepolo L.M., Rodrigues L.F. & Almeida L.B. 2012. Avaliação do risco de extinção do Veado-catingueiro. *Mazama gouazoubira* G. Fischer [von Waldheim], 1814, no Brasil. Biodiversidade Brasileira, 2(3):50-58.

Esteban J.G., Aguirre C., Angles R., Ash L.R. & Mas-Coma S. 1998. Balantidiasis in Aymara children from the northern Bolivian Altiplano. Am. J. Trop. Med. Hyg. 59(6):922-927. <https://dx.doi.org/10.4269/ajtmh.1998.59.922> <PMid:9886201>

Farret M.H., Fanfa V.R., Silva A.S. & Monteiro S.G. 2010. Gastrointestinal protozoa in *Tayassu pecari* kept in captivity in Brazil. Semina: Ciências Agrárias, 31(4):1041-1044. <https://dx.doi.org/10.5433/1679-0359.2010v31n4p1041>

Gordon H.M. & Whitlock H.V. 1939. A new technique for counting nematode eggs in sheep faeces. J. Counc. Scient. Ind. Res. 12(1):50-52.

Hinde K. 2007. Milk composition varies in relation to the presence and abundance of *Balantidium coli* in the mother in captive Rhesus macaques (*Macaca mulatta*). Am. J. Primatol. 69(6):625-634. <https://dx.doi.org/10.1002/ajp.20373> <PMid:17245767>

Honer M.R. & Neitz W.O. 1981. *Buxtonella sulcata* in black buffalo and cattle in northen Brazil. Pesq. Vet. Bras. 1(3):99-100.

Huber F., Bonfim T.C. & Gomes R.S. 2003. Comparação da eficiência da técnica de sedimentação pelo formaldeído-éter e da técnica de centrífugo-flutuação modificada na detecção de cistos de *Giardia* sp. e oocistos de *Cryptosporidium* sp. em amostras fecais de bezerros. Revta Bras. Parasitol. Vet. 12(2):135-137.

Keuroghlian A., Desbiez A., Reyna-Hurtado R., Altrichter M., Beck H., Taber A. & Fragoso J.M.V. 2013. *Tayassu pecari*. The IUCN Red List of Threatened Species. 2023:e.T41778A44051115. Available at <https://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T41778A44051115.en> Accessed on Mar. 21, 2022.

Landis J.R. & Koch G.G. 1977. The measurement of observer agreement for categorical data. Biometrics 33(1):159-174. <https://dx.doi.org/10.2307/2529310> <PMid:843571>

Lee R.V., Prowten A.W., Anthone S., Satchidanand S.K., Fisher J.E. & Anthone R. 1990. Typhitis due to *Balantidium coli* in captive lowland gorillas. Rev. Infect. Dis. 12(6):1052-1059. <https://dx.doi.org/10.1093/clinids/12.6.1052> <PMid:2267484>

Lima V.F.S., Cringoli G., Rinaldi L., Monteiro M.F.M., Calado A.M.C., Ramos R.A.N., Meira-Santos P.O. & Alves L.C. 2015. A comparison of mini-FLOTAC and FLOTAC with classic methods to diagnosing intestinal parasites of dogs from Brazil. Parasitol. Res. 114(9):3529-3533. <https://dx.doi.org/10.1007/s00436-015-4605-x> <PMid:26122998>

Lutz A. 1919. O *Schistosomum mansoni* e a schistosomatose segundo observações feitas no Brasil. Mem. Inst. Oswaldo Cruz 11(1):121-155. <https://dx.doi.org/10.1590/S0074-02761919000100006>

Marchini S., Cavalcanti S. & Paula R.C. 2011. Predadores Silvestres e Animais Domésticos: guia prático de convivência. Atibaia, São Paulo, p.1835-1844.

Ministério do Meio Ambiente 2017. Plano nacional de prevenção, controle e monitoramento do javali (*Sus scrofa*) no Brasil. Ministério da Agricutura, Pecuária e Abastecimento, Ministério do Meio Ambiente, Brasília. 119p.

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. <https://dx.doi.org/10.1016/j.jevs.2016.09.006>

Omeragić J. & Crnkić C. 2015. Diarrhoea in cattle caused by *Buxtonella sulcata* in Sarajevo area. Veterinaria 64(2):50-54.

Parques Estaduais 2017. Parques Estaduais Rio de Janeiro. Available at <http://parquesestaduais.inea.rj.gov.br/inea/peps_s.php> Accessed on Mar. 21, 2022.

Pomajbíková K., Oboromik M., Horák A., Petrželková K.J., Grim J.N., Levecke B., Todd A., Mulama M., Kiyang J. & Modrý D. 2013. Novel Insights into the genetic diversity of *Balantidium* and *Balantidium*-like cyst – forming ciliates. Plos Negl. Trop. Dis. 7(3): e2140. <https://dx.doi.org/10.1371/journal.pntd.0002140> <PMid:23556024>

Ritchie L.S. 1948. An ether sedimentation technique for routine stool examinations. Bull. U.S. Army Med. Dep. 8(4):326. <PMid:18911509>

Schuster F.L. & Ramirez-Avila L. 2008. Current world status of *Balantidium coli*. Clin. Microbiol. Rev. 21(4):626-638. <https://dx.doi.org/10.1128/CMR.00021-08> <PMid:18854484>

Sheather A.L. 1923. The detection of intestinal protozoa and mange parasites by a flotation technique. J. Comp. Pathol. Therap. 36:266-275. <https://dx.doi.org/10.1016/S0368-1742(23)80052-2>

Tiepolo L.M. & Tomas W.M. 2006. Ordem Artiodactyla, p.283-304. In: Reis N.R., Peracchi A.L., Pedro A.W. & Lima I.P. (Eds), Mamíferos do Brasil. Nelio R. dos Reis, Londrina.

Young K.H., Bullock S.L., Melvin D.M. & Spruill C.L. 1979. Ethyl Acetate as a substitute for diethyl ether in the formalin-ether sedimentation technique. J. Clin. Microbiol. 10(6):852-853. <https://dx.doi.org/10.1128/jcm.10.6.852-853.1979> <PMid:574877>