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PREVALENCE OF SARCOCYSTIS SPP. IN CATTLE IN ELMADAĞ, ANKARA, TURKEY*

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Elmadağ Yöresinde Sığırlarda Sarcosporidiose'un Yayılışı

Özet: Bu çalışma Elmadağ mezbahasında kesilen sığırlarda sarcosporidiose'un yayılışını incelemek amacıyla yürütülmüştür. Mayıs-Aralık 1988 tarihleri arasında kesilen 155 sığırdan alınan yemek borusu, diyafram ve çene kasları tripsin ile muamele edilmiş ve mikroskobik olarak Sarcocystis türleri aranmıştır. Araştırma sonunda 155 sığırdan % 94.84 ünün enfekte olduğu belirlenmiştir. Mikroskobik bakıda görülen kistler ince ve kalın kenarlı olarak sınıflandırılmış, ayrıca cinsiyet, yaş, ırk faktörlerinin enfeksiyon üzerindeki etkileri araştırılmıştır.

Aynı yörede bulunan 83 köpekten alınan dışkı örneklerinde parazitolojik yoklama yapılmış, Sarcocystis türlerine % 43.3 oranında rastlanmıştır.

Summary: An examination of Sarcocystis spp. in cattle slaughtered in Elmadağ, Ankara, Turkey was undertaken during May to December 1988. The muscle samples of oesophagus, diaphragm and masseter were subjected to tryptic digestion prior to the microscopic examination. Results indicated that 94.84 % of 155 cattle were infected. The cysts seen were grouped as thin- and thick-walled. The effect of sex, age and breed on the prevalence of infection were also determined.

Faecal samples of 83 dogs were examined to expose the parasitological pattern of the same region. The results indicated that 43.3 % of the dogs were infected with Sarcocystis spp.

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Introduction

Sarcocystis spp. have been recognized in cattle throughout the world for many years. Three species of Sarcocystis, each with an obligative two-host life-cycle in different hosts are found in cattle: Sarcocystis bovicanis which uses dogs, Sarcocystis bovifelis which uses cats and Sarcocystis bovihominis which uses man as definitive hosts (7, 23).

Sarcocystis bovicanis is the most common and severe pathogen in cattle (5, 13). Sarcocystis are found in the intermediate hosts both as schizonts in the endothelium of the blood vessels and as the cysts in the striated muscles (27). The mature sarcocyst in the muscle contains hundreds of banana-shaped bradyzoites (13). These are infectious for the definitive host (12).

The intermediate hosts acquire the infection by ingesting the sporocysts dispersed in the environment by the infective dogs (8, 27). Sporulated sporocysts are shed on the 9 th day of infection and they are immediately infective to cattle (7). There is a strong correlation between the number of sporocyst ingested and the severity of disease in cattle. For example, a dose of about 50.000 *Sarcocystis bovicanis* sporocysts is mildly pathogenic or nonpathogenic but 200,000 sporocysts is the lethal dose (12).

Sporozoites are released from the sporocysts in the intestine of the intermediate hosts and invade many tissues. Schizogony occurs in endothelial cells of blood vessels in most organs of the cattle, preceding the development of typical cysts in the muscles (7, 20). Cysts vary in length from a few micrometers to several centimeters. The thickness and structure of the cyst walls may vary with each Sarcocystis species (2, 12).

In cattle muscle cysts are distinguished readily by the structure of the cyst wall (11, 21). Under LM two morphologically distinct types of cyst are common (4, 9). Sarcocystis bovicanis cysts appear to be thinwalled with very slender protrusions around (4, 23). The cysts of Sarcocystis bovifelis and Sarcocystis bovihominis appear to be thick-walled with hair- like layer of protrusions on their outer surfaces under LM (3, 17).

Clinical signs of acute bovine sarcocystosis are: anorexia, pyrexia (42°C or more), anemia, cachexia, enlarged palpable lymph nodes, excessive salivation and loss of hair at the tip of the tail. These signs are evident at the time of vascular endothelium is parasitized by schizonts. The schizonts disappear in about one month and cysts are formed in the muscles. The cystic phase of sarcocystosis is virtually nonpathogenic (7).

A number of workers have contributed data to the prevalence and the identity of Sarcocystis spp. infections in cattle in several countries (4, 16, 18, 19, 20, 22). Thé finding of cysts in muscles prompted several investigations to determine the prevalence of intestinal protozoa of dogs (1, 6, 7, 15, 25, 26).

In this paper we report the prevalence of microscopic sarcocystis in muscle of 155 beef cattle from Elmadağ local abattoir in Ankara, and the intestinal parasitological patterns of 83 dogs of the same district.

Materials and Methods

The muscle samples of oesophagus, diaphragm and masseter from 155 cattle of both sexes and of various ages and breed were collected from Elmadağ abattoir in Ankara between May and December 1988. The samples were subjected to tryptic digestion to release the sarcocysts from muscle samples.

For the digestion technique approximately 15 g of muscle was finely chopped and digested for 20 min at 22 °C in 50 ml of digestion medium containing 100 mg tripsin in 50 ml PBS (pH: 7.5). After digestion the digestate was sieved to a beaker to remove the coarse particles and the filtrate was centrifuged at 1000 rpm for 5 min. The supernatant was removed by leaving 10 ml of the sediment. The reaction was stopped by adding 600 μ l of 1 % CuSO₄ solution and 666 μ l of mixed sediment was dropped on a slide and then examined for individual cysts at 10x magnification by LM.

The cysts examined were recorded as thin- or thick- walled and counts were given as number of sarcocysts per gram of muscle.

Faecal samples from 83 stray dogs from the same district were collected and examined by centrifugal-flotation methods. The solutions used were saturated sodium chloride, zinc sulphate and sodium chloride plus zinc chloride with densities of 1.20, 1.28 and 1.50 respectively. The parasites seen under LM were recorded.

Results

Intact sarcocysts were recovered from 147 of the 155 animals examined (Table 1). The effect of sex, age, breed on the prevalence of Sarcocystis spp. infections are shown in Table 1, 2 and 3 respectively.

| <u></u> | Animals | s examin | ned | | Infected | d anima | ls | Te | otal |
|---------|---------|----------|-------|-----|----------|---------|----------|-----|-------|
| . N | 1ale | Fe | male | м | ale | Fen | nale | | ! |
| No. | ~~~~ | No. | % | No. | % | No. | 0/ /0 | No | % |
| 48 | 30.97 | 107 | 69.03 | 47 | 97.9 | 100 . | 93.4 | 147 | 94.84 |

Table 1: Prevalence of Sarcocystis spp. infections in cattle according to sex.

Thin -and thick- walled cysts were easily distinguished by LM (Fig. 1 and Fig. 2). The prevalence and distribution of these cysts are shown in Table 4. The banana-shaped bradyzoites of a ruptured cyst is shown on Fig. 3.



Fig. 1. Thin-walled cyst

| Number of animals examined | | Infected animals | | | | | | | | | | | |
|-------------------------------|------------|------------------|------|-----|-----------|-----|------|-----|-----|-----|------|-----|-----|
| 155 | Age | ! | 1 | | 2 | | 3 | . 4 | | 5 | 5 | 6 | |
| | Prevalence | No. | % | No. | °∕₀ ~ | No. | _% | No. | % | No. | ~~~~ | No. | % |
| · | | 37 | 23.8 | 36 | 23.2 | 37 | 23.8 | 10 | 6.4 | 21 | 13.5 | 6 | 3.8 |

Table 3. Prevalence of Sarcocystis spp. infections in cattle according to breed.

| Number of animals examined | ł | : | | | Infected | d animals | | | |
|----------------------------|------------|-----|------|------|----------|-----------|-------|------|------|
| ļ··· | Breed | Nat | live | Hols | tein | Swiss | Brown | Jers | ey |
| 155 | Prevalence | No. | % | No. | % | No. | % | No. | % |
| l | <u></u> | 104 | 67.0 | 28 | 18.0 | 12 | 7.74 | 3 | 1.93 |

Table 4. Prevalence of thin- and thick-walled sarcocystis cysts in the oesophagus, diaphragm and masseter muscle of 155 cattle.

| | | Oesophagus | | Diap | hragm | Masseter muscle | | Total | |
|--------------------|-------------------|------------|------|------|-------|-----------------|------|-------|------|
| | | No. | % | No. | % | No. | % | No. | % |
| Thin walled cysts | No. of cyst / g | 4382 | 51.1 | 1035 | 11.4 | 3315 | 37.5 | 8732 | 100 |
| Thin-waned Cysis | No. of inf. anim. | 136 | 87.7 | 112 | 72.2 | 108 | 69.6 | 146 | 94.1 |
| Thick walled cysts | No. of cyst/g | 282 | 62.3 | 78 | 17.2 | 93 | 20.5 | 453 | 100 |
| | No. of inf. anim. | 50 | 32.2 | 38 | 24.5 | 24 | 15.4 | 79 | 50.9 |



Fig. 2. Thick-walled cyst with hair-like protrusions around



Fig. 3. Bradyzoites

Thin-walled cysts were found significantly (p < 0.001) more often in the oesophagus than that of diaphragm but not in the masseter muscle (51.1 %, 11.4 % and 37.5 % respectively). The prevalence of thickwalled cysts in the oesophagus was significantly (p < 0.05) higher than in the diaphragm and masseter muscles (62.3 %, 17.2 %, and 20.5 % respectively). During the study 79 of the 147 animals have shown mixed infections (Table 4).

Cyst lengths are ranged from 0–1900 μ m (mean is 686.31 \pm 72.45 for thin-walled cysts and 644. 25 \pm 60.74 for thick-walled cysts). Cyst widths are ranged from 0 to 160 μ m (mean is 71.18 \pm 7.12 for thin-walled cysts and 64.0 \pm 5.5 for thick-walled cysts). Protrusion widths are grouped into two populations: 2.5 μ m or 5.0 μ m.

The results of examinations in dog faeces are summarised in Table 5 and 6. No unsporulated sporocysts were seen in the faeces (Fig. 6).



Lengths of thick-walled cysts

[]] Lengths of thin-walled cysts



Fig. 5. Distribution of widths of thin-and thick-walled cysts Widths of thick-walled cysts

Table 5: Prevalence of intestinal protozoa of 83 stray dogs.

| | Infec | cted dogs | | |
|------------------|-------|-----------|--|--|
| Species | No. |) % | | |
| Sarcocystis spp. | 36 | 43.37 | | |
| Isospora spp. | 15 | 18.07 | | |
| Giardia canis | 2 | j 2.40 | | |
| Eimeria canis | 2 | 2.40 | | |

Table 6. The helmintological findings of 83 dogs

| | Infected dogs | | | | |
|--------------------|---------------|------|--|--|--|
| Species | No. | % | | | |
| Toxascaris leonina | 37 | 44.5 | | | |
| Taenia spp. | 36 | 43.3 | | | |
| Ancylostoma spp. | 19 | 22.8 | | | |
| Toxocara canis | i 11 | 13.2 | | | |
| Trichuris vulpis | 8 | 9.6 | | | |



Fig. 6. Sporulated sporocysts in dog faeces

Discussion and Conclusion

The geographic distribution of Sarcocystis spp. of cattle is worldwide and the prevalence of infection is high. Infection is important in meat inspection for being affective in muscle tissue in cattle.

To our knowledge, there are no published figures for the actual prevalence of sarcosporidiosis in cattle in Turkey though there are some survey studies in sheep and goats (14, 24).

Observed prevalence of Sarcocystis infection is 94.84 % in this study. During the study the muscle samples were examined by tryptic digestion technique for it is proved to be practical and reliable (19). The thin-walled cysts are morphologically indistinguishable from those of *Sarcocystis bovicanis* described by the others (2, 4, 23, 27). Virtually all cattle had been exposed to *Sarcocystis bovicanis* infection (94.1 %). This is comparable with the prevalences recorded in Australia (22) and India (16, 17).

Thick-walled cysts were recovered 50.9 % of animals but these cysts couldn't be identified as *Sarcocystis bovifelis* and / or *Sarcocystis*

bovihominis on morphological grounds. The continuous examination and the measurements of the thickness of cyst walls provided no basis for the seperation of the cysts into two types in terms of species as in agreement with the findings of Böttner et al. (3).

No significant differences in the prevalence of Sarcocystis spp. infections between male and female cattle were found. In the present study age and breed were seem to have a significant influence on the prevalence of infection. It was observed the more the age the less the tendency to infection and the native breed is more open to infection than the other breeds.

Protozoal patterns of dog facces revealed higher prevalence on Sarcocystis spp. than the prevalences found in Austria (15, 26), Switzerland (25) and California (1). However, less than the prevalence recorded in Elazığ, Turkey (10). The environment in Elmadağ must be heavily loaded with infective sporocysts as a result of many dogs and cats passing sporocysts on the pasture. The appearance and the size of sporocyst seen in the faeces resembled to those observed by Mahrt, J.L. (21) and Jain, P.C. and Shah, H.L. (18).

At the faecal examinations of the dogs, the methods involving flotations in three saturated salt solutions did not give different results. However, the saturated zinc sulphate solution seemed preferable since it deforms the Giardia cysts and enables the identification. This finding is in accordance with Burgu's (6).

Sarcosporidiosis have negative effect on the economies of nations and of breeders as well as on the health of people. There is a need to control parasitosis in order to safeguard both human health and animal productivity, but unfortunately diagnosis is not yet simple and accurate in live animals. What seems clear is that the identification of cysts in meat samples should be the point of approach and it is necessary to pay more attention to the occurrence of sarcosporidiosis.

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