

EXPERIMENTAL INFECTION OF DOGS WITH *SARCOCYSTIS* SP. USING INFECTED EQUINE MUSCULAR TISSUE.

G. L. BONESI¹, M. H. YAMAMURA² & A. B. L. PEREIRA²

(1) Ministério da Agricultura e do Abastecimento - Serviço de Inspeção Federal Londrina-PR, e Faculdade de Medicina Veterinária - Universidade do Oeste Paulista - Presidente Prudente - SP; (2) Departamento de Medicina Veterinária/CCA - Universidade Estadual de Londrina, C.P. 6001 - Campus Universitário - Londrina PR.

SUMMARY: Three crossbred dogs, aged 3 months, were inoculated with equine muscular tissues infected with sarcocysts (bradyzoites), obtained from a slaughterhouse. A fourth dog (control) received a sarcocyst-free inoculum. The three infected dogs passed sporocysts in the feces from the 13th day (pre-patent period) and continued to eliminate them for an average of 35.6 days (patent period). The control animal did not eliminate sporocysts. All animals remained asymptomatic and showed no temperature variations. Morphological and biometric observations showed that the sporocysts were found free, with an ellipsoid shape and surrounded by a thin membrane, without color, and measuring 16-11 µm long and 14-11 µm wide, (mean=12,2 (m x 8,28 (m). Dogs are definitive hosts for equine sarcocystosis in Brazil.

KEY WORDS: *Sarcocystis*, cysts, sarcocysts, experimental infection, sporocysts, dogs, equine.

INTRODUCTION

Protozoans of the genus *Sarcocystis* (LANKESTER, 1882) apud LEVINE, (1977), are heteroxenic coccidia, needing two hosts in order to complete their life cycle (DUBEY, 1977).

The non-sexual stage takes place in the intermediate hosts (herbivores) after the ingestion of the infective forms – the sporocysts, which are eliminated by the definitive hosts (carnivores). Once inside the intermediate host, the production of meronts will occur after which muscular cysts will be formed. Carnivorous animals, the definitive hosts, will produce oocysts and sporocysts (sexual stage) in their small intestines after ingestion of contaminated herbivores muscles (FAYER, 1972; HEYDORN & ROMMEL, 1972; ROMMEL *et alii* 1972; DUBEY, 1976).

The domestic dog is a definitive host of equine *Sarcocystis* species and eliminates sporocysts in the feces, which will contaminate the environment (ROMMEL & GEISEL, 1975; DUBEY *et alii*, 1977).

The present study evaluates the experimental infection of dogs orally inoculated with equine muscles, as well as the pre-

patent and patent periods, and the biometric and morphological characteristics of the sporocysts passed out in the feces.

MATERIALS AND METHODS

Animals

Four crossbred dogs 3 months old were used. All were examined for *Sarcocystis* oocysts and sporocysts before inoculation, and found to be negative.

The animals were housed throughout the experiment in individual pens, in the isolation area of the division of parasitic and infectious diseases, Universidade Estadual de Londrina Veterinary Hospital. Pens were washed daily with water and soap. Water and a commercial diet were fed *ad libitum* and the animals were normally vaccinated. Clinical examinations were performed daily and temperature was recorded.

Inoculum preparation and experimental inoculation.

The infective material consisted of pieces of equine muscles (including the tongue and esophagus) containing sarcocysts with bradyzoites. From horses in a slaughterhouse

in the municipality of Apucarana, Paraná State, Brazil. Tissue cysts were diagnosed using the muscle grinding technique. A feed processor from the Parasitology Laboratory of the Universidade Estadual de Londrina was used. The weight of each inoculum was 120 grams, given in a single dose to each one of three dogs. Another inoculum also made of tongue and esophagus muscles, but free of sarcocysts, was given to the control dog.

Fecal examination for sporocysts

After inoculation, the total fecal contents of each day were collected and examined using saturated sugar solution (density 1.15) technique were performed (DUBEY *et alii*, 1972).

RESULTS AND DISCUSSION

The three infected dogs passed sporocysts in the feces from the 13th day (pre-patent period) (Fig. 1).

Dog nº 1 eliminated sporocysts during 38 days, dog nº 2 for 37 days and the last one for 32 days. The average elimination time, therefore considered as the patent period was 35.6 days. The control dog did not eliminate sporocysts throughout the trial, *i.e.* during 61 days (Table 1). All animals remained asymptomatic, and showed no oscillations in the temperature during the observation period.

According to ROMMEL & GEISEL (1975) and DUBEY *et alii* (1977) dogs are definitive hosts of equine *Sarcocystis* species and eliminate sporulated sporocysts in the feces, as was confirmed in dogs experimentally infected equine muscles contaminated with sarcocysts. HEYDORN & ROMMEL (1972) stated that dogs are infected through the ingestion of cysts found in equine muscular tissues, confirming the results of the present study.

The pre-patent period found was 13 days, similar to the results of DUBEY *et alii* (1977), who found a 12-15 day period, and of ERBER & GEISEL (1981) with 11-17 days, but lower than those obtained by ROMMEL & GEISEL (1975) - 8 days, on by MATUSCHKA (1983) - 9 - 10 days.

For the present trial, due to the patency period of 35.6 days, one can conclude that on environmental contamination with sporocysts occurred, what probably leading to water and pasture contamination, resulting in a potential infection source for equines.

Regarding the morphological and biometric observations, free and isolated sporocysts were found, without the presence of sporulated oocysts, which presented an ellipsoid shape, were surrounded by a thin, colorless membrane (Fig. 1). In 31 measurements using a Nikon ocular micrometer, the sporocysts were 16-11 µm long and 14-11 µm wide (mean=12.2 µm x 8.28 µm).



Figure 1. Sarcocystis sporocyst (arrow) in saturate sugar solution. Obtained from dogs experimentally infested with equine muscular tissue (tongue and esophagus) (5000x).

Table 1. Experimental oral inoculation of 3 dogs, using sarcocysts contaminated equine muscular tissue.

Age Month	Inoculum Sporocysts (g)	Composition of inoculum	Rectal average temperature after inoculation (°C)	Feces exams *(d.a.i.)	Period of elimination *(d.a.i.)
3	120	pool of tongue + esophagus	38,26	1° - 61°	13° - 51°
3	120	..	38,26	1° - 61°	13° - 50°
3	120	..	38,25	1° - 61°	13° - 45°
3**	120	..	38,26	1° - 61°	Negative

* dai - day after inoculation

** control animal

The sporocyst shape was similar to that of other species of the *Sarcocystis* genus, but than those found by ROMMEL & GEISEL (1975), i.e. 16.3 - 15 μm length and 11.3 - 8.8 μm wide, and those of DUBEY *et alii* (1977) with 13-11 μm length and 8,5 - 7 μm wide. According to DUBEY *et alii* (1989), sporocysts have similar structures and do not present huge variations in size, although they do present a greater variation during the pre-patent and patent periods.

The lack of clinical signs for the three dogs inoculated with infested muscle confirmed the observations made by DUBEY (1977) and MARKUS (1978) who did not found characteristic signs or any other alterations except for a gut wall inflammation and mucus production, for the intestinal phase of the infection in carnivorous hosts.

The founding of sporocysts in dog feces confirms the role of the dog as a definitive host in equine sarcocystosis epidemiology in Brazil. Through the ingestion of mature sarcocysts, i.e. with bradyzoites, found in equine muscular tissues, the development of parasite sexual stages in dogs small intestines took place, culminating with the elimination of sporocysts in feces.

SUMMARY

Foram utilizados 3 cães sem raça definida, faixa etária de 3 meses, que receberam inóculo de tecidos musculares contendo sarcocistos (bradizoítas) de equídeos abatidos em matadouro-frigorífico. Um quarto cão (animal controle) também recebeu um inóculo de tecido muscular de equídeos porém, isento de *Sarcocystis*. Após a inoculação, os três cães iniciaram a eliminação de esporocistos fecais a partir do 13º dia (período pré-patente) com período médio de eliminação de 35,6 dias (período de patência). O animal-controle não eliminou esporocistos. Os 4 cães permaneceram assintomáticos e sem variação de temperatura. Quanto às observações morfológicas e biométricas, os esporocistos apresentaram-se livres, isolados, na forma elipsoidal, envolvidos por uma delgada membrana, incolores, medindo 16-11 μm comprimento X 14-11 μm largura, ($X=12,2 \mu\text{m} \times 8,28 \mu\text{m}$). Os cães são hospedeiros definitivos da sarcocistose na espécie equina no Brasil.

PALAVRAS-CHAVE: *Sarcocystis*, cistos, sarcocistos, infecção experimental, esporocistos, cães, equídeos.

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