

Flotation solution influence on the quantification of *Ascaris suum* eggs in pig feces using McMaster technique

Influência da solução de flutuação na quantificação de ovos de *Ascaris suum* em fezes de suínos utilizando a técnica de McMaster

Mariana Costa Fausto^{1,2} , Luís Henrique Gouvêa Saraiva³, Guilherme Costa Fausto^{1,2} , Lorendane Millena de Carvalho* , Fabrício Luciani Valente⁴ , Marisa Caixeta Valadão⁵ , Artur Kanadani Campos⁴  & Jackson Victor de Araújo⁴ 

¹ Veterinarian, DSc. Programa de Pós-Graduação em Medicina Veterinária (PPGMV), Laboratório de Parasitologia e Doenças Parasitárias, Departamento de Veterinária (DVT), Universidade Federal de Viçosa (UFV), *Campus* Viçosa, MG, Brazil.

² Veterinarian, DSc., Faculdade de Ciências Biológicas e da Saúde, Univiçosa, Viçosa, MG, Brazil.

³ Undergraduate in Veterinary Medicine, Faculdade de Ciências Biológicas e da Saúde, Univiçosa, Viçosa, MG, Brazil.

⁴ Veterinarian, DSc., Laboratório de Parasitologia e Doenças Parasitárias, DVT, UFV, *Campus* Viçosa, MG, Brazil.

⁵ Veterinarian, Laboratório de Parasitologia e Doenças Parasitárias, DVT, UFV, *Campus* Viçosa, MG, Brazil.

Abstract

Parasitic diseases are responsible for economic losses in the pig production industry. It is necessary to monitor the parasite load through stool ova and parasite analysis to avoid losses to the producer. Among analytical tests, the McMaster technique is the most widely used method; however, several variations of this method have been described, and, therefore, there is a need for standardization. The aim of this study was to evaluate the use of different saturated solutions prepared from four different salts (NaCl, MgSO₄, NaNO₃, ZnSO₄), a sugar solution, and Polysorbate Tween 80, to perform the McMaster technique. Pig feces containing *Ascaris suum* eggs were homogenized in Griffin beakers and divided into 2g aliquots for the exams with different solutions. All solutions evaluated were able to float *A. suum* eggs. However, eggs per gram of feces (EPG) values using the sodium nitrate solution were higher ($p < 0.05$) than for the other solutions. Magnesium sulfate and sodium chloride solutions provided intermediate values for EPG. Lower values were obtained with zinc sulfate and sugar solutions. Polysorbate Tween 80 was added to the sodium nitrate solution at a concentration of 0.2%, and this proved to be more efficient ($p < 0.05$) in flotation of the eggs.

Keywords: parasitological techniques, sodium nitrate, Tween 80.

Resumo

As doenças parasitárias são responsáveis por perdas econômicas na indústria suinícola. É necessário monitorar a carga parasitária por meio de análises de ovos de parasitos nas fezes, para evitar perdas ao produtor. Dentre os testes analíticos, a técnica de McMaster é o método mais utilizado; entretanto, diversas variações desse método foram descritas e, portanto, há necessidade de padronização. O objetivo deste estudo foi avaliar a utilização de diferentes soluções saturadas preparadas a partir de quatro diferentes sais (NaCl, MgSO₄, NaNO₃, ZnSO₄), uma solução de açúcar e Polissorbato Tween 80, para a realização da técnica de McMaster. Fezes de suínos contendo ovos de *Ascaris suum* foram homogeneizadas em béqueres e divididas em alíquotas de 2g para os exames com diferentes soluções. Todas as soluções avaliadas foram capazes de flutuar ovos de *A. suum*. No entanto, os valores de ovos por grama de fezes (OPG) com a solução de nitrato de sódio foram maiores ($p < 0,05$) do que com as demais soluções. As soluções de sulfato de magnésio e cloreto de sódio forneceram valores intermediários para OPG. Valores mais baixos de OPG foram obtidos com soluções de sulfato de zinco e açúcar. O polissorbato Tween 80 foi adicionado à solução de nitrato de sódio na concentração de 0,2%, e isso aumentou a eficiência ($p < 0,05$) na flutuação dos ovos.

Palavras-chave: técnicas parasitológicas, nitrato de sódio, Tween 80.



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*Correspondence

Lorendane Millena de Carvalho
Laboratório de Parasitologia e Doenças Parasitárias, Departamento de Veterinária, Universidade Federal de Viçosa – UFV
Av. P.H. Rolfs, s/n
CEP 36570-900 - Viçosa (MG), Brasil
E-mail: lorendane@gmail.com

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Introduction

Parasitic diseases can cause economic losses for the swine production industry. Parasites can damage blood vessels and internal organs (including the intestinal epithelium), resulting in malabsorption and decreased assimilation of nutrients, reduction in average daily gain (ADG), reproductive failure, failures in vaccine response, increases in the fattening period, decreases in meat quality and economic losses due to condemnation of affected organs (Fausto et al., 2015; Knecht et al., 2011).

Monitoring the parasite load is an essential method of maintaining livestock health. Stool ova and parasite analyses to detect the presence of eggs, larvae, cysts and oocysts are widely used in veterinary parasitology. Among these analyses, flotation techniques such as the McMaster technique, used to count the eggs present in feces, are the most routine method for detecting and quantifying the parasitic load in different animal species (Foreyt, 2005). The McMaster technique has the advantage of being practical and delivering results quickly. However, many variations have been described in the literature, and therefore standardization is needed (Cringoli et al., 2004). This standardization is important to avoid false negative results, which could lead to the neglect of parasitic problems in animal production.

The aim of this study was to evaluate different saturated solutions prepared from four different salts (NaCl , MgSO_4 , NaNO_3 , ZnSO_4), a sugar solution and the use of Polysorbate Tween 80 for parasite detection using the McMaster technique, in feces of pigs from commercial farms.

Materials and methods

Solutions of sugar ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$), sodium chloride (NaCl), sodium nitrate (NaNO_3), magnesium sulfate (MgSO_4) and zinc sulfate (ZnSO_4) were prepared following the protocol described in Foreyt (2005). The viscosity of the solutions was measured at 25 °C, based on a 100 sec⁻¹ shear rate using a concentric cylinder Searle-type rheometer (Brookfield model R/S Plus). Polysorbate Tween 80 at 0.2% was added to the solution that had the greatest recovery of eggs by fecal flotation, according to the protocol described by Santarém et al. (2009).

The sugar solution was composed of $\text{C}_{12}\text{H}_{22}\text{O}_{11}$, 454 g + H_2O , 355 mL, with specific gravity of 1.27 and viscosity of 3.42mPas (Millipascal). The sodium chloride solution was composed of NaCl , 400 g + H_2O , 1000 mL, with specific gravity of 1.2 and viscosity of 2.14mPas. The sodium nitrate solution was composed of NaNO_3 , 400 g + H_2O , 1000 mL, with specific gravity of 1.2 and viscosity of 1.37mPas. The zinc sulfate solution was composed of ZnSO_4 , 371 g + H_2O , 1000 mL, with specific gravity of 1.18 and viscosity of 1.92mPas. The magnesium sulfate solution was composed of MgSO_4 , 400 g + H_2O , 1000 mL, with specific gravity of 1.2 and viscosity of 3.65mPas.

Feces were directly collected from the rectal ampoule of pigs. Samples were collected from both parasite-free pigs as well as naturally infected pigs, which were previously checked by the feces eggs per gram (EPG) technique, conducted in a McMaster chamber according to the following methodology: Dilution of 2g of feces in 29 mL of saturated solution. Homogenization of the material, followed by filtration in sieve. Then, a mixture containing 14.5 mL of saturated solution and 14.5 mL of tap water was passed through the sieve. The material was homogenized with a pipette and aliquots were collected to fill the McMaster chamber. *Ascaris suum* eggs were obtained by dissection of adult female worms, which were collected from naturally infected pigs.

The fresh fecal samples were inoculated with an aqueous solution containing *A. suum* eggs, in the proportion of 2000 fertile unembryonated eggs per gram of feces. The material was then homogenized in Griffin beakers and divided into 2g aliquots for the assay with different solutions. Feces of naturally infected animals were also divided into 2 g aliquots. The EPG method was performed using the previously mentioned solutions. For artificially infected feces, 15 replications for each of the evaluated solutions were performed. In naturally infected feces, 20 replications for each of the evaluated solutions were performed. Polysorbate Tween 80 at 0.2% was added to the solution that recovered the most eggs by flotation. All analyses were performed at the standard laboratory ambient temperature of 25 °C.

The methods used in this study was approved by the Ethics Committee for the Use of Animals of the of the School of Biological and Health Sciences - CEPEUA/FACISA 025/2015-I. The data

were tabulated and analyzed by ANOVA followed by Tukey's test, with a 5% significance level, by using the Sigma Plot software (version 11.0).

Results

All solutions evaluated were able to float *A. suum* eggs. However, the type of solution significantly influenced the number of eggs found in the McMaster chamber.

In artificially infected feces, the EPG values using the sodium nitrate solution were higher ($p < 0.05$) than the other solutions (1533.33 ± 409.99). Magnesium sulfate and sodium chloride solutions provided intermediate values for EPG (926.66 ± 321.75 and 720 ± 174.02 , respectively). Lower values were obtained with zinc sulfate and sugar solutions (526.66 ± 284.01 and 480 ± 227.40 , respectively). It was observed that in artificially infected feces, the results obtained with sodium nitrate and sodium chloride solutions were statistically similar ($p > 0.05$). The sugar, zinc sulfate, magnesium sulfate and sodium chloride solutions also did not differ significantly (Table 1).

Table 1. Mean values of eggs per gram of feces (EPG) obtained in McMaster chamber using the sodium nitrate, sodium chloride, magnesium sulfate, zinc sulfate and sugar solution in artificially infected pig faeces with 2.000 *Ascaris suum* eggs per gram of feces.

Solution	Specific gravity	Viscosity (mPas)	EPG results Artificially infected feces
Sodium nitrate	1.2	1.37	1533.33 ± 409.99 a
Sodium chloride	1.2	2.14	926.66 ± 321.75 ab
Magnesium sulfate	1.2	3.65	720 ± 174.02 b
Zinc sulfate	1.18	1.92	526.66 ± 284.01 b
Sugar	1.27	3.42	480 ± 227.40 b

The averages followed by the same letter in the column do not differ statistically by Tukey test ($p < 0.05$).

The results for naturally infected feces were similar. The EPG values found by using the sodium nitrate solution were higher ($p < 0.05$) than the other solutions (3930 ± 1237.6). Sodium chloride and magnesium sulfate solutions showed intermediate values for EPG (2755 ± 856.8 and 2750 ± 1100.5 , respectively). Lower values were obtained with the zinc sulfate and sugar solutions (1600 ± 857.8 and 1735 ± 752.7 , respectively).

For naturally infected feces, the results demonstrated that the sodium chloride and magnesium sulfate solutions were not different ($p > 0.05$), and the zinc sulfate and sugar solutions were also equivalent ($p > 0.05$). However, all four solutions obtained lower results compared to the sodium nitrate solution (Table 2).

Table 2. Mean values of eggs per gram of feces (EPG) obtained in McMaster chamber using the sodium nitrate, sodium chloride, magnesium sulfate, zinc sulfate and sugar solution in naturally infected pig feces with *Ascaris suum* eggs.

Solution	Specific gravity	Viscosity (mPas)	EPG results Naturally infected feces
Sodium nitrate	1.2	1.37	3930 ± 1237.6 ^a
Sodium chloride	1.2	2.14	2755 ± 856.8 ^b
Magnesium sulfate	1.2	3.65	2750 ± 1100.5 ^b
Zinc sulfate	1.18	1.92	1600 ± 857.8 ^c
Sugar	1.27	3.42	1735 ± 752.7 ^c

The averages followed by the same letter in the column do not differ statistically by Tukey test ($p < 0.05$).

Discussion

Differences between the results obtained with the artificially and naturally infected stool may be attributed to variations in the outer egg layer, called the uterine layer, since this layer is responsible for the adherence of eggs to the fecal debris (Methanitikorn et al., 2003).

These variations may have occurred because the methodology used to artificially infect the feces was based on the dissection of adult females to collect eggs that were later added to the stool. Thus, these eggs might not have received their outer layer, which is secreted by the female's uterus before oviposition (Souza et al., 2011).

Comparisons between the results obtained with different floating solutions in naturally infected swine feces are important to the practice of routine field detection. Ballweber et al. (2014) mention that there are several factors that influence stool examination techniques. Floating solutions with high gravity can favor the detection of parasite eggs, but they can also be difficult to read due to the amount of debris in the preparation. Dryden et al. (2005) demonstrated that the process of centrifugation was able to increase the recovery and detection of nematode eggs in dog feces. However, Ballweber et al. (2014) mention that the centrifugation process can improve the ability to detect some parasites, but not all.

Pereckiene et al. (2007), analyzing 7 modifications to the McMaster technique found in the literature, describe that the technique proposed by Henriksen & Aagaard (1976) was the technique with the highest sensitivity for detecting *A. suum* eggs in swine feces. The technique evaluated by these authors uses 4g of feces, 56 mL of floating solution, which is composed of NaCl + sugar and had a specific gravity of 1.27, and centrifugation of the material for 7 minutes at 1200 rpm.

Pouillevet et al. (2017) evaluated 3 different solutions (sugar, sodium chloride and zinc sulfate) for the McMaster technique, analyzing mandrill stools and researching the best results with the use of zinc sulfate. These variations reinforce the fact that many variables are involved in choosing the best stool ova and parasite diagnostic technique.

The analysis of naturally infected swine feces showed that the sodium nitrate solution had higher efficiency floating eggs ($p < 0.05$). Corroborating this result, Menezes et al. (1999) demonstrated that the sodium nitrate solution is the most efficient and the most appropriate for the recovery of avian nematode and tapeworm eggs and coccidian oocysts by the McMaster technique. Guimarães et al. (2005) used the centrifugation-flotation technique with saturated sugar, sodium dichromate or sodium nitrate solutions to assess contamination with *Ancylostoma* sp. eggs in soil samples collected from public parks and children's play areas, and found that the three solutions presented the same efficiency in the recovery of *Ancylostoma* sp. eggs.

Xavier et al. (2010) evaluated the influence of different saturated solutions on centrifugal flotation techniques of soil samples artificially infected with *Toxocara canis* eggs, and they found that there was no significant difference between the zinc sulfate and sodium nitrate solutions. However, the zinc sulfate solution showed greater sensitivity for detecting positive samples containing ≥ 10 eggs, while the sodium nitrate solution only effectively detected positive samples containing a minimum of 25 eggs.

Traditionally, sodium chloride is used for egg count in the McMaster chamber (Elsheikha & Khan, 2011). Comparing the effect of sodium chloride with sodium nitrate in naturally infected feces, there was a difference ($p < 0.05$) in this study. In absolute values, the recovery with the sodium chloride solution was 1.42 times lower. This result can probably be attributed to viscosity, since both solutions have the same specific gravity. Schramm (2006) referred to viscosity resistance of a fluid as any irreversible change of its elements, and according to Bretas & D'Ávila (2000), the greater the viscosity of the fluid, the greater its resistance to changes. The higher viscosity of the sodium chloride solution may therefore be responsible for an increased resistance to fluctuation of eggs in this solution. Although in the present study this solution was the second most effective in the recovery of eggs, it is not recommended for flotation techniques of *A. suum* eggs because it showed a low detection sensitivity, which easily enables occurrence of false negative results.

Viscosity may also be one of the factors responsible for the low recovery of eggs observed with the magnesium sulfate solution. The solution made with this salt showed the highest viscosity, and therefore, greatest resistance to fluctuating eggs. There was a significant difference ($p < 0.05$) between this solution and the sodium nitrate solution. In absolute values, the recovery efficiency

of the magnesium sulfate solution in feces naturally infected with *A. suum* eggs was 1.42 times lower compared to the sodium nitrate solution.

The viscosity of the zinc sulfate solution was higher than that of the sodium nitrate solution. Additionally, the two solutions had different specific gravities, with sodium nitrate having the highest value. According to Ruoti et al. (2000), specific gravity refers to the relationship between the density of a substance and the density of a reference material, which is usually water. When a substance of lower specific gravity is added to a solution with greater specific gravity, the substance tends to float. Therefore, the greater the specific gravity of the float solution used, the greater the fluctuation of the eggs. The zinc sulfate solution had lower specific gravity and higher viscosity, so there was a significant difference ($p < 0.05$) between this solution and sodium nitrate, and absolute values showed a recovery rate of 2.45 times lower.

Cringoli et al. (2004) used a zinc sulfate solution with specific gravity of 1.35 for floating strongyle eggs in sheep, which resulted in low egg counts when compared to other solutions. Whereas for floating eggs of the trematode *Dicrocoelium dendriticum*, increasing the specific gravity from 1.2 to 1.35 resulted in a better performance in the fecal tests (Rinaldi et al., 2011). Thus, adjustments to the solutions, such as increased concentration and thus increased specific gravity, may influence the fluctuation rate of *A. suum* eggs.

There was a significant difference ($p < 0.05$) between the sugar solution and the sodium nitrate solution, and in absolute values, the sugar solution obtained a number of eggs 2.26 times lower if compared to the sodium nitrate solution.

According to Great Britain (1986), ascarid eggs, as well as some trematode eggs, are heavier and larger than strongyle eggs, and require solutions with higher specific gravity to float. However, other properties of the solution aside from specific gravity can interfere with fluctuation such as temperature, viscosity and especially the ability to exert effects on the surface of the eggs (Quinn et al., 1980).

For these reasons, lower specific gravity solutions can sometimes, in certain situations, have greater flotation capacity than solutions with higher specific gravities (Menezes et al., 1999).

Pereckiene et al. (2007) reported better performance of sugar solutions with 1.27 specific gravity when compared to saline solutions with 1.20 specific gravity for floating *Ascaris* eggs. However, these data do not corroborate the results obtained in the present study because, despite having higher specific gravity, the lowest egg counts per gram of feces were obtained with the sugar solution.

The addition of Polysorbate Tween 80 at 0.2% to the sodium nitrate solution caused an increase in viscosity of the solution, from 1.30 to 1.824 mPas. Besides increasing the viscosity, the sodium nitrate solution with Tween 80 at 0.2% was more efficient ($p < 0.05$) in the fluctuation of eggs ($1330 + 447.3312678$) compared to the nitrate solution without the addition of Polysorbate Tween 80 (1000 ± 316.27766) (Figure 1).

Ascaris eggs have great adhesiveness capacity (Massara et al., 2003) because the outermost layer consists of mucopolysaccharides and proteins (Souza et al., 2011). Capizzi & Schwartzbrod (2001) suggested that the surface of *Ascaris* eggs has hydrophobic characteristics. These factors may favor the link between eggs and fecal material, hindering their recovery in the floating solutions.

Polysorbate Tween 80 is a nonionic surfactant with both a hydrophobic and a hydrophilic region (Maniasso, 2001). Due to this characteristic, it has the ability to lower the interfacial tension between two immiscible phases and solubilize species of low solubility. Surfactants help move and disperse nonpolar particles in water (Gomes, 2010; Moura, 2009). In this case, the hydrophobic regions of the Polysorbate Tween 80 molecules can bind to the nonpolar surface of the uterine layer of the eggshell, keeping the hydrophilic regions in contact with water. Thus, by using Polysorbate Tween 80 in the float solution, the eggs can move with the water flow and separate more easily from the fecal debris, favoring their fluctuation (Methanitikorn et al., 2003).

However, eggs of different helminth species have different sizes, shapes, weights and shell constitutions, and cannot simply be considered as inert floating elements in solution (Cringoli et al., 2004). Because of these differences it is not feasible to use the same fecal examinations to diagnose parasites of various species. Therefore, the results of this study should not be extrapolated to eggs of other helminth species.

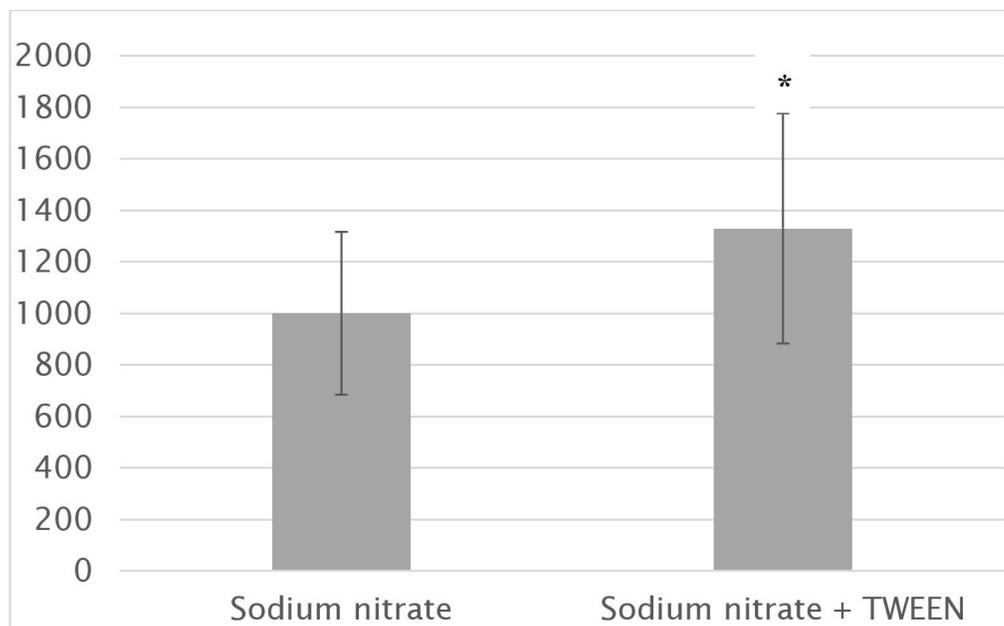


Figure 1. Mean values OPG obtained in McMaster chamber using sodium nitrate solution added or not Polysorbate Tween 80 in infected pigs. *Statistical difference.

It is important to standardize the flotation solutions used in the diagnosis of other parasites of different animal species. Only then will it be possible to optimize the techniques and improve results, combining optimal specificity and sensitivity with affordable costs.

Conclusion

Considering the convenience and accessibility of the McMaster technique, and according to the results obtained in the present study, the use of a sodium nitrate solution with Polysorbate Tween 80 added at 0.2% is indicated for the diagnosis of infection with *A. suum* in pigs.

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Ethics statement

The methods used in this study was approved by the Ethics Committee for the Use of Animals of the School of Biological and Health Sciences - CEPEUA/FACISA 025/2015-I.

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Conflict of interests

MCF, LHGS, GCF, LMC, FLV, MCV, AKC and JVA - No conflict of interest.

Authors' contributions

MCF, LHGS, GCF and LMC - Development of methodology; preparation and writing the initial draft. FLV - Application of statistical study data, Review and Editing manuscript. MCF, LMC, AKC and JVA - Writing, Review and Editing manuscript. JVA - Acquisition of the financial support for the project leading to this publication.

Availability of complementary results

The authors must identify where readers can access any complementary information available, such as in an online repository or from the authors on request. We suggest consulting: https://wp.scielo.org/wp-content/uploads/Lista-de-Repositorios-Recomendados_pt.pdf

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