

# EVALUATION OF VITALITY OF SARCOCYSTS IN BEEF BY THE DAPI FLUORESCENCE TEST

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## Abstract

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Vitality of *Sarcocystis* sp. cystozoites in beef was evaluated by means of the DAPI fluorescence test. Results were verified by biological assays in definitive hosts. Sarcocysts remained vital in beef kept at refrigerator temperature over a period of 20 days after slaughter. They did not survive in frozen ( $-18^{\circ}\text{C}$ , 48 hours), cooked ( $70^{\circ}\text{C}$ , 10 min.), and pickled (12 % sol. curing mixture,  $12^{\circ}\text{C}$ , 72 hours) meat.

Preliminary experiments with other protozoa indicate that the DAPI fluorescence test will meet application in different parasitological topics.

*Cystozoite, fresh meat, chilled meat, frozen meat, cooked meat, pickled meat.*

There are three species of the genus *Sarcocystis* known in muscle tissue of cattle. Their definitive hosts are different and so are apparently their pathogenic effects on the intermediate host: *S. cruzi* has the dog as definitive host and acts as pathogenic organism for cattle, *S. hirsuta* with the cat as definitive host is less pathogenic for the ox; *S. hominis* who has the man as definitive host, seems not to be pathogenic for the intermediate host.

Several ways have been designed how to evaluate vitality and infectivity of sarcocysts in beef meat. Gestrich and Heydorn (1974), Fayer (1975), Golubkov (1978), and Leek and Fayer (1978) performed assays in definitive hosts. Beside biological experiments, in vitro cultivation and dye-colouring by methylen or trypan blue were recommended (Bergler et al., 1980).

Our goal was to examine the availability of the DAPI fluorescence test for the same purpose.

DAPI (4,6-diamidino-2-phenylindol) (SERVA FEINBIOCHEMICA) is a substance with soecific affinity to intracellular DNA and emits intensive fluorescence in the UV light (Russel et al., 1975). Schilling et al. (1979) and Niemann et al. (1981) worked with the DAPI fluorescence test to determine vitality of early embryonic stages in cattle and rabbit. After incubation of embryos with DAPI, nuclei of dead cells emitted fluorescence whereas the live ones did not. Russel et al. (1975) and Hyman and MacInnes (1979) based detecting of intracellular parasites, mycoplasmas and viruses on the specific activity of DAPI to DNA.

## Materials and Methods

### Meat samples

From 10 at random chosen cows aged 3 to 5 years, samples of diaphragmal muscles and m. longissimus dorsi were collected at each stage of meat processing:

#### A. Fresh meat

Samples of 100–150 g were collected one hour after slaughter.

#### B. Chilled meat

As the next step in meat processing, beef halves were cooled down to  $16^{\circ}\text{C}$  in the core by a stream of cold air in a quick-chilling tunnel for 10 hours, then cut to quarters and stored in the chilling

room at 12 °C. To register the progress of meat ageing, pH-values were measured by a needle electrode (ORION pH/temp., model 221). Meat samples were collected 6, 24, 48, 72 and 120 hours after slaughter. From day 5 to 20, the samples were stored in a refrigerator at 4 °C, and, examined in intervals of 3 days.

#### C. Frozen meat

Meat samples of 200–300 g each were sealed in PE-bags, frozen to –18 °C and kept at this temperature for 48 hours. Thawing took place in a refrigerator at 4 °C.

#### D. Heat-processed meat

Samples were considered cooked when temperature in the core remained at 70 °C for at least 10 minutes.

#### E. Pickled meat

Meat samples of 50–100 g each were pickled in a 12% solution of the standard curing mixture (94 % NaCl, 0.5 NaNO<sub>2</sub>) over 72 hours at 12 °C.

### Isolation and purification of *Sarcocystis* sp. cystozoites

In order to isolate cystozoites, meat samples were exposed to digestion in artificial peptic juice (Sharma and Dubey 1981) for 10 minutes, and, purified by means of the chromatographic gel Spheron (Koudela in press).

### DAPI fluorescence test

As recommended by Niemann et al. (1981), DAPI was diluted with PBS (1 : 100,000). One drop of diluted DAPI was added to one drop of purified cystozoites suspended in phosphate buffered saline (NaCl 8.0 g, KCl 0.2 g, Na<sub>2</sub>HPO<sub>4</sub> · 12 H<sub>2</sub>O, 3.21 g, KH<sub>2</sub>PO<sub>4</sub>, 0.2 g, H<sub>2</sub>O dest. ad 1 000 ml), (pH 7.2) and covered by a cover glass. Fluorescence was evaluated with the Soviet-made fluorescence microscope ML-2 in reflected light, at magnifications 200 × and 450 ×, with filters FS 1–2, S3S 7–2 (UV light 350 nm). Emission was observed with the filter ŽS 18 (480 nm and more). Cystozoites with an intensively fluorescent nucleus were classified as dead, those showing no fluorescence as live ones.

### Effect of peptic juice on *Sarcocystis* sp. cystozoites

Vitality of cystozoites was evaluated after 60, 90, and 120 minutes of exposure to artificial peptic juice.

### Effect of meat processing on isolated cystozoites

Cystozoites isolated from fresh meat were suspended in PBS and exposed to conditions imitating meat processing. They were examined after 5, 10, 15, 20, 25, 30, 45, 60, and 90 minutes of exposure to 55 °C or 70 °C. Isolated cystozoites were kept at –18 °C for 48 hours and then examined. The effect of the nitrite curing mixture was evaluated after 72 hours of exposure.

### Verification of vitality of cystozoites by assays in definitive hosts

To verify our classification of cystozoites, assays were carried out with four whelps aged two months and three kittens aged three months. Two pups and two kittens were fed meat from which isolated cystozoites displayed no fluorescence. Two pups and one kitten were fed meat from which isolated cystozoites emitted fluorescence. Parasitological examinations of their faeces were carried out daily over the period of 20 days.

## Results

The evaluation of vitality of *Sarcocystis* sp. cystozoites is demonstrated in Table 1. Living cystozoites emitted no fluorescence, while there was an intensive fluorescence of nuclei in dead cystozoites. Fragments of cystozoites only could be isolated from frozen, cooked, or pickled meat samples. As far as there was a cell nucleus within the fragment, it emitted intensive fluorescence.

The process of isolation and purification had no effect on cystozoites. Their exposure to peptic juice for 120 minutes resulted in no alteration in vitality.

The effect of meat processing was illustrated by experiments with isolated cysto-

zoites. Exposure to the temperature of  $-18^{\circ}\text{C}$  for 48 hours affected the destruction of cystozoites. Likewise destroyed were cystozoites treated by the nitrite curing mixture. The effect of high temperature on isolated cystozoites is shown in Table 2.

Table 1  
Vitality of *Sarcocystis* sp. cystozoites in differently processed beef

Meat	No. of samples	Cystozoites of <i>Sarcocystis</i> sp.	
		vital	dead
Fresh	10	+	0
Chilled	10	+	0
Frozen	10	0	+
Cooked	10	0	+
Pickled	10	0	+

Table 2  
Effect of temperature on vitality of isolated *Sarcocysts* sp. cystozoites from beef

Exposure to temperature (minutes)	Percentage of dead cystozoites	
	at $55^{\circ}\text{C}$	at $70^{\circ}\text{C}$
5	$3.4 \pm 4.87$	$89.7 \pm 8.79$ 100
10	$15.6 \pm 3.65$	
15	$22.4 \pm 4.89$	
20	$27.6 \pm 5.23$	
25	$50.6 \pm 8.79$	
30	$78.9 \pm 9.79$	
45	$84.7 \pm 15.87$	
60	$96.7 \pm 8.96$	
90	100	

Whelps and kittens fed meat from which isolated cystozoites emitted no fluorescence, began elimination by day 11. Animals fed meat from which isolated cystozoites emitted fluorescence, eliminated no sporocysts.

## Discussion

The results of our evaluating vitality of sarcocysts by means of the DAPI fluorescence test are in agreement with the results achieved by biological assays carried out by Gestrich and Heydorn (1974), Fayer (1975), Golubkov (1978), and Leek and Fayer (1978).

Morphological and biological similarities of sarcocysts and *Toxoplasma gondii* suggest comparison with analogical assays on vitality and infectivity of toxoplasma cysts (Jacobs et al., 1960; Sommer et al., 1965; Dubey 1974). The vitality of toxoplasma cysts and sarcocysts in fresh, chilled, frozen and cooked meat is similar, and so is the survival of bradyzoites of *Toxoplasma gondii* and cystozoites of *Sarcocystis* sp. in peptic juice (Sharma and Dubey, 1981).

Determining vitality of cystozoites in beef after different processing is of considerable hygienic and epizootiological importance.

According to practical use of DAPI fluorescence test it is important to mention

that nonspecific fluorescence was not observed. The only fragments of cystozoites with broken structure of cell components emitted weak fluorescence.

The intensity of fluorescence depends on the concentration of DAPI. While testing various concentrations of DAPI the concentration recommended by Niemann et al. (1981) (1 : 100 000) was found optimum.

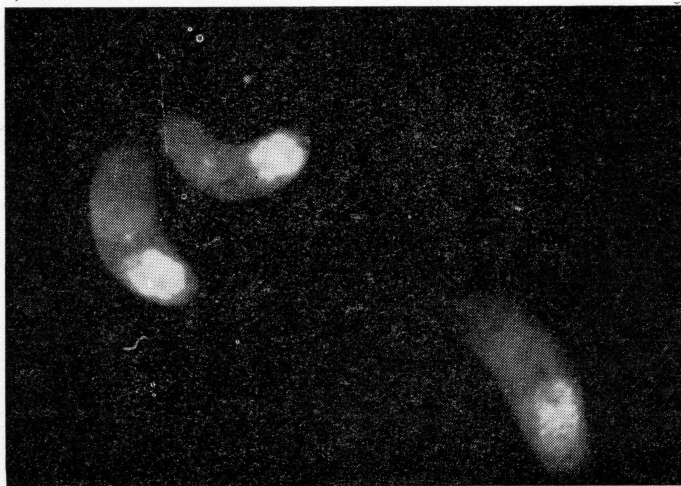


Fig. 1. Dead cystozoites with intensively fluorescent nuclei

The biological trials we presented here as well as assays reported by other authors confirmed the results achieved by the DAPI fluorescence test. Tentative assays with sporozoites of different coccidia (*Eimeria tenella*, *Hammondia heydorni*) and with cystozoites of *Sarcocystis dispersa* made it obvious that the DAPI fluorescence test might meet ample employment in parasitology.

### **Posouzení životaschopnosti sarkocyst v hovězím masě fluorescenčním testem DAPI**

Posuzovali jsme životaschopnost cystozoitů *Sarcocystis* sp. v hovězím masě fluorescenčním testem DAPI. Výsledky posouzení jsme ověřovali biologickým pokusem na definitivních hostitelích. Sarkocysty přežívají v hovězím masě do 20. dne po poražení. V masě mraženém ( $-18^{\circ}\text{C}$ , 48 hodin), tepelně opracovaném ( $70^{\circ}\text{C}$ , 10 minut) a v masě lákovaném (12% roztok solící směsi,  $12^{\circ}\text{C}$ , 72 hodin) sarkocysty nepřežívají.

Orientační posouzení životaschopnosti jiných protozoí ukazují na možnosti využití fluorescenčního testu DAPI v parazitologii.

## Оценка жизнеспособности саркокист в говядине флуоресцентным тестом ДАПИ

Проводилась оценка жизнеспособности цистозоитов *Sarcocystis* sp., в говяжьем мясе флуоресцентным тестом ДАПИ. Результаты оценки проверялись биологическим экспериментом на окончательном хозяине. Саркокисты переживают в говядине до 20 суток после убоя. В замороженном мясе ( $-18^{\circ}\text{C}$ , 48 часов), термически обработанном ( $70^{\circ}\text{C}$ , 10 минут) и в мясе в рассоле (12 % рассол,  $12^{\circ}\text{C}$ , 72 часа) саркокисты не переживают.

Ориентировочная оценка жизнеспособности других протозов свидетельствует о возможности использования флуоресцентного теста ДАПИ в паразитологии.

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