EFFECTIVENESS OF METHODS USED FOR THE DETECTION OF SARCOSPORIDIOSIS IN FARM ANIMALS

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Abstract. Trypanozoon and stereoscopy were found to be the most suitable methods for the detection of sarcosporidiosis in farm animals. Sarcocystis crusti, S. hitrata and S. hominis were detected in 87 % of 200 cattle and S. oesoinus and S. tenella in 92 % of 100 sheep examined. The examinations of 200 pigs were negative.

In the seventies, the knowledge of coccidians of the genus Sarcocystis was much deepened. These pathogenic parasites are widely distributed and have been reported from almost all species of farm animals. They form cyst-like structures in the muscles of their hosts and cause great economic losses due to a low increase in weight of the infected animals (Frelier et al. 1979, Erber and Geiæl 1979), abortion (Erber et al. 1978, Fayer et al. 1978) and even death in case of massive infection (Schmitz and Wolf 1977, Stalheim et al. 1977).

The presence of cysts in the muscles and of antibodies in the blood of infected animals was used for the detection of muscle sarcocystosis. Serological methods are not quite suitable for this purpose and their results are not always comparable. The examination of animals is limited to microscopical methods. Various organs, most often oesophagus, heart muscle, diaphragm, straight ventral muscle and others, have been reported as preferential sites of cyst occurrence. Asteriades and Haralambides (1978) recorded the bulbocavernous muscle to be the preferential site in ram and stallion.

The muscle sarcocystosis has been widely distributed among the farm animals; the recent papers have recorded mostly 89–90 % of cattle to be infected. The occurrence of this disease is lower in younger animals and increases with the age. The percentage of occurrence reported by various authors is as follows: 94.4 % in Austria (Hinaidy et al. 1979), 98 % in cattle slaughtered at the abattoir in Vienna (Hinaidy 1980), 94.3 % in cows at the abattoir in Steiermark (Oesterreicher 1979) and 90.7 % in slaughter cattle in F.R.G. (Boeh et al. 1978). A. i. east and Brackmann (1978) detected sarcocysts in by trichina in 77 % of the 1,000 cattle examined at sanitary abattoir in G.D.R. Raju and Munro (1978) recorded this disease in 78.1 % of cows in Fiji Islands and Rico and Caldone (1979) in 83.2 % of cattle slaughtered in El Salvador.

The records of muscle sarcocystosis in pigs are very different. Raju and Munro (1978) detected it in 82.8 % of pigs slaughtered in Fiji Islands, Masker et al. (1977) in 57.0 %, of pigs diaphragms examined at the abattoir in Istanbul, Mannowitz (1978) in 35.5 % of pigs in F.R.G., Heydorn et al. (1978) in 1.7 % of pigs in F.R.G. and in 41.3 % of pigs in G.D.R., Dubey (1979) in 3.4 % of pigs slaughtered at the abattoir in Ohio (USA), Balutes (1975) in 1.67 % of pigs in the Voronezh Region (USSR). Derylo and Kinka (1978), who studied the occurrence of sarcocystosis at the abattoir in Poland for ten years, found the infection in 0.037 % of pigs. Sarcocystosis occurs frequently also in sheep, as reported, for example, by Boch et al. (1979) from Bavaria (F.R.G.) (85.4 %).

Kapelkova et al. (1972) studied the occurrence of sarcocystosis in Czechoslovakia and found low infections in cattle. Sarcycysta were detected by trichina not in 17.8 % of cattle and in 63.33 % of animals when histological preparations were examined. The examinations of pigs, sheep and goats were negative. Nevoile and Luksova (1979) found sarcocystosis in 93.3 % of cows and 23.3 % of calves. They examined histological preparations of muscle tissues which contained cysts of two types— thick-walled and thin-walled ones, but the authors did not determine them. Černá and Mechaňová (1980) examined oesophagus of 99 cattle and 57 sheep at Prague abattoir. They detected sarcocystosis in 78 % of cattle and 81 % of sheep. In 1981, the same authors found sarcocystosis in 85 % of cattle and 80 % of sheep.
in 84% of cattle and 85% of sheep while examining oesophagi of 154 cattle and 154 sheep at Prague abattoir (Cernik and Merhartová 1981). They used the method of homogenization without digestion. In both cases the species S. cruzi, S. hirera and S. hominis were found in cattle and S. tenella and S. ovina in sheep.

The aim of the present paper is to compare various methods used for the detection of sarcocystosis in farm animals and to prepare a simple, rapid and reliable method for the detection of this disease.

**MATERIAL AND METHODS**

The material used for the examination originated from various places in Central Bohemia. A total of 100 sheep, 200 cattle and 200 pigs were examined in the period from December 1979 to June 1980; 150 animals originated from large-scale breedings and 50 from private breeders. The animals (both males and females) were of various ages. The oesophagus was found to be the most suitable for the examination, as it is easily available and muscle cysts occur very frequently in its wall, as reported by a majority of authors. According to Borch et al. (1978), the muscle cysts can be with certainty detected in the oesophagus even if they are formed in muscles. For this reason, this organ is also used for the detection of bovine sarcocystosis in F.R.G., where this disease occurs in 99.7% of cattle.

The following methods used for the detection of muscle stages (cysts and zoites) were compared:

a) **Direct macroscopical observation.** Large macroscopic cysts of even several mm are often found on the oesophagi of sheep. The species forming microscopic cysts form sometimes larger cysts visible with the naked eye as minute white lines in the muscles.

b) **Stereoscopy.** Direct observations of muscles under the stereoscopic preparation microscope after Gut (1986) were carried out as follows: The oesophagus was cut longitudinally and trimmed of all membranous parts to get only the transversely striated muscles. A sample of 10 x 10 cm and max. 5 mm thick was put on the lid of Petri dish (15 cm in diameter), pressed with the bottom of the dish and examined under the stereoscopic microscope at the magnification of 10-60 x. In the oblique light the muscle cysts appeared like whitish slender spindle-shaped structures inside the muscle fibres. If observed in the passing light, the muscle cysts were darker than the surrounding healthy muscles. The great depth of field of the preparation microscope enables to find easily even the minute cysts embedded in the muscles which escape attention during trichinoscopy.

c) **Trichinoscopy.** Three muscle samples (c.a. 1 x 1 cm) from each oesophagus were examined at the magnification of 40-80 x.

d) **Trypsinization after Erber (1977).** Three oesophagus samples (10 g each) were cut into small pieces (c.a. 3 x 3 cm), mixed in 150 ml of 0.25% solution of trypsin in PBS pH 7.4 and incubated in the mixer at 22-23°C for 30 min. The sample was then centrifuged, washed in PBS and the sediment was examined for the presence of zoites and walls of muscle cysts.

**RESULTS**

Macroscopical observations. Minute cysts visible with the naked eye were detected in 88 (29.0%) of the 300 bovine oesophagi examined. Large macroscopic cysts measuring several mm and corresponding to S. tenella were found in 37 of the 100 sheep examined (Fig. 1). All examinations of pigs were negative. This method of examination is unsuitable for the detection of sarcocystosis. Each finding of small cysts, though distinctly visible with the naked eye, is not quite reliable and requires a microscopic verification that really Sarcocystis cysts are involved.

Stereoscopy. This method revealed 172 (86.0%) positive cases among 200 bovine oesophagi and 92 among 100 sheep oesophagi examined. Examinations of all pigs were negative. This method does not require a large material, enables to examine a large piece of muscles during a relatively short time and the results obtained are reliable and comparable with those obtained by trypsinization which is much more laborious.

Trichinoscopy. Of the 300 bovine oesophagi examined, 325 (92.5%) were positive and of the 100 sheep oesophagi 73 were positive. Examinations of pigs were negative. This method requires few material and time, but it is less reliable than trypsinization or stereoscopy.
Trypsinization. Of the 200 cattle examined, 174 (87.0 %) were positive and of the 100 sheep examined, 92 were positive. The examinations of pigs were negative. Trypsinization is a reliable method, but it is time consuming and requires a large amount of material. The results are shown in Table 1.

Table 1. Results of examination of cattle, sheep and pigs by various methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Cattle</th>
<th>Pig</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Examined</td>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>Trypsinization</td>
<td>200</td>
<td>174</td>
<td>87.0</td>
</tr>
<tr>
<td>Trichinoscopy</td>
<td>200</td>
<td>125</td>
<td>62.5</td>
</tr>
<tr>
<td>Stereoscopy</td>
<td>200</td>
<td>172</td>
<td>86.0</td>
</tr>
<tr>
<td>Macroscopic examination</td>
<td>200</td>
<td>88</td>
<td>29.0</td>
</tr>
</tbody>
</table>

The species representation and possibility of a mixed infection were studied in 100 infected cattle. The morphology of muscle cysts, as reported by Boch et al. (1978), was used for the differentiation of species. Three types of muscle cysts corresponding to the species *S. cruzi* (Hasselman, 1926) Wenyon, 1936, *S. hirsuta* Mould, 1888 and *S. hominis* (Bailliet et Lucet, 1891) Dubey, 1976 were found in cattle. The structure of the muscle cyst wall can be used as a differentiating character.

The cyst of *S. cruzi* has a thin wall with hair-like cytoplasmic filaments on its surface (Fig. 2), whereas the cysts of *S. hirsuta* and *S. hominis* possess a thick wall with finger-like cytoplasmic filaments adjacent to one another. In *S. hirsuta* they measure ca. 7 μm (5—8 μm) × 1.5 μm (1.3—1.6 μm) (Figs. 3, 4) and in *S. hominis* ca. 5 μm (4—7 μm) × 0.7 μm (0.6—8 μm).

Table 2. Occurrence of *Sarcocystis* species in 100 heads of infected cattle

<table>
<thead>
<tr>
<th>Single infection</th>
<th>Mixed infection with two species</th>
<th>Mixed infection with three species</th>
</tr>
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<tbody>
<tr>
<td>Species</td>
<td>No. of animals</td>
<td>%</td>
</tr>
<tr>
<td><em>S. cruzi</em></td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td><em>S. hirsuta</em></td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

The mixed infection was found in a majority of animals. Only 40 % of the infected cattle were infected with one species; 42 % with two species and 18 % with three species of parasites. The results are summarized in Table 2.

Among the sheep, 37 % of animals possessed macroscopic cysts consistent with *S. tenella* and all positive animals possessed also minute microscopical cysts, most probably *S. bovis*.

**DISCUSSION**

Trypsinization gave best results with a fresh material in which the muscle cysts did not start to decay and the cystozoites were not dead. This method is very laborious and requires a good laboratory equipment. The examination of one sample lasts about one hour. Trypsin digestion recommended by Boch and McGuiness (1978) was not tested; the method is neither faster nor easier, but the results are not better than those obtained by trypsinization and the quality of the used peptic is variable. The digestion released a large number of zoites which can be easily found in the sediment after centrifugation, even in very light infections. This method cannot be applied for old or frozen samples, because the zoites are damaged during the digestion. Almost identical results were obtained with stereoscopy, which is less time consuming and does not require a special equipment. Since large samples of muscles can be examined, it is possible to detect even very light infections. In contrast to trypsinization, this method can be successfully used for the examination of old, partly disintegrated or frozen samples, where the microscopic identification of zoites is impossible after trypsinization. Both methods give the same good results, but stereoscopy is more advantageous, since the examination is more simple and rapid. The preparation and examination of one sample lasts maximally 5 min and up to 5 mm thick muscle samples of any size can be examined. The stereoscopy can become a routine method widely used for the detection of sarcocystosis.

Hinairy (1980) described a simplified method of homogenization by which a muscle sample can be examined within 8—12 min. The muscles are homogenized in a food mixer, and the homogenate is twice filtered and centrifuged. The fresh preparation is then examined under a stereoscopic microscope. The method is effective, but compared to the stereoscopy, it is more laborious and time consuming. Cerne and Horluka (1980, 1981) used homogenization for the detection of sarcocystosis in cattle and sheep. By this method they detected free zoites and fragments of cyst walls in the sediment after centrifugation. The method is effective, but more laborious than the stereoscopy.

The trichinoscopy and macroscopic observations are unsuitable for the detection of sarcocystosis due to their little effectivity.

Our results are consistent with the literary data on the occurrence of sarcocystosis. Only the pig examinations were negative in all cases. This is probably due to the very hygienic way of feeding and stabling in modern large-scale breeding farms where the animals are fed with practically sterile food mixtures and the probability of food infection with *Sarcocystis* is minimum. It is of interest that sarcocystosis was not found even in pigs from private breeders where the food could be contaminated. The examined material, however, was not large enough and it would be necessary to examine more animals to obtain a more exact information about the distribution of sarcocystosis in pigs.

In cattle and sheep, the occurrence of sarcocystosis is very high, which is due to their mode of feeding. The animals are kept out at grass or they are fed with grass from meadows where the definitive hosts of *Sarcocystis*, dogs, cats or man, can get and eliminate infective sarcocysts.

Of particular importance from the hygienic view is the high occurrence of *S. bovis* in cattle. This species was found in 66 % of slaughter cattle infected. Similar data were
reported by Boch et al. (1978) who detected S. hominis in 63.7% of cattle examined in the F.R.G. The definitive host of S. hominis is man and the wide distribution of this species is probably due to the liking for underdone or raw beef.

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EFEKTIVNOST MÉTOĐOV, PRIMENĚNYCH PRI OBSAHOVANÍ SARCOSSPORIDIOZ SCELJÍŠČÍCH HÔVÍ

Я. Гут

Renzemě. Triposkocení a streptocéka okašněly sejmy důzdebnými metody pro obnařízení sarcozopridií v domácím zvířeti. Sarcozystis family, S. vitilis a S. hominis obnařízeny u 87% z 200 obšetřených holců krátkého růžového skota a S. meleagris a S. tenella u 92% z 100 ovcí. Obšetření 200 bylin byl ohrožený.

REFERENCES


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