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Efficiency of peptide digestion and scarification techniques for detecting *Sarcocystis* spp. in beef cattle

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Abstract Mangas TP, Rocha HPC, Filho ES, Serra-Freire NM, Benigno RNM. 2014. **Efficiency of peptide digestion and scarification techniques for detecting spp. in beef cattle** [Eficiência das técnicas de digestão peptídica e escarificação para a detecção de spp. em bovinos de corte] *Coccidia* 2, 52-57. Universidade Federal Rural da Amazônia, Belém-PA Brasil. E-mail: tmangas2008@hotmail.com

The objective of this study was to compare two techniques used for testing cattle for spp. Samples of heart and tongue were collected from 200 slaughtered cattle in Belém - Pará, totaling 400 samples, which were analyzed by peptide digestion and scarification. The results demonstrated that peptide digestion was better than scarification for tongue samples, where infection was detected with a sensitivity of 83 versus 59.5%, respectively (odds ratio= 3.32, p<0.0001). However, the difference in sensitivity between the techniques for heart samples was not statistically significant, with 92% positive for digestion and 90% for scarification (odds ratio= 1.28, p= 0.6). With scarification, the parasite indicators mean parasite index, mean parasite abundance and parasite range were higher for heart than tongue, and the examination of the first slide detected 82% and 60% positive samples in heart and tongue, respectively. The results suggest that scarification of the heart can be used as an alternative to the digestion technique for the diagnosis of infection of cattle by spp., with the advantage of being easy to perform and inexpensive.

Keywords sarcocysts, diagnostic techniques, evaluation of infection, state of Pará, beef cattle.

Resumo Com o objetivo de comparar duas técnicas utilizadas na pesquisa de spp. em bovinos, foram coletadas alíquotas de coração e língua de 200 bovinos abatidos em Belém, Pará, totalizando 400 amostras, que foram analisadas pelas técnicas de digestão peptídica e escarificação. Os resultados demonstraram que a digestão peptídica foi superior a escarificação nas alíquotas de língua, com sensibilidade de infecção de 83% contra 59,5%, respectivamente (odds ratio= 3,32; p<0,0001). No entanto, a diferença de sensibilidade entre as técnicas para alíquotas do coração foi estatisticamente não significativa, com 92% de positividade à digestão e 90% à escarificação (odds ratio= 1,28; p = 0,6). Através da escarificação, os valores dos indicadores parasitários mostraram que o índice médio parasitário, a abundância média parasitária e a amplitude parasitária nas amostras de coração foram superiores as de língua, sendo que o exame da primeira lâmina detectou 82% e 60% das amostras positivas no coração e língua. Os resultados sugerem que a escarificação do coração pode ser utilizada como técnica alternativa à digestão para o diagnóstico de infecção por spp. em bovinos, com a vantagem

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de ser de fácil execução e baixo custo.

Palavras-chave sarcocistos, técnicas de diagnóstico, avaliação de infecção, estado do Pará, gado bovino.

Introduction

The protozoa of the genus (Phylum Apicomplexa) are some of the most common parasites that affect animals, with more than 100 recognized species (Dubey & Lindsay 2006). It is an obligatory intracellular heterogeneous parasite that is involved in a predator-prey relationship (CDC 2012, Esposito et al. 2012). There are three species of the genus that infect cattle, namely *cruzi*, *S. hominis* and *S. hirsuta*, and their definitive hosts are canids, felines and primates, respectively (Dubey & Lindsay 2006).

Several studies have demonstrated the importance of infection by *S. cruzi* in animal production, since it can cause considerable economic losses due to abortions, decreased milk production, weight loss, growth retardation and death of calves (Fayer et al. 1976, Fayer et al. 1983, Botelho 1985, Hettiarachchi & Rajapakse 2008). In public health, S. hominis is the most important because primates, including humans, are its definitive hosts (Fayer 2004).

The concern about spp. in food safety has been highlight by the European Food Safety Authority (EFSA), due to the lack of reliable methods for epidemiological studies of infection in animals and derived products (Taylor et al. 2010). The objective of this study was to compare the efficiency of two techniques used in the investigation of spp. in beef cattle, to determine the feasibility of their application in epidemiological studies.

Materials and methods

Collection of samples

In the period of June to August 2013, during the slaughter of cattle at the Tapanã slaughterhouse, Icoaraci district, Belém – Pará, aliquots of about 50 grams of heart and tongue were collect from two hundred cattle. Thus, 400 samples were obtain for analysis. They were from 57 males and 143 females, originating from five of the six Pará mesoregions, with 11 from the metropolitan region of Belém, 17 from Northeast Pará, 50 from the archipelago of Marajó, 59 from Southwest Pará and 63 from Southeast Pará. The age of the cattle was estimate by examining their teeth. Each sample was place individually in a plastic bag and taken in a Styrofoam box containing ice to the Laboratory of Animal Parasitology, of Universidade Federal Rural da Amazônia (UFRA), Belém campus.

Analysis and processing of samples

Scarification

In the laboratory, the samples were scarified with the aid of a scalpel blade, pressed between two slides and examined by light microscopy at 100X and 200X for viewing sarcocysts. Samples were only consider negative after examination of four slides without the detection of cysts or bradyzoites.

The parasite indicators calculated were using the average weight of the scarified material per slide (0.01 g), where the number of cysts in the first positive slide recorded.

Peptide digestion

A 10-g portion of tissue obtained from each organ, which was cut into small pieces and transferred to an 80-ml beaker containing 50 ml of the following solution: 1.3 g pepsin (1:10,000), 2.5 g NaCl, 3.5 mL of HCl, and 500 ml of distilled water. The preparation was keep in an incubator at 40°C
for 20 minutes for digestion of muscle fibers. Afterwards, the preparation was subjected to filtration and then centrifuged at 2,000 rpm. The supernatant was discard, and the pellet was placed on a slide, cover slipped and examined with a light microscope at 400X magnification. Samples were consider negative
only after examination of four slides without the detection of cysts or bradyzoites.

Statistical Analysis

Frequencies were subject to odds ratio analysis for two independent samples, and
calculations of parasite indices were perform according to Serra-Freire (2002).

Results and discussion

The results obtained with peptide digestion and scarification revealed different sensitivities (Table 1). With scarification, whole cysts and bradyzoites were detected, while only bradyzoites with digestion.

The most sensitive technique for diagnosing infection by spp. was peptide digestion with 92% (n= 184) of cases in the heart and 83% (n= 166) in the tongue. With the scarification technique, the highest efficiency was the examination of the heart with 180 cases (90%), while the examination of tongue revealed 118 cases (59%). These results, when subjected to odds ratio analysis (Table 2), demonstrated that peptide digestion and scarification of the heart was more efficient for detecting infection by spp. (odds ratio = 2.35 ($p = 0.01$) and odds ratio = 6.25 ($p = 0.001$), respectively).

Comparing the results for the two organs, odds ratio analysis (Table 2) revealed that the probability of finding a positive in the tongue was approximately three times higher by peptide digestion than scarification (odds ratio= 3.39, $p<0.0001$). However, the data for the heart showed that there was no significant statistical difference between techniques (odds ratio = 1.28, $p = 0.6$).

The high rates of infection detected by peptide digestion in the two organs indicate high efficiency of the technique, corroborating reports of maximum infection rate of Pereira & Carvalho (1989), Shekarforoush et al. (2004), Fard et al. (2009) and Hamidinejat et al. (2010). Compared with other techniques, digestion also showed greater efficiency in the detection of *Sarcocytis* spp. (Fard et al. 2009, Hamidinejat et al. 2010).

However, peptide digestion is not consider the most appropriate method for large-scale monitoring programs, because it is costly and time-consuming (SAVINE et al. 1994). In addition, work with spp. should allow differentiation between species of economic importance and/or public health (Taylor et al. 2010), which is not possible when the slide preparation does not show intact cysts (Moré et al. 2011), which occurred in this study.

The visualization of only bradizoites by the digestion method was also reported by Pereira & Carvalho (1989), Shekarforoush et al. (2004), Hamidinejat et al. (2010) and Obijiaku et al. (2013), but Latif et al. (1999) and Fard et al. (2009) reported the presence of cysts.

The positive results for the scarification method were much higher with heart than tongue (odds ratio= 6.25, $p<0.0001$). This parasitic behavior, according to Dubey et al. (1989) may be relate to the digestive tract infection, since in its intravenous route, the first site of infection is the heart. Another related factor may be the type of muscles, as stated by Puebla et al. (2013), explaining that the musculature that has a large amount of hemoglobin, such as heart muscle, has special features to capture, store and provide oxygen, which is passed on to the parasite in the host.

In addition to allowing the identification of *S. cruzi* by the characteristics of its wall, tissue tearing proved easy to perform, quick and economical when compare to peptide digestion and could be applied extensively to the detection of spp., including epidemiological studies of sarcocystosis using the heart as reference. Nevertheless, the inability to differentiate between *S. hominis* and *S. hirsuta* is a limiting factor of the technique.

The values of the parasite indicators (Table 3, Fig. 1) showed that the mean parasite index (MPI), mean parasite abundance (MPA) and

Table 1. Frequency of *Sarcocystis* spp. in tongue and heart of slaughtered cattle in the period June to August 2013 in Belém – PA.

Technique / Organ	Only by digestion		Only by scarification		By digestion and scarification		Total	
	n	(%)	n	(%)	n	(%)	n	(%)
Heart	20	**10**	16	**8**	164	**82**	200	**100**
Tongue	64	**32**	16	**8**	102	**51**	182	**91**
Both	84	**21**	32	**8**	266	**66.5**	382	**95.5**

Table 2. Detection of *Sarcocystis* spp. in tongue and heart of slaughtered cattle in the state of Pará, analyzed by the peptide digestion and scarification, in the period of June to August 2013.

Organ and parasitological technique	Infected organs		Odds ratio	Probability (p)	Confidence interval (95%)
	n	(%)			
Organ					
TONGUE					
Peptide digestion	166	83	3.39	<0.0001	2.13 - 5.39
Scarification	118	59			
HEART					
Peptide digestion	184	92	1.28	0.6	0.64 - 2.54
Scarification	180	90			
Parasitological technique					
PEPTIDE DIGESTION					
Heart	184	92	2.35	0.01	1.25 - 4.42
Tongue	166	83			
SCARIFICATION					
Heart	180	90	6.25	<0.0001	3.64 - 10.74
Tongue	118	59			

Table 3. Parasitic indicators of *Sarcocystis* spp. in the heart and tongue of slaughtered cattle in the state of Pará, in the period of June to August 2013.

Organs and parasite indicators	parasite indicators/slide examined				
	General	1st slide	2nd slide	3rd slide	4th slide
HEART					
N	200	200	53	32	22
P (%)	90	73.5	39.62	31.25	9.09
MPI	3.26	3.65	1.62	1.3	2.0
MPA	2.94	2.68	0.64	0.41	0.23
PR	1-35	1-35	1-5	1-3	2
P (1st + 2nd slide) (%)	-	84		-	-
P (1st + 2nd + 3rd slide) (%)	-		89		-
TONGUE					
N	200	200	129	103	91
P (%)	59	36	20.16	11.65	7.69
MPI	1.54	1.64	1.23	1.58	1.86
MPA	0.91	0.59	0.25	0.18	0.14
PR	1-8	1-8	1-3	1-5	1-3
P (1st + 2nd slide) (%)	-	49		-	-
P (1st + 2nd + 3rd slide) (%)	-		55		-

N: number of aliquots; P: prevalence; MPI: mean parasite index; MPA: mean parasite abundance; PR: parasite range.

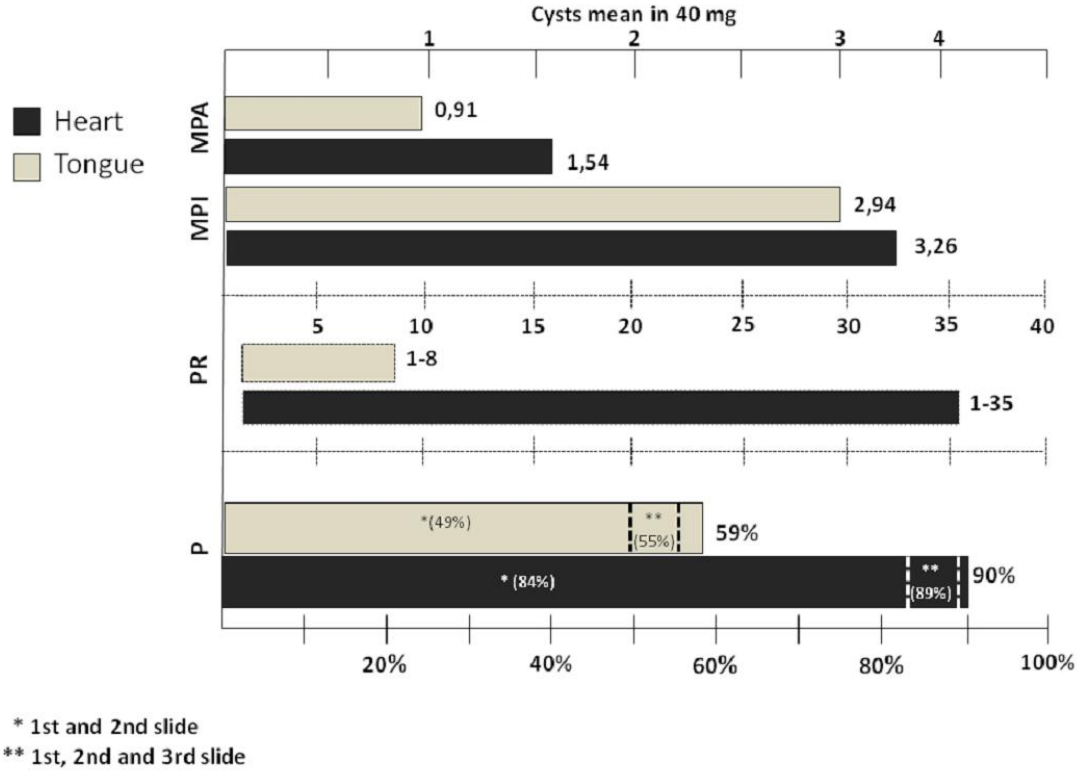

Fig. 1. Parasite indices for cysts of *Sarcocystis* spp. in the heart and tongue of slaughtered cattle in the state of Pará, in the period of June-August 2013. P: prevalence; MPI: mean parasite index; MPA: mean parasite abundance; PR: parasite range.

parasite range (PR) of the samples were higher for the heart than tongue. These data may explain the higher rates of infection in samples of heart obtained with both techniques, because higher values of these indicators were found in the myocardium and because detecting positive samples on the first slide was more likely, which was supported by the prevalence rate being very close to zero in the last slide examined.

According to the MPI values, it can be assumed that an aliquot of 100 g of heart and tongue would contain 32,600 and 15,400 cysts, respectively, considered a high degree of infectivity of these organs in the definitive hosts. The reasoning is based on that Xiang et al. (2011) managed to reproduce the cycle of *S. cruzi* in dogs fed about 600 cysts derived from heart tissue, and Chen et al. (2003) were able to reproduce the *S. hominis* cycle in two human volunteers after providing 5,000 cysts of the parasite.

Conclusion

The technique of scarification is statistically equivalent to peptide digestion for heart examination. Scarification is worth considering as an efficient technique for epidemiological studies of spp. using heart tissue as a biological indicator.

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