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RESEARCH ARTICLE

Investigation on abortion mechanism of ovine sarcosporidiosis

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Özet

Aldemir OS, Seyrek K, Yenisey Ç, Eren H, Ünlü H. Koyun sarcosporidiozis enfeksiyonunda abort oluşum mekanizmasının araştırılması.

Amaç: Bu çalışmada koyun sarcosporidiosis enfeksiyonu ile prostaglandin F2α F2 düzeyi aralarındaki ilişki incelenerek abort mekanizması üzerindeki etkisi açıklanmaya çalışılmıştır.

Gereç ve Yöntem: Materyaller Aydın bölgesi mezbahanelerinden elde edildi. Parazit izolasyonu amacıyla PCR ve modifiye tripsin tekniği kullanıldı. Ayrıca kan örneklerindeki prostaglandin F2α düzeyleri ticari kit yardımı ile enzimimmunoassay (EIA) yöntemiyle belirlendi.

Bulgular: İncelenen 114 koyunun 9 (%7.89)'unda *Sarcocystis* spp. makro kistleri, 58 (%55.23)'inde mikro kistler tespit edildi. Saptanan mikro kistlerin dağılımı ise 32 (%30.47)'sinde *S. tenella*, 17 (%16.19)'sinde *S. gigantae* ve 9 (%8.57)'unda *S. arieticanis* olarak belirlendi. Ayrıca 114 koyundan alınan kan örneklerinden 9 (%7.89) örnekte 300 ile 402 pg/mL, 47 (%41.22) örnekte 200 ile 300 pg/mL ve geri kalan örneklerde ise 53 ile 200 pg/mL arasında değişen değerlerde prostaglandin F2α düzeyi belirlendi. Gruplar arası fark istatistiksel olarak anlamlı bulundu (P<0.001).

Öneri: Özefaguslarında makrokist ve mikrokist tespit edilen koyunların prostaglandin F2α düzeyleri arasındaki ilişki incelendiğinde aborta neden oluşturacak türe özgü farklı değerlerde prostaglandin F2α saptanmıştır. Sonuç olarak sarcosporidiozis enfeksiyonunda abort oluşumunun prostaglandin F2α ile ilişkili olabileceği kanaatine varılıştır.

Anahtar kelimeler: Koyun sarcosporidiosis, abort, prostaglandin F2α, PCR

Abstract

Aldemir OS, Seyrek K, Yenisey Ç, Eren H, Unlu H. Investigation on abortion mechanism of ovine sarcosporidiosis.

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Aim: In this study, the relationship between the level of prostaglandin F2α and ovine sarcosproridiosis infection were investigated for abortion mechanism.

Materials and Methods: Materials were collected at slaughterhouse of Aydın region. In order to identification of parasite, macroscopical and microscopical cysts were used for *S. gigantea* and *S. arieticanis* or *S. tenella* by the PCR and Modified Trypcine Technique. Also in order to determination of the level of prostaglandin F2α in blood samples were used the methods of enzimmunoassay using a commercial kit (EIA).

Results: A total number of 114 sheep oesophageal muscle were found in 9 (7.89%) to be infected with Sarcocystis macroscopic cysts. Examinations of a total number of 114 sheep were found in 58 (55.23%) of the sheep with microscopic cyst. These microscopic cyst species corresponding values were as follows: 32 (30.47%), 17 (16.19%) and 9 (8.57%) for *S. tenella*, *S. gigantae* and *S. arieticanis*, respectively. In addition a total number of 114 sheep blood examinations were determined in 9 (7.89%) samples at 300 and 402 pg/mL, 47 (41.22%) samples at 200 to 300 pg/mL and 58 (50.87%) samples at 53-200 pg/mL ranging between the levels of prostaglandin F2α. The differences between groups were found statistically significant (P<0.001).

Conclusions: In this study were detected in different values of the level of prostaglandin F2α, when the relationship between the level of prostaglandin F2α and macrocyst and microcyst in the oesophageal muscle of sheep were assessed. In conclusion, the aborts formation in Sarcosporidiosis infection may be associated with prostaglandin F2α identified.

Keywords: Ovine sarcosporidiosis, aborts, prostaglandin F2α, PCR

Introduction

Sarcocystis spp. are obligatorily intracellular parasites with a typical coccidian life cycle consisting of merogony, gamogony and sporogony that are prevalent in domestic animals, humans, wild animals, birds and some cold-blooded animals throughout the world. The protozoon parasite utilizes vertebrates as both its intermediate and its definitive host. The intermediate host becomes infected by food or water contaminated with sporocysts (Erber 1980, Barker 1984).

Today the intermediate and final hosts are known for 56 of 122 named species. The genus Sarcocystis belongs to the phlum apicomplexa with differences in life cycle and pathogenicity. Pathogenic *Sarcocystis* spp. can cause disease in their intermediate host, in particular in cattle, sheep, pigs and wild cervids (Dubey et al 1986, Tenter 1995). Some animals show neurological signs. In pregnant ewes, experimental infection with microscopic *Sarcocystis* spp. can lead to abortion, fetal death, and stillbirth. During the chronic phase of the infection, weight gain and wool growth can be affected. But the most pathogenic effect of *Sarcocystis* spp. is abortion in pregnant ewes (Ellis et al 1995, Aldemir and Dik 2003, Aldemir and Güçlü 2004).

There are a lot of cases of abortion in pregnant ewes in world. The abortions may be different source for example noninfectious or infectious agents. The infectious agents caused abortions are especially Campylobacteriosis, Salmonellosis, Listeriosis and Chlamidia (Muday 1984, Latif et al 1999).

Although both macroscopic and microscopic cysts of *Sarcocystis* spp. are not found to fetus or reproductive tract (Macro and micro) the abortion of sarcosporidiosis have been still unexplored which caused by the mechanism. However, some authors (Daugschies et al 1990, Tenter 1995) stated that may be related to prostanoids levels.

Therefore, in this study has been investigated to relationship between the level of prostaglandin F2α and *Sarcocystis* species of sheep the first time in the world. So, abortion mechanism of sarcosporidiosis has been asked to be explained.

Materials and Methods

Material

Materials were collected at slaughterhouse of Aydın region. The entire length of each esophagus was examined by close visual inspection for infections by macroscopic cysts and the materials were investigated by modified trypcine and PCR technique.

Modified Trypcine Technique

The trypcine technique described by Gut, (1982) was slightly modified as follows; a total of 114 sheep were examined for Sarcosporidiosis infection. In order to identification of parasite, macroscopical and microscopical cysts were used for *Sarcocystis gigantea* and *S. arieticanis* or *S. tenella.* Pieces of esophagi (15 g each) were minced stirred for 20 min at 25ºC in magnetic water bath and then the suspension was decanted and filtered through gauze. The cystozoits were isolated by density gradient centrifugation as described in detail (Owen et al 1998, Latif et al 1999).

PCR technique

The protocol described by Joachim (1994) was followed with minor modifications to isolation of parasites, extraction of DNA and optimize PCR conditions in the study.

Isolation of parasites

Sarcocystis spp: Materials were obtained from the oesophagi, hearts and diaphragms of sheep. Macroscopically visible tissue cysts were excised and manually disrupted with a glass homogenizer. The suspension was filtered through 3 layers of gauze and cells were isolated by density gradient centrifugation as described by Tenter et al (1995). The purified cystozoites were resuspended in PBS (pH 7.2). Each suspension was stirred at medium speed on a magnetic stirrer, its pH was gradually reduced to 5.5 over 45 min by dispensing drops of 1 N HCl and then restored to 7.2 by dispensing drops of 7.5% $NaHCO_3$. The suspensions of cystozoits were stored in liquid nitrogen until required.

Extraction of DNA

Cystozoites (*Sarcocystis* spp.) were carefully resuspended in 10 ml of cold buffer (10 mM Tris-HCl, 10 mM EDTA, pH 8.0). The solution was incubated for 1 hour at 37°C and then lysed with sodium dodecyl sulphate at a final concentration of 1%. This was followed by proteinase K treatment (100 µg/mL). Using a glass rod, the enzyme was gently mixed into the viscous solution and incubated for 5 hours at 56°C with some movement. The suspension of lysed cells was left in a water bath for 3 hours at 50° C and the viscous solution was swirled periodically. The protein was precipitated with 2 ml of sodium perchlorate and an equal volume of phenol equilibrated with 0.5 M Tris (pH 8.0) was added and mixed gently for 10 minutes for the extraction of DNA. To improve the specificity of the RAPD-PCR, the of parasite, macroscopic and microscopic cysts were used for *Sarcocystis gigantea* and *S. arieticanis* or *S.tenella.* A total number of 114 sheep oesophageal muscle were found in 9 (7.89%) to be infected with Sarcocystis macroscopic cysts. A total number of 114 sheep were found in 58 (55.23%) of the sheep with microscopic

cyst. These microscopic cyst species corresponding values were as follows: 32 (30.47%), 17 (16.19%) and 9 (8.57%) for *S. tenella* (Figure 1), *S. gigantae* (Figure 2) and *S. arieticanis* (Figure 3), respectively. Also the numbers of DNA fragments amplified by SAT-1 primer was obtained for *S. tenella*, *S. arieticanis* and *S. gigantae* (Figure 4).

Plasma prostaglandin F2α concentrations of 9 (7.89%) sheep infected with Sarcocystis macroscopic cysts, 58 (55.23%) sheep with microscopic cyst and 47 (%41.2) noninfected sheep were given in Table 1.

Discussion

Sarcocystis species have a predator-prey, 2 host life cycle. Herbivores (prey) acquire infection by ingesting sporocysts that are shed in the feces of infected carnivores (predator) (Dubey et al 1986). Carnivores become infected with *Sarcocystis* spp. by ingesting the encysted form of the parasite in the musculature of herbivores. Both domestic and wild carnivores are hosts for Sarcocystis infecting cattle and sheep. *Sarcocystis* spp. are prevalent parasites in livestock and a world-wide distribution. Nearly, 100% cattle and sheep are infected (Dubey et al 1986, Aldemir 2006).

Four species of Sarcocystis are known to infect sheep. *Sarcocystis tenella* and *S. arieticanis* are transmited by canids and form microscopic cyst in sheep (Güçlü et al 2004). Their first and second generation schizonts are pathogenic. The cat-borne species *S. gigantea* and *S. medusiformis* form macroscopic cysts and have no observed pathogenic effects in sheep (Munday 1984, Sevinç et al 2000). Sarcosporidiosis has been diagnosed by several methods, digestion, trichinoscope, staining with methylene blue, histological techniques

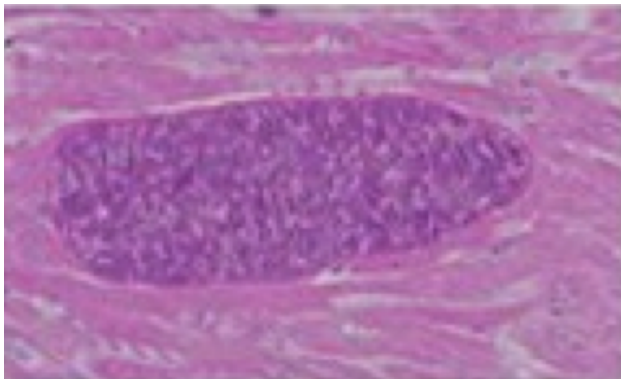

Figure 1. *S. tenella* (Giemsa stain X375)

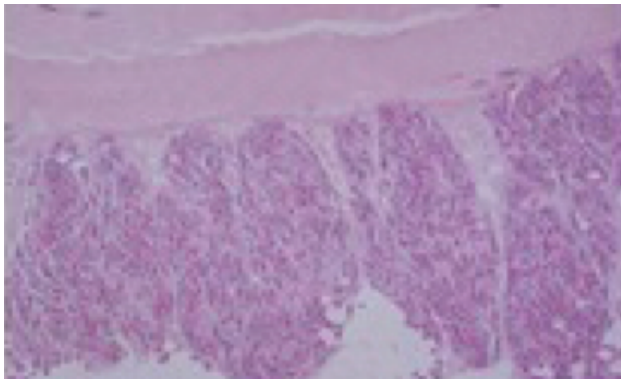

Figure 2. *S. gigantae* (Giemsa stain X375)

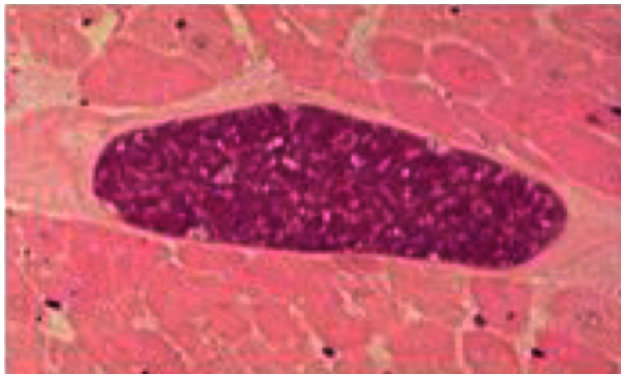

Figure 3. *S. arieticanis* (Giemsa stain X375)

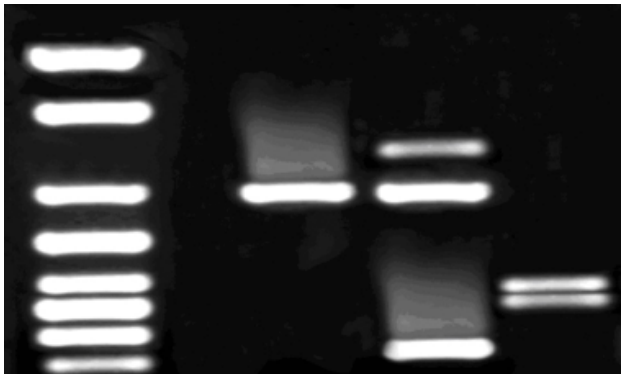

Figure 4. Amplification of genomic DNA, Ω: Marker, Nc: Negative control, St: *Sarcocystis tenella*, Sa: *Sarcocystis arieticanis*, Sg: *Sarcocystis gigantae*

Tablo 1. Plasma prostaglandin F2α concentrations of sheep (mean±SE).

Sheep infected with Sarcocystis macroscopic cysts (n:9)	Sheep with microscopic cyst (n:58)	Nonifected sheep (n:47)	P
339±1.22[a]	248±5.61[b]	124±6.27[c]	***

***: P <0.001, Mean in row with letters shown significantly (P<0.05)

and PCR. In addition, IFAT, ELISA, Agglutination and hemagglutination methods are used for the serological diagnosis of sarcosporidiosis (Munday 1975, Joachim 1994, Aldemir and Dik 2003).

In the present study, modified trypcine and PCR technique were used macroscopic and microscopic cysts. Also for blood samples were used the methoths of enzimmunoassay using a commerical kit (EIA). Infections of sheep by *Sarcocystis* spp. are cause of concern to the meat industry mainly (Sevinç et al 2000). The macroscopic cysts in the stiated muscles and microscopic cysts in the musculature of sheep are distributed world-wide (Taşcı and Değer 1989, Aldemir and Dik 2003, Aldemir and Güçlü 2004, Aldemir 2006). The prevalence of microscopic cysts and macroscopic cysts is reported to be 92.95% and 19.76% respectively, in Turkey (Taşcı and Değer 1989, Sevinç et al 2000, Aldemir and Dik 2003, Aldemir and Güçlü 2004, Aldemir 2006).

In the present study, a total number of 114 sheep were found in 58 (55.23%) of the sheep. The prevalence of Sarcocystis (55.23%) in Turkey in ovine is in agreements with studies Turkey (Taşcı and Değer 1989, Sevinç et al 2000, Aldemir and Dik 2003, Aldemir and Güçlü 2004, Aldemir 2006).

Three types of microscopic cysts were detected with differences in their cyst walls morphologically. These microscopic cyst species corresponding values were as follows: 32 (30.47%), 17 (16.19%) and 9 (8.57%) for *S. tenella*, *S. gigantae* and *S. arieticanis*, respectively.

This study infers that *S. tenella* is the dominant species in sheep in the Aydın region. In this study were detected in different values of species specific of the level of prostaglandin F2α, when the relationship between the level of prostaglandin F2α and macrocys and microcyst in the oesophageal muscle of sheep were assessed.

A total number of 114 sheep blood examinations were determined in 9 (7.89%) samples at 300 and 402 pg/mL 47 (41.22%) samples at 200 to 300 pg/mL and 58 (50.87%) samples at 53-200 pg/mL ranging between the levels of prostaglandin F2α. The differences between groups were found statistically significant ($P<0.001$). High values of prostaglandin F2α are regularly found in the blood of animals suffering from acute inflammation and they have also been reported to occur in the course of parturition or abortion (Owen et al 1998). High values of the level of prostaglandin F2α are thought to reflect increased prostaglandin F2α release by the uterine tissue. So we are assumed that the high the level of prostaglandin F2α measured in infected animals are the trigger of *Sarcocystis* spp. induced abortion.

Conclusion

When it was investigated the relationship between the level of prostaglandin F2α and *Sarcocystis* spp, there was a direct correlation relationship between macrocyst and microcyst formation and the level of prostaglandin F2α. At the end of this study, obtained results explain that the abortion mechanism of *Sarcocystis* spp. have a relationship with the level of prostaglandin F2α.

Contribution of the present author

This study was supported by the Commission for the Scientific Research Projects of Adnan Menderes University. So coordinator of the study is conceived and designed by Assoc. Prof. Dr. Osman Selcuk Aldemir. Parasitological examination was studied by Aldemir OS, Eren H and Ünlü H. Biochemical examination were studied by Seyrek K and Yenisey Ç. In addition all researchers are responsible for all data, material and method and whole manuscript.

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