



DETECTION OF BOVINE *Sarcocystis cruzi* CYSTS IN CARDIAC MUSCLES: A NEW TECHNIQUE OF CONCENTRATION FOR DIAGNOSTIC*

NILTON ROGÉRIO SANTOS DA SILVA¹, REGINALDO JOSÉ DÍAZ RODRIGUES¹,
FLÁVIO ANTÔNIO PACHECO DE ARAÚJO¹, CRISTIANE BECK² & ADRIANA TARNOWSKI OLICHESKI²

ABSTRACT

The parasites of genus *Sarcocystis* are among the most commonly found parasites in domestic ruminants and some species of *Sarcocystis* can generate important economic loss when causing clinical and subclinical diseases. The acute form of sarcocystosis is caused by intravascular development of *Sarcocystis* while chronic sarcocystosis is apparently related to the encystment stage that occurs when immunity takes place. This work aimed to describe an improved method for diagnosis of sarcocystosis together with monitoring other prevalent diseases in cattle. In order to pursue our goals, 50 grams of myocardium (longitudinal region of left ventricle) were obtained from each one of 50 animals coming from three different counties of Rio Grande do Sul state and killed for human consume. Each one of the cardiac muscle samples was cut in fragments smaller than 2 cm³ and put into a blender with 100mL of saline during 10 to 15 seconds and filtered using a strainer with a double layer of sterile gauze. The filtered was poured into sterile glass tubes and centrifuged for 5 minutes at 2800 rpm at environment temperature (20 to 25°C). After pouring off the supernatant, one drop of the sediment was put on a slide and covered by a coverslip to prepare each one of the 10 fresh slides that were examined per sample at the optic microscope. The results of 500 slides were all positive for the presence of sarcocysts. The newly developed technique is extremely sensitive, simple, fast, and inexpensive and its use matched former results obtained in the same geographical region that showed the presence of *Sarcocystis cruzi* in 100% of the samples of bovine heart.

Key words: *Sarcocystis cruzi*, bovine, cardiac muscle, new technique, cysts concentration.

* Artigo originado da Dissertação de Mestrado do segundo autor. ¹Setor de Protozoologia, Departamento de Patologia Clínica Veterinária, Faculdade de Veterinária (FAVET), Universidade Federal do Rio Grande do Sul (UFRGS) ²Programa de Pós-Graduação em Ciências Veterinárias, UFRGS.

CORRESPONDÊNCIA: F.A.P. Araújo [e-mail: faraujo@vortex.ufrgs.br ; FAX: +55 51 3316 7305]. Setor de Protozoologia, FAVET-UFRGS. Caixa Postal 15094; 91501-970, Porto Alegre, RS - Brasil.

INTRODUCTION

Hasselmann [4,5], a pioneer about *Sarcocystis* research in Brazil, gave the name *Miescheria cruzi* to describe a parasite encircled by a thin membrane he found in bovine cardiac muscle. This new protozoa, according to Hasselmann, was found in 100% of the myocardium of the animals he examined and his initial studies have been followed by other works reporting similar numbers in Brazil [2,7,8].

In the other hand, a lower number of positive samples but yet a significant index of 41% of parasitism was found in Rio Grande do Sul state [9] and 66% in Boa Vista, Roraima state [10].

Heart, diaphragm, and skeletal muscles are the preferred organs for *Sarcocystis sp* location in the intermediate host and can persist through life in the hosts but many start to disappear after three months of inoculation [6].

The parasites of genus *Sarcocystis* are among the most commonly found in domestic ruminants and some species can generate important economic loss when causing clinical and subclinical diseases [6]. The acute form of sarcocystosis is linked to the intravascular development of *Sarcocystis* while chronic sarcocystosis is apparently related to the encystment stage that occurs when immunity takes place.

This work aimed to describe an improved method for diagnosis of sarcocystosis together with monitoring other prevalent diseases in cattle.

MATERIALS AND METHODS

A muscle section of 50 grams of myocardium was taken from the longitudinal region of the left ventricle from each bovine heart. This region was chosen to minimize the deleterious changes inflicted by the research procedure in the appearance of the hearts that were later commercialized. The samples were put in individual plastic bags labeled according to the origin of the animal and conveyed to the laboratory within a Styrofoam box and without refrigeration. The origin of the 50 samples were 10 from São Sepé, 10 from Vila Nova do Sul and 30 from Caçapava do Sul, counties of Rio Grande do Sul state.

Description of the Technique of Sarcocysts Concentration

A) Each one of the 50 grams samples was cut in small fragments of 2cm³ that were cut in smaller pieces and put into a blender with 100mL of saline during 10 to 15 seconds.

B) The smashed material was filtered using a strainer with a double layer of sterile gauze and the filtered was poured into sterile glass tubes.

C) The material was then centrifuged for 5 minutes at 2800rpm at environment temperature (20 to 25°C).

D) After pouring off the supernatant, 10 drops of the sediment were used per sample to make 10 different fresh slides with one drop of sediment put on a slide and covered by a coverslip and 10 fresh preparations per sample were examined using optic microscopy at 100x magnification to look for sarcocysts. Each slide was examined for the presence of sarcocysts and the slide was recorded as a positive slide when sarcocysts were detected.

RESULTS

The results of the microscopic examination of the 500 slides made from the 50 hearts were all positive for the presence of sarcocysts (*Sarcocysts spp*) using the technique described for concentration of sarcocysts in fresh samples. The authors think it is important to bring the attention to the fact that there were no young animals among the 50 animals used.

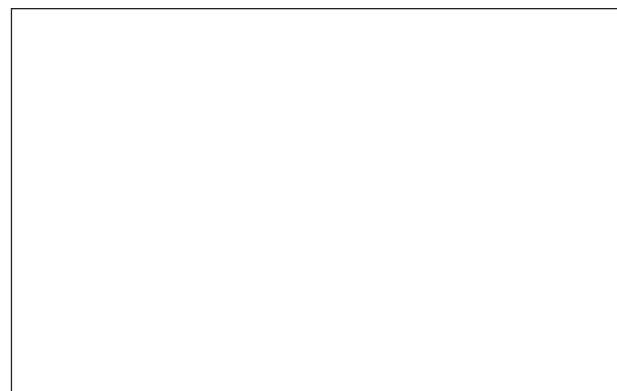


Figure 1. *Sarcocystis cruzi* cist isolated using the new technique of concentration of cists from a fresh tissue sample of the bovine myocardium (optic microscopy, 400x).

DISCUSSION

Cysts of *Sarcocystis hirsute* and *S. hominis* were very similar in morphology when examined at optic microscope. The specific diagnose is only possible through transmission electron microscopy [1,6,11,12]. The diagnose of *S. cruzi* was based on the fact that *S. cruzi* produces only microscopic cysts found in the present study against *S. hirsute* that produces only macroscopic cysts which were not found in the present study. Besides this, *S. hominis* that produces only microscopic cysts similarly to *S. cruzi* does not occur in cardiac muscle [3].

The technique of concentration developed by the authors is easy to apply and the necessary equipment can be found in most of the Parasitology diagnose and research laboratories besides it the whole method does not take more than a few minutes. And it is important to point out that since the first report of *S. cruzi* in Rio Grande do Sul state [7] until to the results reported in 1994 [8] there was no alteration in the prevalence of the disease that is still a 100%.

CONCLUSIONS

Sarcocystis cruzi is still present in 100% of bovine hearts in Rio Grande do Sul and the new technique of concentration using fresh tissue preparations is extremely sensitive, simple, fast, and it has a low cost to detect the presence of the parasite in the myocardium.

REFERENCES

- 1 **Bottner A., Charleston W. A., Pomroy W.E. & Rommel M. 1987.** The prevalence and identify of *Sarcocystis* in beef cattle in New Zealand. *Veterinary Parasitologic*. 24: 157-168.
- 2 **Carneiro J. R. & Cardoso V.M. 1982/3.** Incidência de *Sarcocystis* (Lankester, 1882) em bovinos azebuados procedentes de matadouros de Goiânia - GO. *Revista da Escola de Agronomia Veterinária*. 12/13: 53-57.
- 3 **Dubey J. P., Speer C.A. & Fayer R. 1989.** *Sarcocystosis from animals and man*. Boca Ratón: CRC Press, 215 p.

- 4 **Hasselmann G. 1923.** Parasitose das carnes de consumo (4ª Nota Prévia). *Brasil-médica*. 2: 341.
- 5 **Hasselmann G. 1926.** Alterações patológicas do miocárdio na sarcosporidiose. *Boletim do Instituto Brasileiro de Ciência*. 2: 310-326.
- 6 **Mitchell M. A. 1988.** The prevalence of macroscopic sarcocysts in New Zealand cattle at slaughter. *New Zealand Veterinary Journal*. 36: 35-38.
- 7 **Nishikawa H., Arnoni J.N., Silva S.S., Pivato I. & Rassier D.S.S. 1984.** Prevalência de *Sarcocystis* spp. (Lankester, 1882) no Rio Grande do Sul. In: *Anais do XIX Congresso Brasileiro de Medicina Veterinária* (Belém, Brasil). p. 129.
- 8 **Ruas J.L. & Silva S.S. 1994.** Ocorrência de *Sarcocystis* spp (Lankester, 1882) em bovinos clinicamente sadios abatidos em Pelotas, RS, BR. In: *Anais do I Congresso de Medicina Veterinária do Cone Sul e XII Congresso Estadual de Medicina Veterinária* (Porto Alegre, Brasil). p.64.
- 9 **Santurio J.M., Silveira A.F. & Santos A.F. 1984.** Ocorrência de sarcocistose em coração de bovinos do Estado do Rio Grande do Sul, Brasil. *Revista do Centro de Ciências Rurais*. 14: 51-54.
- 10 **Santurio J.M., Silveira A. F., Santos A.F., Beck A.A. & Weiblen A.M. 1986.** Sarcocistose em coração de bovinos do território federal de Roraima, Brasil. *Revista do Centro de Ciências Rurais*. 16: 79-83.
- 11 **Savini G., Dunsmore J.D., Robertson I. D. & Seneviratna P. 1992.** The epidemiology of *Sarcocystis* spp. in cattle of Western Australia. *Epidemiologic Infection*. 108: 107-113.
- 12 **Vercruyse J., Franssen J. & Van Goubergen M. 1989.** The prevalence and identity of *Sarcocystis* cyst in cattle in Belgium. *Zentralblein Veterinarmed [B]*. 36:148-153.



