TÜBİTAK

Short Communication

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Encephalitic Sarcocystosis and its Prophylactic Treatment in Sheep

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Abstract: The aim of this study was to examine the clinical and pathological findings in sheep naturally infected with severe encephalitic sarcocystosis and to evaluate the prophylactic effect of amprolium on the disease. From a flock of approximately 350 animals, 10 sheep were referred to the Veterinary Faculty Clinic with neurological symptoms that developed during the previous 2 weeks. These 10 sheep were clinically and pathologically examined, and the remaining animals in the flock without neurological symptoms were treated. All 10 animals were hypothermic, weak, and unable to stand. Complete blood count and serum biochemical analysis revealed mild leukocytosis, hypoglycemia, hyponatremia, hypochloremia, hypocalcemia, and hypoalbuminemia. Gross lesions were not observed during postmortem examination of the central nervous system (CNS). On histopathological examination focal areas of perivascular cuffing in the brainstem, cerebellum, and medulla spinalis that were comprised mainly of lymphocytes, monocytes, and plasma cells were observed. The lesions were associated with Sarcocystis sp. schizonts. Serological analysis for *Toxoplasma gondii* was negative. The animals in the flock that did not show neurological signs were prophylactically treated with amprolium 10 mg kg^{-1} day^{-1} for 5 days. No new neurological symptoms were seen in the flock after treatment.

Key Words: Encephalitic sarcocystosis, clinical findings, pathology, prophylaxis

Koyunlarda Ensefalitik Sarkosistozis ve Profilaktik Tedavisi

Özet: Bu çalışmanın amacı, koyunlarda gözlenen şiddetli ensefalitik sarkosistozis hastalığında klinik ve patolojik bulguları incelemek ve hastalığın proflaktik tedavisinde amprolium'un etkisini değerlendirmektir. Veteriner Fakültesi Kliniklerine iki haftalık bir sürede, 350 koyunluk bir sürüden sinirsel semptom göstererek getirilen 10 hayvan klinik ve patolojik olarak incelendi, diğer hayvanlara ise tedavi uygulandı. On hayvanın tümü hipotermik, zayıf ve yatar pozisyondaydı. Tam kan ve serum biyokimyası sonuçlarına göre hafif löykositozis, hipoglisemi, hipokloremi, hipokalsemi ve hipoalbuminemi saptandı. Postmortal incelemede merkezi sinir sisteminde lezyon saptanmadı, histopatolojik yoklamada; beyinde fokal perivasküler hücre infiltrasyonları, beyincik ve özellikle medulla spinaliste lenfosit, monosit ve plazma hücrelerinden oluşan infiltrasyonlar dikkati çekti. Histopatolojik incelemede lezyonların bulunduğu bölgelerde Sarcocystis sp. şizontları gözlendi. *Toxoplasma gondii* için yapılan serolojik inceleme negatifti. Sürüdeki sinirsel semptom göstermeyen hayvanlara proflaktik amaçla 10 mg/kg/gün dozunda amprolium 5 gün boyunca uygulandı. Tedaviden sonra sürüde sinirsel semptom gösteren hayvan gözlenmedi.

Anahtar Sözcükler: Ensefalitik sarkosistozis, klinik bulgular, patoloji, profilaksi

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Introduction

Sarcocystis comprise protozoan parasites that require carnivores and ruminants as definitive and secondary hosts, respectively (1,2). After ingestion of sporulated oocysts by the intermediate host, the released sporozoites pass through the intestinal tract and first multiply into groups of merozoites by endopolygony in the endothelial cells of arteries. Multiplication by endopolygony causes a rosette of merozoites, a distinguishing feature that differentiates Sarcocystis from other Sarcocystidae. Released merozoites undergo a second cycle in capillary endothelium in many tissues. The second generation of meronts releases merozoites that invade circulating mononuclear cells, undergo endodyogeny (division into 2), and are freed to enter type I and type II myofibers of skeletal muscle or the heart, where the metrocytes divide into large numbers of bradyzoites, forming the typical sarcocyst. The cycle is completed when the definitive host ingests infected muscle and the released bradyzoites develop directly into micro- and macrogamonts that in turn, form oocysts to be released in the feces as infective sporulated oocysts. The sporulated Sarcocystis oocysts are identical to those of Isospora, with 2 sporocysts, each containing 4 sporozoites. Variations in morphology, tissue distribution, and pathogenicity of certain Sarcosporidia spp. have been described (2,3).

Sarcocystis spp. were once considered of little pathologic significance; however, they are now recognized as important pathogens that can cause encephalitis, generalized disease, and abortion (2). Infestation of carnivores is asymptomatic, but ruminants may show numerous signs. Most infections are subclinical; however, animals become visibly ill after ingesting a sufficiently large quantity of parasites. Clinical signs of Sarcocystis infection usually begin 4-11 weeks after ingestion of infectious sporocysts. Acute disease is characterized by fever (> 39.5 °C), anorexia, weight loss, poor growth performance, bilateral lameness, muscular weakness, tremors, and diarrhea. Animals with chronic infections may develop limb edema, poor weight gain, hyperexcitability, hypersalivation, muscular atrophy, and pallor. Neurological signs include ataxia, recumbency, tonic clonic seizures, leg biting, blindness, head tremors, opisthotonus, flaccid forelimb paralysis, and nystagmus (1,3-8).

Ovine protozoal myeloencephalitis (OPM) is a neurological condition of sheep associated with Sarcocystis-like organisms (4,5). The identity of the sporozoa is in doubt, as discrimination between the intermediate host stages of *Toxoplasma gondii* and Sarcocystis spp., the 2 most likely etiological agents reported in earlier studies, has been difficult. Reports of some cases previously described as toxoplasma encephalitis are now thought to be descriptions of acute sarcocystosis (8,9). The aim of the present study was to describe the pathological findings of encephalitic sarcocystosis and evaluate the prophylactic effect of amprolium.

From a flock of approximately 350 animals, 10 sheep were brought to the Veterinary Faculty Clinic with neurological symptoms that developed during the previous 2 weeks. Due to poor prognoses they were presented to the Department of Pathology for euthanasia and diagnosis. After clinical examination blood samples were collected in EDTA tubes from all the animals prior to euthanasia. An MS9 blood counting device was used for hematological analysis of the blood. An IDEXX Vet-Test and reagents were used for biochemical analysis of the sera. All samples were tested for antibodies against *T. gondii* using an Abbott AxSYM autoanalyzer.

Following euthanasia, the organs, including CNS organs, were removed and examined grossly. Tissue samples were taken from all organs, including CNS organs, during necropsy and were fixed in 10% buffered formaldehyde. Using standard methods, tissues were embedded in paraffin and cut to sections 5 µm thick. Tissue sections were stained with hematoxylin-eosin (H&E) and examined microscopically.

All 10 sheep showed neurological symptoms on clinical examination, such as depression, incoordination, hind leg paralysis, and coma. Weakness and hind leg paralysis were typical clinical signs. All of the sheep fell into lateral recumbency, showed bilateral posterior ataxia, and were euthanized after blood sampling due to poor prognoses.

Slight to mild leukocytosis, hypoglycemia, hyponatremia, hypochloremia, hypocalcemia, and hypoalbuminemia were observed upon hematological and biochemical examination.

Gross lesions were not observed during postmortem examination of the organs and CNS. Histological examination showed that CNS lesions were present in all 10 sheep. The most severe lesions were in the gray and white matter of the caudal region of the cerebral cortex, and the spinal cord. Typical CNS findings were asymmetric, multifocal inflammatory cell infiltration and gliosis. Non-suppurative encephalitis and myelitis associated with Sarcocystis spp. were the prominent lesions (Figures 1 and 2). A small number of circular to oval protozoan cysts were present in the brain and spinal cord. Sporozoan cysts and meronts were widely distributed throughout the CNS, though with low density. The cysts varied in diameter, had thin eosinophilic walls, and contained numerous spindle-shaped basophilic merozoites. Numerous immature and mature schizonts, and individual merozoites were scattered freely in the cytoplasm, without a parasitophorus vacuole. The parasite was located in the cytoplasm, and rhoptries were absent in the merozoites. Focal gliosis and necroses were also prominent in the medulla spinalis. There was moderate to marked multifocal perivascular cuffing throughout the brain and spinal cord. Perivascular cuffing consisted primarily of lymphocytes, monocytes, and plasma cells, and was 4-10 cells thick. Microvacuolization of the neuropile near the areas of cuffing was present. Protozoa were observed in the center of the lesions. Lesions were much more frequently observed and prominent in the caudal region of the brain than in the middle and cranial regions. Spinal cord lesions were much more severe than those in the brain. Changes in the spinal cord were more severe than those in the ventral horns. The meninges and choroid plexus contained mild diffuse lymphocytic infiltrates.

Numerous round to oval protozoan cysts, including numerous merozoites, were also present in the heart and skeletal muscle (Figure 3). There were mild multifocal lymphocytic and plasmacytic infiltrates, together with cysts observed in the tongue, esophagus, and diaphragm.

Bacteriological and toxicological analyses were negative and no viral inclusion was observed in the CNS according to histopathological examination. Serological analysis was also negative for *T. gondii* antibodies in the sera of the affected animals.

The animals in the flock that did not have neurological signs were treated with amprolium 10 mg kg^{-1} day^{-1} for 5 days. No new neurological symptoms were seen in the flock after treatment.

Although lesions occurred throughout the CNS, there was a definite predilection for sarcocystic lesions in the spinal cord. Microscopic lesions, as described in the literature, include prominent necrosis, hemorrhage, perivascular cuffing with lymphocytes and macrophages, the presence of parasitic stages in the neuropile associated with gitter cells (lipid-laden macrophages), eosinophils, multinucleate giant cells, and varying numbers of neutrophils (9). Additionally, the presence of gemistocytic

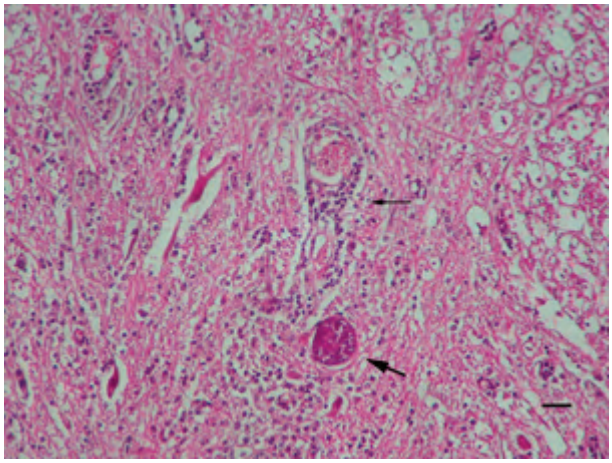

Figure 1. Perivascular cuffing (thin arrow) and a Sarcocystis sp. schizont (thick arrow) in the medulla spinalis. Bar = 50 mm.

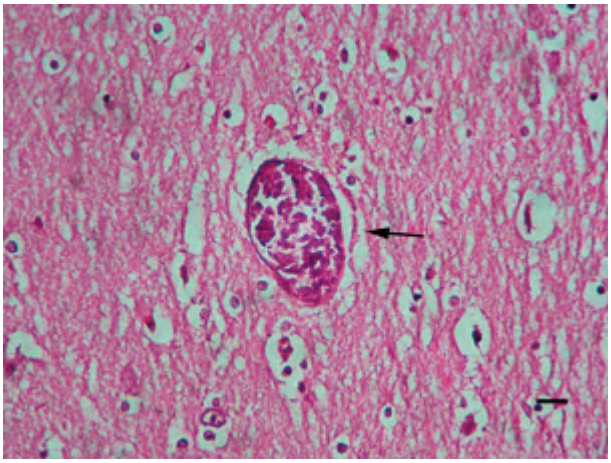

Figure 2. A Sarcocystis sp. schizont in the brain (arrow). Bar = 100 mm.

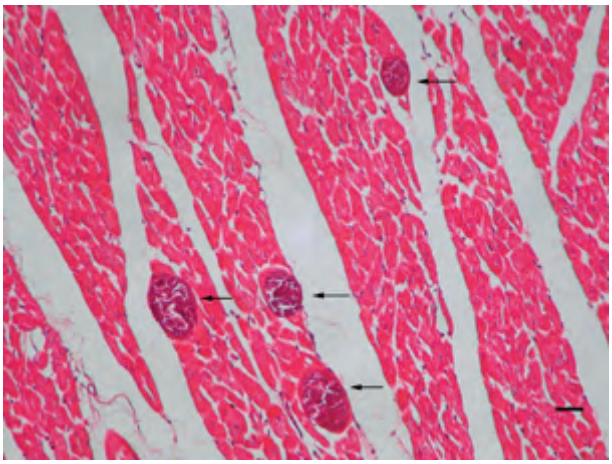

Figure 3. Numerous Sarcocystis sp. schizonts in the heart muscle (arrows). Bar = 100 mm.

astrocytosis and neuronal degeneration with axonal swelling, and sometimes leptomeningitis are reported. The etiologic protozoan agent is small and crescent-shaped to round, and has a well-defined nucleus (10). Histopathologically confirmed lesions in all the ewes in the present study closely resembled those previously reported. In the present study sarcocystis structures in the CNS were similar to those in muscle. There were more parasitic stages in the spinal cord than in the cerebrum and cerebellum. Although severe infiltration and gliosis were seen in the CNS of all the animals, myelomalacia was not detected in any of the ewes. Some authors reported that in naturally occurring cases of sarcocystosis schizogony might be completed by the time neurological signs develop. Various reports describe protozoan schizonts throughout the brain and spinal cord (5,10,11). Similar findings were observed in the present study. The absence of *T. gondii* antibodies was an important finding that aided diagnosis. Sarcocystosis is diagnosed according to parasite morphology and characteristic histopathological findings.

Sarcocystis spp. are obligate 2-host parasites. The final host is a carnivore in which sexual reproduction takes place in the intestinal wall before oocysts are produced and passed in the feces. Fecal contamination of the food of the intermediate host leads to infection and the 3 asexual reproductive cycles then take place to form infective cysts. These cycles, from the time of ingestion until cysts are found in muscle, last about 5-7 weeks and by 11 weeks the cysts are infective to the final host, in this case the dog or fox (4). Sheep are kept together with sheepdogs in Turkey; therefore, the incidence of sarcocystosis is relatively high, but the agent is generally localized in muscle. There is only one previous report of encephalitic sarcocystosis in sheep in Turkey (12). In contrast to previously reported cases, in the present study clinical signs were severe and the disease caused mortality (3,12).

The parasite in sheep is thought to be a Sarcocystis sp., because it is located in the cytoplasm without a parasitophorus vacuole and rhoptries are absent in merozoites. These findings are specific to Sarcocystis spp. (4). A similar morphology was observed in the present study.

There is no effective treatment for sarcocystosis described in the literature (1). In the present study we also observed that none of the sheep recovered after nervous system symptoms began. Following amprolium treatment, no new cases with nervous signs were seen, and although we did not prove the other animals were indeed infected, we think that amprolium can be effective in preventing the development of clinical encephalitic sarcocystosis. It is advisable to use this drug for prophylactic purposes when clinical signs are observed in some animals in a flock. This could help to prevent the spread of the disease, which has a high incidence in Turkey.

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