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TÜBİTAK

Case Report

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Massive toxoplasma abortions in a dairy sheep flock and therapeutic approach with different doses of sulfadimidine

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Abstract: In a 500 head dairy sheep flock in Northern Greece, massive abortions (60%) were observed at 110-130 days of gestation. Attempts were made to treat the abortions with oxytetracyclin L.A. 20% but they were not successful. Toxoplasmosis was confirmed by laboratory examination of blood sera of ewes, aborted fetuses, and fetal brain samples. The remaining 200 pregnant sheep were divided in 2 groups of 100 ewes each. Animals of Group 1 were treated with 20 mg/kg b.w. sulfadimidine every other day 4 times, while the animals in Group 2 received 33 mg/kg b.w. sulfadimidine every other day 4 times via i.m. route. Considerable reductions in abortions were observed. The treatments were more effective in Group 2. Lactation started in both groups after treatment and satisfactory milk was produced.

Key words: sheep, toxoplasmosis, abortions, treatment, sulfadimidine

Bir süt koyunculuğu işletmesinde toxoplazmozis'e bağlı toplu abortlar ve buna değişik dozlarda sulfadimidine ile tedavisel yaklaşım

Özet: Kuzey Yunanistan'da süt tipi koyunculuk yapan 500 başlık bir işletmede, gebeliğin 110-130. günlerinde toplu abortus vakası gözlemlenmiştir. İşletmeyi ziyaretimizde, sürüde bulunan toplam 500 baş koyundan 300'ünde (% 60) abortus şekillenmişti. Öncenden, Oxytetracyclin % 20 L.A. ile yapılan tedavi denemesinin sonuçsuz kaldığı gözlenmiştir. Toxoplazmozis, anne ve atık fötüslerin kan serumu muayenesi (testi) ve aynı zamanda fötüslerin beyinlerinden yapılan frotilerle teşhis edilmiştir. Tedavi sonrası her iki grupta da abortus vakalarında belirgin azalma görülmüştür. İkinci grupta tedavi etkinliği daha başarılı bulunmuştur. Her iki tedavi uygulaması sonrasında hayvanlarda normal laktasyon başlamış ve yeterli süt verimi elde edilmiştir.

Anahtar sözcükler: Koyun, toxoplazmozis, abortus, tedavi, sulfadimidine

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Introduction

Toxoplasmosis is a zoonosis caused by the protozoan parasite *Toxoplasma gondii*. Felids are the definitive hosts, while endothermic animals are the intermediate hosts of the parasite (1). In pregnant sheep toxoplasmosis may cause embryonic death and resorption, abortion, fetal mummification, stillbirth and neonatal death (1,2). Immunosuppressed sheep may also develop a nervous form of the disease (3). To date, toxoplasmosis has not been diagnosed in Greek sheep or goat flocks, although many seropositive animals have been found in recent seroprevalence studies (4).

A rapid diagnosis of toxoplasmosis can be made following the examination of impression smears from the lesions (1). Furthermore, diagnosis may be established based on mother and fetus serum antibody detection, placental and fetal brain histopathology, immunohistochemistry, and PCR (2).

Toxoplasmosis seems to be controlled effectively with the use of live vaccines applied before the mating period, although their use may have some disadvantages (1,2). However, vaccines cannot be used in flocks with pregnant sheep that abort due to toxoplasmosis; in these cases, the administration of appropriate treatment is necessary to stop the abortions. To date, studies have been conducted to control experimental infections with *T. gondii* in non-dairy sheep using monensin (5), combination of sulfadimidine and pyrimethamine (6), decoquinate (7), and combination of sulfadimidine and baquiloprim (2). Furthermore, in other animal species and man spiramycin, clindamycin, atavaquone, arithromycin, clarithromycin, roxithromycin, and dapsone have been used with various results (1).

In the present study a massive outbreak of toxoplasmic abortions in a Greek dairy sheep flock is described. Moreover, the therapeutic efficacy of 2 different doses of sulfadimidine were evaluated and compared and their effect on subsequent milk production is described. To the best of the authors' knowledge, this is the first evaluation of a therapeutic approach for the disease under field conditions with 2 different dose schemes and the first report of reduced milk production in a dairy flock attributed to toxoplasmosis abortions.

Case history

Massive abortions were observed at 110-130 days of pregnancy in a sheep flock in northern Greece that consisted of 500 dairy Lacaune ewes. All the sheep were regularly de-wormed and vaccinated against chlamydiosis, brucellosis, contagious agalactia, and enterotoxemia. All the sheep of the flock were at the same stage of gestation, as they had been subjected to estrus synchronization. At the time of our initial visit to the farm, about 300 ewes (60%) had aborted, while empirical treatment by local veterinarians with long acting oxytetracycline had been ineffective. Although no deaths were observed among the ewes that had aborted, the problem was further compounded by the fact that the animals that had aborted did not produce milk, probably because the short length of their gestation did not allow their udders to develop adequately. According to the owner, the flock had no history of abortions in the previous years, while he noted that a litter of kittens were found in the farm 2 months earlier.

On the day of our initial visit to the farm, 5 sheep had aborted a total of 8 fetuses. Five of these fetuses had been mummified (Figure 1), while the remaining 3 were grossly normal. Fetal membranes were not found. The aborting ewes were febrile (>40 °C) and inappetent.

Vaginal swabs taken in duplicate from the 5 ewes on abortion day, as well as liver and the abomasal contents from the 3 normal in appearance aborted fetuses were submitted to the Laboratory of Microbiology and Infectious Diseases for bacteriological testing.

Figure 1. A mummified ovine fetus.

Vaginal swabs were examined for *Chlamydophila abortus* and *Coxiella burnetii* with microscopic examination after staining with modified Ziehl-Neelsen (MZN). In addition, the Clearview® kit (Unipath Ltd., Bedford, UK) and PCR assays were used for the detection of chlamydial antigen or DNA (8), respectively. On the other hand, examinations for *Brucella*, *Salmonella*, *Listeria*, *Campylobacter*, and *Mycoplasma* were conducted by culturing fetus materials according to standard isolation and identification methods.

The brains of the 8 aborted fetuses (5 mummified and 3 grossly normal), blood serum from the 3 grossly normal fetuses, as well as blood serum from the 5 ewes that had aborted the same day and 6 ewes that had aborted 15 days earlier were submitted for examination to the Laboratory of Parasitology and Parasitic Diseases.

The impression smears from the brains were examined according to a method described by Dubey (9). A cerebral portion (enough to fill an area under 22 mm cover slip) was crashed between a glass slide and a cover slip and examined unstained under light microscope for tissue cysts (3-4 smears for each sampled brain) at 1000× magnification. Also, one half of the brain of each animal was homogenized with a manual glass homogenizer in 0.5 mL of normal saline. A small portion of the above was spread on 2-3 slides and allowed to air dried. Samples were stained with Giemsa and examined for *T. gondii* tissue cysts at a magnification of 1000×.

ELISA was performed in serum samples as previously described (10), using soluble *T. gondii* antigen (from in vivo cultured parasites) in a final concentration of 3 μg/mL. Sera were tested at the concentration of 1/300 and the secondary antibody used was Anti-Sheep IgG (A-5187 Sigma®). The substrate used was p-nirophenyl phosphate (Sigma®) and optical densities were measured with a 405 nm measurement and a 630 nm reference filter, using the microplate reader HUMANREADER (HUMAN Diagnostic Systems, Germany). Cut-off value was determined by the mean of negative controls plus 3 standard deviations.

Once the diagnosis of toxoplasmosis was confirmed, a treatment with sulfadimidine was undertaken. One group of 100 pregnant sheep (Group 1) was treated with intramuscular injections of sulfadimidine (Sulphadimidin®-CEVA) in dose 20 mg/kg (1 injection every 2 days, 4 times in total). The other group of 100 animals (Group 2) was treated with intramuscular injections of sulfadimidine (Sulphadimidin®-CEVA) in dose 33 mg/kg (1 injection every 2 days, 4 times in total).

Results and discussion

Examinations for *Chlamydophila abortus*, *Coxiella burnetii*, *Brucella*, *Salmonella*, *Listeria*, *Campylobacter*, and *Mycoplasma* were negative.

The impression smears from the brains revealed cysts morphologically consistent with *T. gondii* cysts (Figure 2). High *T. gondii* antibody titers (IgG) for all the examined ewes were detected. Moreover, the blood serum of 1 fetus was found positive to *T. gondii*, while the other 2 grossly normal aborted fetuses were found seronegative. ELISA was also performed in the same samples for *Neospora caninum* and all were found negative.

From the 1st injection abortion rate started to decline in both groups. Abortions stopped totally after the 4th injection in the animals of Group 2, while they continued with a lower rate in Group 1. During the treatment 7 animals in Group 2 (7%) aborted, while no abortions were observed subsequently (total abortion rate 7%). The lambs that were born in this group were lighter from normal, but many of them survived (survived 90 out of 120 neonatal

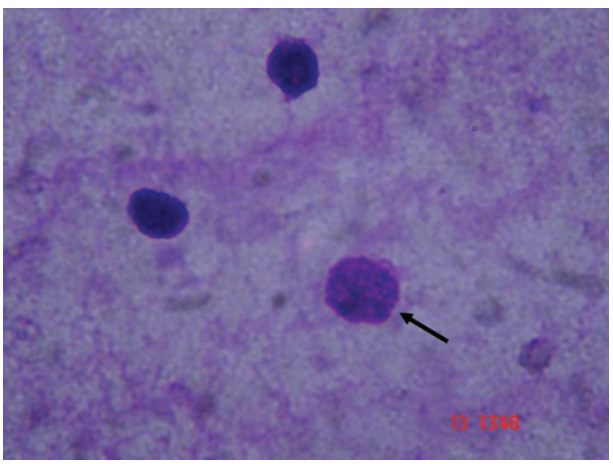

Figure 2. *Toxoplasma* cyst (arrow) in a Giemsa stained brain smear (1000×).

lambs-neonatal viability 75%). The gestation period of the treated animals was normal and they had also normal milk production. In Group 1 ten sheep aborted during the treatment (10%) and 15 after the treatment (total abortion rate 25%). However, 10 out of 15 animals had long enough gestation periods to reach their udder size in milk production levels. The rest of the ewes gave birth normally, but most of their lambs were stillbirths and did not survive (died 60 out of 98 neonatal lambs-neonatal viability 38.9%). However, the ewes showed normal milk production.

This is the first reported case of abortion attributed to toxoplasmosis in Greece, although data exist for *T. gondii* seroprevalence in many Greek flocks (4). A similar condition seems to exist in other geographical areas, such as New South Wales in Australia, where although many sheep are seropositive in toxoplasmosis, toxoplasmic abortions are rare (11). In endemic areas, the annual abortion rate is low, i.e. up to 2% (12), as sheep usually abort when the ewe is infected for the first time in mid pregnancy (13). In animals that were previously non-infected abortion rate from toxoplasmosis can be high, as has been shown in experimental infections with *T. gondii* in seronegative flocks (6,7). This probably occurred in the flock of the present study, as there was no history of abortions in the previous years. It is possible that the immune protection of the animals against toxoplasmosis was minimal and when the antigen was introduced in the flock (probably with the kitten litter), a storm of abortions appeared (2).

The financial losses in the flock were important, and due to both the large number of the aborted fetuses as well as the loss of milk production, as the abortions took place at 110-130 days of pregnancy, when the udder was not yet developed for the following lactation period (14).

Toxoplasmosis was diagnosed with combination of fetal brain smears along with mother and fetal serology. The cysts found in the brain of all 8 examined aborted fetuses were morphologically consistent with *T. gondii*. Microscopical cyst detection is a rapid way of diagnosing the disease (1). These brain cysts should be differentiated from cysts of *Neospora caninum*. Pathognomonic was that blood serum of 1 normal in appearance fetus (1 out of 3 examined) was seropositive for toxoplasmosis. Diagnosis was also confirmed from serological examination of the sheep that aborted, as they were found seropositive for toxoplasmosis and seronegative for neosporosis. Moreover, neosporosis is not considered a common cause of sheep abortions, although it is a common cause of abortion in cows (2).

To date, the existing treatment protocols for sheep toxoplasmosis were studied in experimental infections (2,5-7). Only one report exists for toxoplasmosis treatment with trimethoprim and sulfadimidine under field conditions, but the doses used were much higher and different to those in the present study (15). From the already existing treatments, sulfadimidine is an easily available substance in the Greek market. It has been used against ovine toxoplasmic abortions together with baquiloprim (2), pyrimethamine (6), and trimethoprim (15). In this emergency condition sulfadimidine was tested alone in 2 different dose schemes, but were used in lower doses and less frequent injections compared to earlier natural (15) and experimental (6) studies.

A disadvantage of the therapeutic approach in the present case report is that no control animals were used, but as the previous losses (60% abortion rate and no milk production) were high, for economic reasons it would be very harmful for the farmer to permit the use of control animals. Also, welfare reasons did not permit us to use control animals. The finding that the higher dose of sulfadimidine (33 mg/kg) was more effective than the lower dose (20 mg/kg) enhances the hypothesis for the efficacy of sulfadimidine against toxoplasma abortions in sheep. The present study could be considered as preliminary results for a useful treatment protocol.

In conclusion, this is the first report of massive ovine toxoplasmosis abortions in Greece. The most probable explanation for the high abortion rate was the immunological incompetence of the animals to the parasite. The treatment with 4 sulfadimidine injections intramuscularly in dose 33 mg/kg, always the same every 2 days seems to be effective, as it reduces the abortion rate and subsequently helps sheep to have a normal length of gestation and subsequently normal milk production.

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