EPIDEMIOLOGY AND ETIOLOGY IN SHEEP SARCOCYSTOSIS

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Abstract: The aim of the present study was to investigate the prevalence, intensity of infection and etiology of sarcocystosis in sheep slaughtered in two abattoirs. The prevalence of intestinal sarcocystosis in naturally infected dogs and cats were also explored. Development of sporocysts in the small intestine of dogs and cats has also been investigated. The presence of macrocysts was observed in 10/38 carcasses being noticed only in esophagus. Concerning the prevalence of microcysts, the highest prevalence was observed in esophagus (81.6%), followed by myocardium (79.9%) and diaphragm (68.8%). In myocardium there was the highest number of microcysts (162 microcysts/g muscle tissue). The prevalence of intestinal sarcocystosis in naturally infected dogs and cats was 1.1%, respectively 1.8%. The pre-patent period in experimentally infected dogs was 12 days and the patent period lasted 60 days. At the microscopic exam (40X) there were noticed a mean of 4 sporocysts/microscopic field at 17 days post-inoculation. The size of sporocysts was 15.14±0.72/10.26±0.69 µm. No sporocysts were shed by cats. The microcysts were identify as Sarcocystis ovicanis (S. tenella).

INTRODUCTION

Sarcocystosis is an enzootic widespread muscle parasitosis, affecting mammals, birds and poikilothermic animals, usually asymptomatic, being observed during postmortem exam of carcasses. In rare cases of a massive infection, the animal may develop an acute syndrome, with nervous signs and abortion (Şuteu et al., 1996). Sarcocystis species are intracellular protozoan parasites with a requisite two-host life cycle based on a prey-predator (intermediate-definitive) host relationship (Fayer, 2004). Sheep are intermediate hosts for four species of Sarcocystis: S. ovicanis (S. tenella; definitive hosts: canides); S. arietcanis (definitive host: dog); S. ovifelis (S. gigantean; definitive host: cat) and S. medusiformis (definitive host: cat). The first two species are pathogenic, while the last two are nonpathogenic species (Dubey et al, 1989).

We performed this study in order (i) to estimate the prevalence of sarcocystosis in slaughtered sheep; (ii) to evaluate the intensity of infection (number of microcyst/gram of muscle tissue); (iii) to evaluate the prevalence of intestinal sarcocystosis in naturally infected dogs and cats and (iv) to reproduce sheep-dog and sheep-cat cycles by experimental infection with infected tissues, in order to identify the species of Sarcocystis.

MATERIAL AND METHODS

Biological samples

Tissue samples: Three different type of tissue samples (esophagus, myocardium and diaphragm) from 48 healthy sheep of 2-3-years-old, females, originating from extensively farms were collected in two abattoirs, S.C. Cetina S.R.L. (38 sheep) from Baia Mare
(Maramures county), respectively S.C. Miacarm S.R.L. (10 sheep) from Miraslău (Alba county).

*Faeces samples:* 406 faeces samples (184 from dogs and 222 from cats) were collected and examined by flotation method with saturated NaCl.

**Macroscopical examination.** At the moment of slaughtering the sheep carcasses were examined for the presence of *Sarcocystis* macrocysts.

**Microscopical examination.** It was performed for *Sarcocystis* microcysts identification in esophagus, myocardium and diaphragm by trichineloscopic method. For each type of tissue sample were examined 28 fields, finally 84 fields/animal. The microcysts in 28 fields/tissue sample were counted. Positive samples were weighted, to calculate the number of microcysts/gram of tissue.

**Experimental infection.** Two 6-month-old female dogs and two 10-month-old cats (a female and a male) were reared free of *Sarcocystis* infection. The dogs and cats were housed individually in cages throughout the study, fed by dry food and water *ad libitum*. Seven days before inoculation, faeces of all experimental animals were daily examined by flotation method. At day 0 the animals were fed with raw meat containing *Sarcocystis* microcysts from infected sheep, but free of macrocysts. Each animal received infected meat two consecutive days as it is described in Table 1.

### Table 1
Experimental infection of dogs and cats with microsarcocysts from sheep: grams of muscle tissue and number of microcysts for each animal

<table>
<thead>
<tr>
<th>Animals</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Total</th>
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<tr>
<td></td>
<td>Grams of muscle tissue (number of microcysts)</td>
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<tr>
<td>Dog 1</td>
<td>81 (2106)</td>
<td>68 (1768)</td>
<td>149 (3874)</td>
</tr>
<tr>
<td>Dog 2</td>
<td>107 (2782)</td>
<td>41.7 (1084)</td>
<td>148.7 (3866)</td>
</tr>
<tr>
<td>Cat 1</td>
<td>40 (1040)</td>
<td>80.66 (2097)</td>
<td>120.66 (3137)</td>
</tr>
<tr>
<td>Cat 2</td>
<td>34.45 (896)</td>
<td>0</td>
<td>34.45 (896)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>452.81 (11773)</strong></td>
<td></td>
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</tbody>
</table>

Faeces of each animal were examined daily for *Sarcocystis* sporocysts by flotation method from day 0 until day 23 post-inoculation (p.i.), and after this at 30 and 60 days p.i., only in dogs. The intensity of shedding was evaluated by notes from 0 to 4. Sporocysts were measured using AdobePhotoshop on microphotographs made with Olympus BX34 microscope, X40 objective.

**Statistical analysis.** The differences on the prevalence of *Sarcocystis* infection among the muscle groups taken in the study (esophagus, myocardium, diaphragm) was assessed using Chi-square test, Fisher's Exact test (GraphPad InStat 3) and t-Student test (Excel). There have been regarded with statistical significance, when the *p* was less than 0.05. The sizes obtained from 23 sporocysts were statistical analyzed using Excel program (arithmetic mean, and standard deviation).

**RESULTS AND DISCUSSIONS**

*Sarcocystis* infection in slaughtered sheep was detected in 44 out of 48 cases (91.7%). Macroscopical examination revealed macrosarcocysts only in oesophagus in 10 out of 38 carcasses (26.3%). Microsarcocysts were found in 81.6%(31/38) oesophagus, 79.9%(35/48) myocardiums and 68.8%(33/48) diaphragms (Table 2). Free spores (bradyzoites) were also noticed, both in meat juice and interfibrilare, in combination or not with the presence of
microcysts (Fig. 1). In the myocardium there were significantly \( p<0.02 \) more microsarcocyst/gram muscle tissue, in comparison with diaphragm and oesophagus (Table 2).

Prevalence of intestinal sarcocystosis in naturally infected dogs and cats was 1.08\%(2/184) (Table 3) and 1.8\%(4/222) respectively (Table 4). The positive dogs and cats were 1-2-year-old.

Following the experimental infection of dogs and cats with microsarcocysts neither changes in animal behaviour, nor clinical symptoms were observed. After a pre-patent period of 12 days, both dogs excreted free sporocysts in the faeces, during the patent period (60 days). The highest number of sporocysts was noticed at 17 days post-inoculation (4 sporocysts/microscopic field at 400X magnification) (Fig. 3). Sporocysts (Fig. 2) measured 15.14 µm X 10.26 µm (range: 13.9-16.6 µm X 9.3-11.7 µm); the wall 1.3 µm (0.9-1.9 µm); the sporozoites 9.2 µm X 3.2 µm (6.8-11.4 µm X 2.1-5 µm); residual body 6.4 µm X 5.4 µm (5.3–7.6 µm X 4.3-6.4 µm). The cats did not shed any sporocysts. Since the cats were fed by the same muscle tissue as the dogs, we estimate that the species of Sarcocystis was S. ovicanis.

<table>
<thead>
<tr>
<th>Prevalence and intensity of infection with Sarcocystis ovicanis in different muscles to sheep</th>
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<tbody>
<tr>
<td>Positive n (%)</td>
</tr>
<tr>
<td>Myocardium</td>
</tr>
<tr>
<td>35 (72.9)</td>
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<tr>
<td>Negative n (%)</td>
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<tr>
<td>Total</td>
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<th>Prevalence of intestinal sarcocystosis in naturally infected dogs from rural area</th>
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<tr>
<td>Positive n (%)</td>
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<tr>
<td>Negative n (%)</td>
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<tr>
<td>Total</td>
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<tr>
<th>Prevalence of intestinal sarcocystosis in naturally infected cats (indoor and outdoor cats)</th>
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<tr>
<td>Positive n (%)</td>
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<tr>
<td>Negative n (%)</td>
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<tr>
<td>Total</td>
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It is known that *S. ovicanis* (*S. tenella*) is one of the two pathogenic *Sarcocystis* species in sheep. However, infection is extremely common throughout the world and follows ingestion of food or water contaminated with sporocysts. Usually the clinical signs are discreet, and when present, include fever, anaemia, inappetance and weight loss or reduced weight gain. Sometimes can cause abortion and central nervous signs (hind limb weakness, ataxia, paresis), acute myopathy and death (Henderson et al., 1997; Buxton, 1998; Fitzgerald et al., 1993).

Our data showed a high prevalence of *S. ovicanis* (*S. tenella*) infection (91.7%) in sheep in Romania. Similar results had previously been found in other countries: 96.9% in Mongolia (Fukuyo et al., 2002); 97.0% in Iraq (Latif et al., 1999); 93% in Ethiopia (Woldemeskel and Gebreab, 1996) and 76% in Italy (Giannetto și col., 2005). In other countries the prevalence is lower as in Algeria 64.4% (Nedjari, 2003); in Turkey 58.9% (Ozkayhan și col., 2007); in Iran 57.7% (Oryan et al., 1996) and 47% in Slovakia (Maľ'a and Baranová, 1995).

The low prevalence of intestinal sarcocystosis in dogs from rural area cannot explain the high prevalence in sheep. It is needed to check the prevalence in dogs from sheep farms, where most likely the infection is more extended. Șuteu et al. (1989), twenty years ago found a higher prevalence of intestinal sarcocystosis in dogs from a caniche, between 3 (on October 1986) and 7% (on March 1987). This can be explained in part by changes in animal food, which is more industrialized. Our results are similar with that from Argentina (0.62%) (Thevenet et al., 2003) and Spain (2.5%) (Martínez-Moreno et al., 2007), but lower then in Switzerland (6.1%) and Germany (9%) (Sager et al., 2006; Barutzki and Schaper, 2003). Also, it was demonstrated that red foxes can be definitive
host for *S. ovicanis* (Dubey, 1983), and in this way a contamination source of pastures. Moreover, the patent period of *S. ovicanis* is longer than 60 days and a dog can shed between 150-200 million sporocysts (Latif et al., 1999).

Regarding the cats, the prevalence of intestinal sarcocystosis was 1.8%, and comparatively in Germany was described a prevalence of 2.2% (Barutzki and Schaper, 2003). The prevalence was noticed in cats older than 2 years, and significantly higher in outdoor cats. This aspect can be explained by the broader spectrum of prey species as potential intermediate hosts in the diet of outdoor cats than in indoor cats.

Better farm-management rules are needed to reduce the high prevalence of sarcocystosis in sheep in Romania. Since most of the sheep farms are extensively ones, a first step is to reduce the number of dogs existing around the flocks of sheep. A second step will be to feed the dogs with thermal treated organs and meat.

**CONCLUSIONS**

The study regarding the epidemiology and etiology of sarcocystosis in 48 sheep slaughtered in two abattoirs, pointed out the following:

- a high prevalence of sarcocystosis in sheep (91.7%);
- macrocysts were identified only in oesophagus
- the highest prevalence of microcysts was observed in esophagus (81.6%), followed by myocardium (79.9%) and diaphragm (68.8%);
- the highest intensity of infection (number of mycro cysts/gram muscle tissue) were noticed in myocardium (162/gram tissue);
- 1.1% of dogs from rural area and 1.8% of cats revealed an intestinal sarcocystosis;
- the *Sarcocystis* species in the present study was indentified as *Sarcocystis ovicanis* (*S. tenella*).

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**BIBLIOGRAPHY**