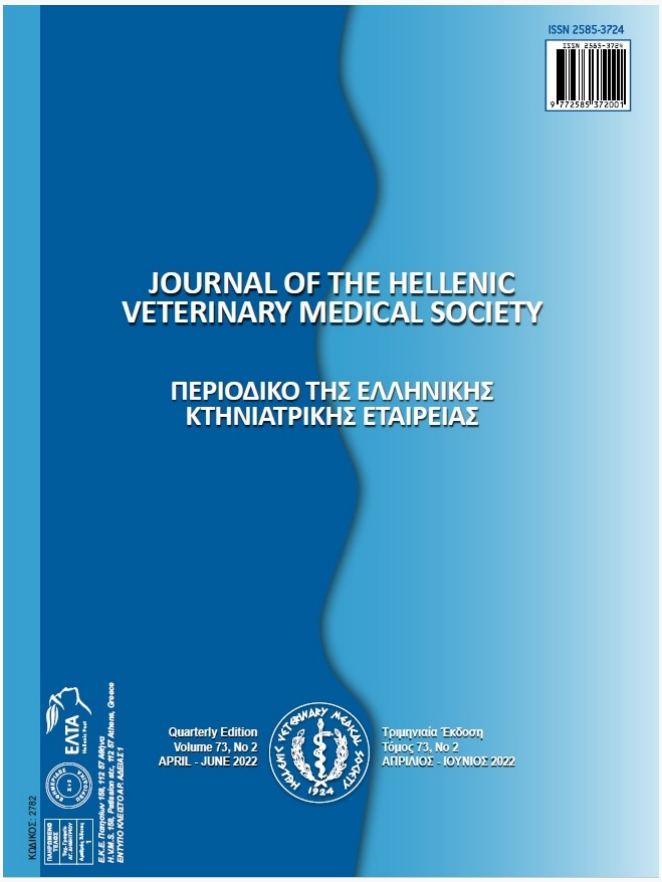
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 2 (2022)



Expression Levels of Some Apoptotic and Oxidative Genes in Sheep with Sarcocystosis

Veysel Yüksek, Özlem Orunç Kılınç, Semiha Dede, Sedat Çetin, Adnan Ayan

doi: 10.12681/jhvms.26702

To cite this article:

Yüksek, V., Orunç Kılınç, Özlem, Dede, S., Çetin, S., & Ayan, A. (2022). Expression Levels of Some Apoptotic and Oxidative Genes in Sheep with Sarcocystosis. *Journal of the Hellenic Veterinary Medical Society*, *73*(2), 4125–4134. https://doi.org/10.12681/jhvms.26702

J HELLENIC VET MED SOC 2022, 73 (2): 4125-4134
ΠΕΚΕ 2022, 73 (2): 4125-4134

Research article
Ερευνητικό άρθρο

Expression Levels of Some Apoptotic and Oxidative Genes in Sheep with Sarcocystosis

V. Yuksek[1*], O.O. Kılınc[1], S. Dede[2], S. Cetin[2], A. Ayan[3]

[1]Department of Medical Laboratory, Ozalp Vocational High School Van Yuzuncu Yil University, Van, Turkey

[2]Department of Biochemistry, Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey

[3]Department of Genetic,Faculty of Veterinary Medicine, Van Yuzuncu Yil University, Van, Turkey

ABSTRACT: Sarcocystosis is a zoonotic protozoon-related disease with a very broad intermediate host spectrum. These protozoon parasites lead to tissue loss in their intermediate hosts. The purpose of this study was to present the mRNA expression levels of some genes belonging to the oxidative stress and apoptosis pathway systems in tissue damage caused by sarcocystosis. In this study, the material consisted of infected tissue taken from sheep esophagus determined to be sarcocystosis-infected and esophageal tissues taken from healthy sheep. The expression levels of the *GPX1, SOD1, SOD2, NCF1*and *Nos2* genes that play a role in the oxidative stress mechanism and the *caspase 3, 8, 9 and BCL-2* genes that play a role in the apoptosis mechanism were determined by RT-qPCR. As a result of the study, it was determined that, with increased oxidative stress, the gene expressions related to the relevant enzyme systems also increased, and in relation to this increase, the caspase enzyme genes that are effective in cell death were up-regulated. These results may shed light on similar studies for understanding and preventing damage mechanisms that may form as a result of sarcocystosis. As a result, it is understood that increased oxidative stress parameters and increased apoptosis in sarcocystic tissue in sheep cause tissue loss. We think that understanding the molecular mechanisms of this disease is clinically important in the treatment of parasitic diseases and in the prevention of economic losses that may occur as a result of the disease.

Keywords: Sarcocystosis, oxidative stress, caspase, apoptosis, gene expression

Corresponding Author:
V, Yuksek Department of Medical Laboratory, Ozalp Vocational High School Van YuzuncuYilUniversity, 65040, Van, Turkey
E-mail address: veyselyuksek@yyu.edu.tr

Date of initial submission: 06-04-2021
Date of acceptance: 13-06-2021

INTRODUCTION

Sarcocystosis is a common infection that is prevalent among mammals, birds, other vertebrates and humans. Parasites from the*Sarcocystis*line are apicomplexan protozoa, and they have an obligate hunter-prey, heteroxenous (two-host) life cycle (Box et al., 1982; Lindsay et al., 1995; Fayer, 2004). The gametogonia and oogonia stages after it where oocysts are shaped take place in the final host, and intermediate hosts are infected by taking in sporulated oocysts orally. The asexual development stage of schizogony occurs in intermedia hosts, and merozoites that are formed as a result of schizogony create sarcocystosis cysts by getting settled in muscle cells. In ultimate hosts (Canidae, Felidae, humans and primates), sarcocystosis is mostly asymptomatic, but mid diarrhoea may be observed (Dubey et al., 1989). However, in intermediate hosts (other vertebrates including humans), the picture may progress more severely. In intermediate hosts, acute sarcocystosis encephalitis, brain and spinal cord inflammation, hemorrhagic diathesis and even deaths may be encountered. It may lead to fetal death, early labor and miscarriages in pregnant animals (Tenter, 1995; Caspari et al., 2011). Sarcocystosis may lead to losses of productivity (Tenter, 1995). Additionally, significant changes have been observed in the behaviors of animals with sarcocystosis (Reiner et al., 2009).

Reactiveoxygenspecies (ROS) arise as a result of the cell metabolism. ROS production and a decreased enzyme activity cause oxidative stress. Low concentrations of ROS are functional for the cell. However, high concentrations of these lead to changes in the structures of cellular molecules such as lipids, proteins and the DNA (Valko et al., 2006; Birben et al., 2012; Arslan et al., 2021). To protect cells and tissues from oxidative damage, living organisms have a highly dynamic antioxidant defense system. This system includes non-enzymatic antioxidants (vitamins, carotenoids, polyphenols, flavonoids and thiol group-containing proteins) and enzymatic antioxidants such as glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT). SOD and CAT areantioxidantenzymesthatplay an essential role againstoxidativestress.Theantioxidantenzymesystemsareinvolved in theprotection of cellsagainstoxidativedamage (Acaroz et al., 2018; Acaroz et al., 2019). Disruption of the balance between oxidants and antioxidants is known as oxidative stress. In infectious diseases, the immune systems of vertebratescoming from birth produce reactive oxygen species in a process known as oxidative burst, and they eliminate pathogens by damaging their lipids and DNAs. ROS and the emerging oxidative stress play a significant role in apoptosis. In parasitic diseases, increased lipid peroxidation is one of the best indicators of the levels of reactive oxygen species (ROS) that lead to systemic biological damage (Dede et al., 2002; Popava et al., 2002).

Apoptosis is a complex serie of protease pathways designed to kill cells in a selective manner (Cruickshanks et., 2013). It is known that intracellular stimulants affecting apoptosis include cytokines, increase in the intracellular calcium concentration, tumor necrosis factor, activation of p53 which is a tumor suppressor gene due to DNA damage, viral and bacterial infections, glucocorticoids and oncogenes (like cmyc) (Akşit and Bildik, 2008).In the apoptosis process, caspases and theBCL-2 / BAX gene family play a role. Caspases, which are the most significant members of the protease family, are activated by several apoptotic stimulants including intracellular and extracellular pathways (SastryandRao, 2000;Danial et al., 2004). Apoptosis, during natural and adoptive immunity, may emerge as an important defense against viral, bacterial and parasitic pathogens (Liles, 1997).

This study was planned with the purpose of revealing the expression levels of some major caspase and oxidative stress genes in the host cell in sheep with sarcocystosis.

MATERIAL AND METHODS

Animal material

3-5-year-old female Akkaraman breed sheep slaughtered at a private slaughterhouse in the center of the province of Van in Turkey constituted the material of the study. In this study, tissues taken from the esophagus of healthy sheep were used for the control group. 12 animal tissues were collected, 6 of which were diseased and 6 were healthy. For the patient group, tissues with macroscopic sarcocystosis cysts in the esophagus and cyststhat were definitively diagnosed with detection of bradyzoites by staining the fluid taken from the cysts with Giemsa (Figure 1) were included, and the diagnosis was confirmed by PCR (Figure 2). Ethical approval was not required since the tissues used in the study were tissues collected after slaughter at a slaughterhouse.

Material collection

The tissues used in the study were collected from the region of the esophagus containing macrocysts.

Approximately 100 mg from the collected tissue samples was added into DNase- and RNase-free sterile 2-ml tubes, and an RNA stabilizer (GenAll, Catalog No: 351-001) was added to cover the material. RNAstable is a preservation product developed to protect RNA from degradation during storage or shipment at ambient temperatures. To prevent RNA damage, the tissues that were collected in a sterile manner were kept at -80°C until RNA isolation.

Microscopic identification of sarcocystosis bradyzoites

By crushing the specimens taken from the macrocysts in the esophagus on a slide, smear preparates were prepared. Afterwards, following fixation of the smears with methanol for 1 minute, they were stained by 5% Giemsa for 30 minutes. They were then examined under a microscope with a 100x lens (Figure 1).

Identification of the parasite by PCR

DNA extraction was carried out on the collected samples based on the kit protocol (Invitrogen PureLink™ Genomic DNA Mini Kit, USA, K182002). Afterwards, the obtained DNAs were stored at -20°C until the next stages.

To amplify the 18s rDNA gene region of *Sarcocystis* spp.,the F (CGCAAATTACCCAATCCTGA) and R (ATTTCTCATAAGGTGCAGGAG) primers were used (Portellaet al., 2016). In 25 µL of final volume, 10 pmol forward and reverse primer, 200 µM dNTPs, 2.5 mM MgCl2, 2.5 U Taq Polymerase and 10X PCR buffer, Nuclease Free Water and DNA were used. The reaction was facilitated in 40 cycles consisting of 15 minutes of pre-denaturation at 95°C, 40 seconds of denaturation at 95°C,30 seconds of binding at 59°C and 1 minute of elongation at 72°C, as well as 6 minutes of final elongation at 72°C. By staining the obtained PCR products with Safe-T-Stain, their images were acquired in 1.5% agarose gel (Figure 2).

Gene expression by RT-qPCR

The tissues that were stored at -80°C were left to thaw at room temperature. After thawing, approximately 70-75 mg of them were taken into sterile Eppendorf tubes. 0.5 ml sterile normal physiological serum was added onto the tissues, and they were homogenized. The homogenized tissues were centrifuged at 1500 rpm at a temperature of 2-8°C. The liquid phase remaining on the top of the tube was removed. Cold sterile phosphate buffer (PBS) was added onto the tissues remaining on the bottom, pipetted

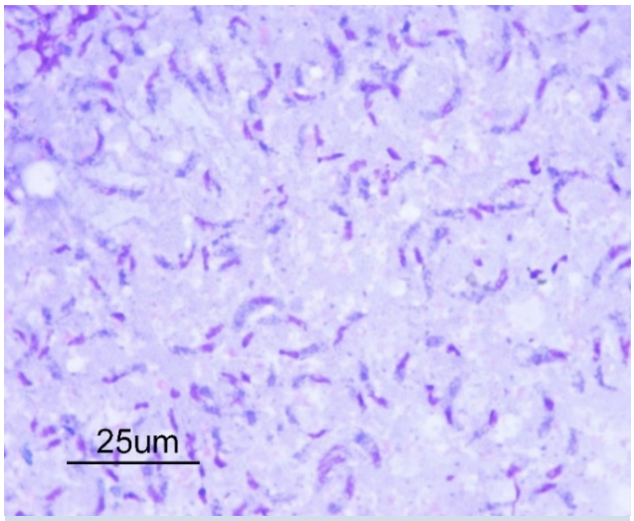


Figure 1. Sarcocystis spp. bradyzoite image

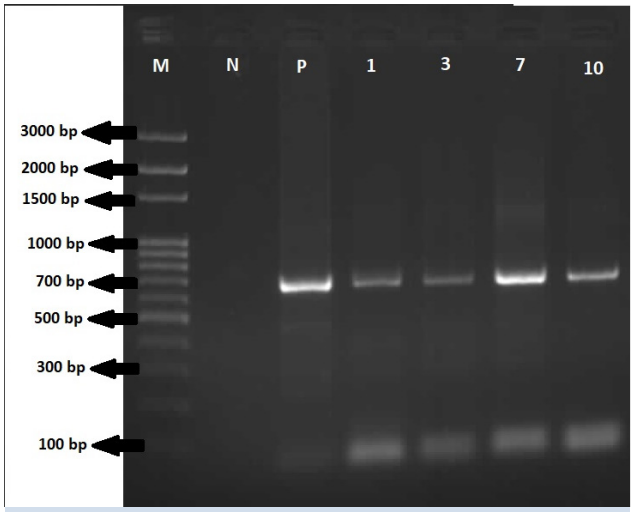


Figure 2. PCR image for Sarcocystis spp. (M: Marker, N: Negative Control, P: PositiveControl, 1, 3, 7, 10: Positive specimens)

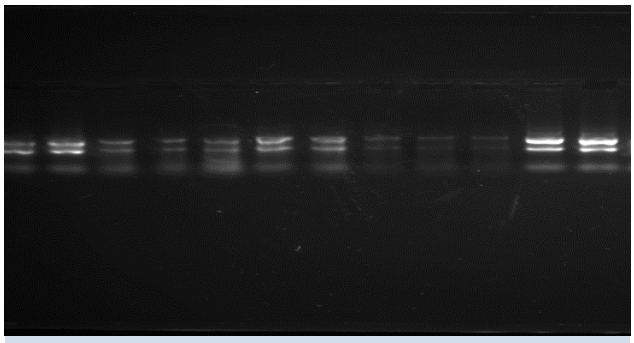


Figure 3. Agarose gel electrophoresis image of total RNA obtained from the study

These images were converted to cDAN for use in RT-qPCR, 6 sick sheep diagnosed with sarcocystosis and 28 h 18s and 5s RNA images of 6 control groups. The fact that these RNA regions are clear indicates that the RNAs used in the study were not broken.

and washed. Afterwards, it was centrifuged again at 1500 rpm, the top part was removed, and washing was carried out. After repeating the washing procedure 3 times, the total RNA isolation stage was started.

The PBS on the top of the centrifuged tissues was removed with the help of a pipette, and the tissue lysate remaining at the bottom was transferred to a new 1.5-ml sterile Eppendorf tube. The total RNA isolation was manually carried out by using the TRIzol reagent (Chomczynski and Mackey, 1995).For purity and quantification, the RNAconcentrationwas measured in a nanodropspectrophotometer (Biodrop, UK).The total RNAconcentration was enoughaccording to the master mix protocol provided by the company.3-5 µl rRNA was ran in GelRed-added 0.7% agarose gel. Its photographs were taken after running. 28S, 18S and 5S RNAimages were clearly observed (Figure 3).cDNA synthesis was carried out based on the protocol of a Wizbio brand (WizScript, cat no: W2211) cDNA synthesis kit. ThecDNA synthesis kit is a complete system for efficient synthesis of high capacity cDNA from RNA.The cDNA kit includes a reaction buffer, dNTP mix, random hexamer, reverse transcriptase, RNase inhibitor and RNase-free water. The reverse transcription reaction mixture was incubated by following the instructions of the synthesis kit.

The forward (F), reverse (R) primers of the target genes were obtained from NCBI (https://www.ncbi.nlm.nih.gov/refseq/) and Primer3web version 4.1.0 (http://primer3.ut.ee/). The RT-qPCR reactions of the target genes were optimized before the study. The primary gene sequences for each gene are given in Table 1.

The expressions of the genes were determined for the oxidative stress mechanism (*GPX1, SOD1, SOD2, NCF1, NOS2*) and the apoptosis mechanism (*Caspase 3, 8, 9 andBCL-2*). For theRT-qPCR reaction, a Wizbio (wizPure, cat no: w1711) sybrgreen master mix kit was used in a RT-qPCR device (Qiagen Rotor Gen Q, UK). The reaction contents were the same for both the target and control (housekeeping) genes. The stages of the method are summarized in Table 2 below based on the kit protocol.

As the control gene,glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used. Onect (cycle threshold) was determined by the start of the logarithmic phase of the amplifications.

By normalizing the expression values of the target genes belonging to the patient and control groups based on the housekeeping gene (GAPDH),ΔCt was calculated. By subtracting the ΔCt value of the control group from theΔCt value of the patient group, the ΔΔCt value was obtained. The expression coefficients of the target genes were determined as theΔΔCt values' logarithm $2^{-\Delta\Delta Ct}$ base (Livak and Schmittgen, 2001). The target gene changes of the patient group were assessed by statistical comparisons based on the expression increase-decrease (logarithm 2-Delta Delta Ct changes) of the control group.

Statistical analysis

For the studied characteristics, the descriptive statistics are expressed Mean ± Standard deviation. Mann-Whitney U test was performed to compare two groups. The statistical significance level was considered as 5%, and the SPSS (ver.: 22) statistical software was used for all statistical computations.

RESULTS AND DISCUSSION

As a result of the microscopic examination for sarcocystosisdiagnosis, *Sarcocystis* bradyzoites were observed (Figure 1).

In all 6 specimens subjected to PCR, bands specific for *Sarcocystis* spp. with a size of 700 bp were obtained (Figure 2).

The peaks show that the primers designed specifically for each target genes bonded accurately and multiplied the correct regions (Figure 4).

The expressions of some apoptotic andoxidative genes were determined by RT-qPCR (Figure 5).

The expressions of the *GPX1* and *SOD2*genes related to the antioxidant system in the patient group were higher in comparison to the control group by respectively 2.2- and 1.6-fold ($p<0.05$). The expressions of the *NCF1* and *NOS2* genes as markers of oxidative stress in the patient group were higher in comparison to the control group by respectively 2.7- and 2.2-fold ($p<0.05$) (Table 3).

It was determined that, in comparison to the control group, all target genes looked at in the apoptosis pathway were up-regulated. Increases were observed in the apoptotic genes of caspase 3, 8 and 9 by respectively 2.5-, 7.9-and 1.3-fold ($p<0.05$) and in the anti-apoptotic gene of Bcl-2 by approximately 1.9-fold (Table 4).

Table 1. Primers used in expression analysis and their sequences

Primers names	Full names	F (5'-3')	R (5'-3')
GPX1	Glutathione Peroxidase 1	TTTGGGCATCAGGAAAACGC	CGTGATGAACTTAGGGTCGG
SOD 1	SuperoxideDismutase 1	GACCAGATGACTTGGGCAGA	GGGAATGTTTACGGGGCAAT
SOD 2	SuperoxideDismutase 2	GGGTTGGCTTGGCTTCAATA	AAGGCTGACGGTTTACTTGC
NCF1	NeutrophilCytosolicFactor 1	AGCCCTACGTCACCATCAAA	GCTCTTGATCTGGCTTTGGG
NOS2	NitricOxideSynthase 2	GCCCTCAGATTACAACAAGTGG	CTGGAGGAGCTGATGGAGTAG
BCL2	BCL2 ApoptosisRegulator	TCTTTGAGTTCGGAGGGGTC	GGCCATACAGCTCCACAAAG
Caspase 3	Apoptosis-RelatedCysteinePeptidase	ACGGAAGCAAATCAGTGGAC	GGTTTCCCTGAGGTTTGCTG
Caspase 8	Apoptosis-RelatedCysteinePeptidase	AGTGAGTTGCAGACATCCGA	AGGTCTTGTCCAAAGCCTCT
Caspase 9	Apoptosis-Related Cysteine Peptidase	AGAGTGATGAAGCAGGACCC	CAGATCGGCATTTCCCTTGG
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase	AGATGGTGAAGGTCGGAGTG	GTTCTCTGCCTTGACTGTGC

Table 2. Reaction content and cycle for RT-qPCR

Reaction content	**For an example**	**Reaction cycle**
Master mix (2X)	10 µl	*95°C 5' denaturation
Primer	Forward: 1 µl	*95°C 20"
	Reverse: 1 µl	*53-55°C 60" 40 **cycle**
dH_2O	7 µl	*MeltingCurve*
cDNA	1 µl	Ramp: 50-99 (1 degree increase)
Total	20 µl	90 ºC 5"

*Initial denaturation was performed according to the master mix protocol. The target genes have a base length of approximately 140-180 bp.
Melting temperature (MT) of all target genes, which were previously optimized, were determined according to the primary binding temperature. Therefore, MT temperatures varied

Table 3. Descriptive statistical results of the measurement values of some genes belonging to the oxidative stress mechanism in the sarcocystosis esophagus sheep tissues

	Control group S (N:6)	Diseased group S±SD (N:6)	***p.**
GPX1	1.00	2.18±0.37	0.001
NCF1	1.00	2.72±1.34	0.026
NOS2	1.00	2.01±0,41	0.002
SOD1	1.00	0.79±0.79	0.64
SOD2	1.00	1.58±0.25	0.030

The significance of the difference between the control and patient groups was assessed based on $p<0.05$

Table 4. Descriptive statistical results of the measurement values of some apoptotic genes in the sarcocystosis esophagus sheep tissues

	Control group S (N:6)	Diseased group S±SD (N:6)	***p.**
Caspase 3	1.00	2.54±0,48	0.001
Caspase 8	1.00	7.85±3.32	0.004
Caspase 9	1.00	1.26±0.21	0.030
BCL2	1.00	1.87±0.37	0.002

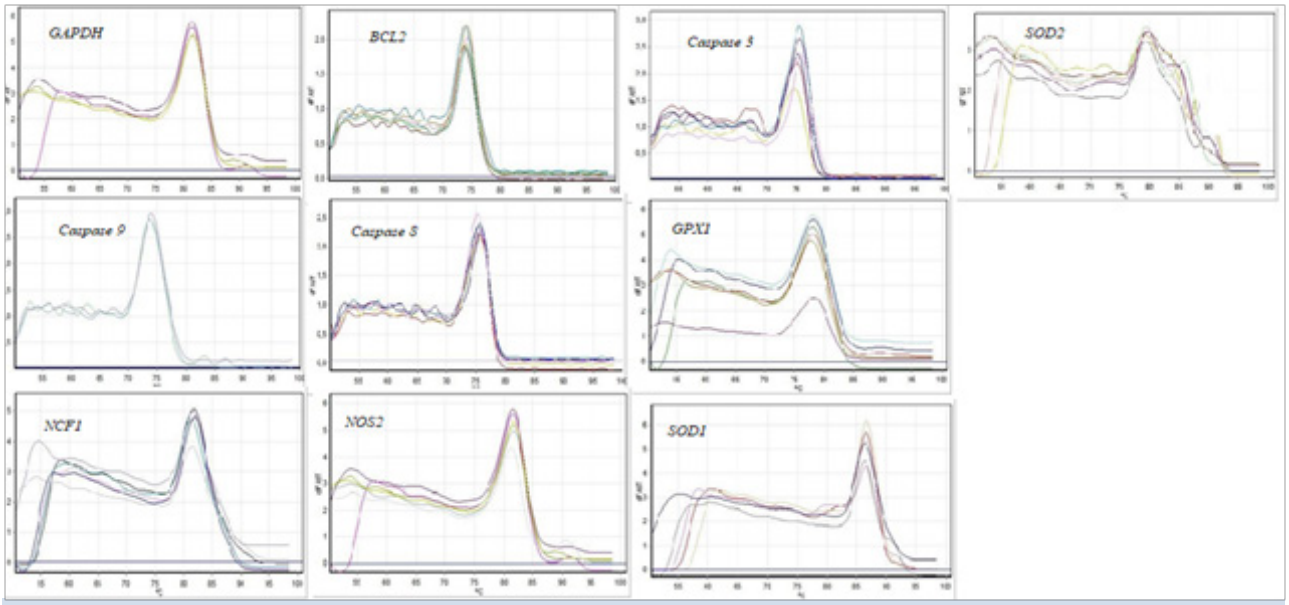


Figure 4. Melting curve plot for the target genes in the RT-qPCR study: An example of the melting curve of each target gene

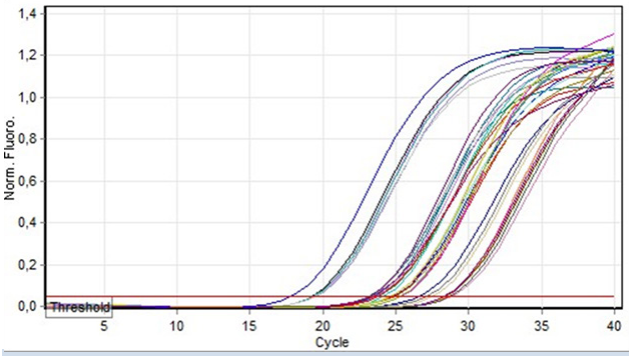


Figure 5. The amplification and standard curve of cDNA-based RT-qPCR; This CT chart is just one example of the RT-qPCR repeats made related to the study

This study selected important junction genes in the apoptotic (*BCL2, Caspase 3, Caspase 8* and *Caspase 9*) and oxidative stress (*GPX1, SOD1, SOD2, NCF1 NOS2*) pathways. It was aimed to demonstrate the role of the apoptotic and oxidative pathways in the pathology ofsarcocystosis by detecting the expression of these genes.Studies have reported on the oxidative stress developing based on increased lipid peroxidation in parasitic diseases and the increased antioxidant levels to cope with this oxidative stress. Lipid peroxidation is one of the best indicators of the level of reactive oxygen species (ROS) that induce systemic biological damage (Popova and Popov, 2002). Antioxidant systems have a protective property against cell and tissue damage forming as a result of parasite invasion (Valko et al., 2006; Deger et al., 2008;Surai, 2016). It was reported that free radicals released from the mitochondria based on oxidative stress are factors that increase apoptosis (Ellerby et al., 1997). Apoptosis may be started or reduced by parasites, and this way, it may contribute to spreading within the host, inhibiting the host immune response or modulating it and making it easier for the pathogen to survive. The apoptosis of the host cell infected with the parasite may show an effect killing the parasite or be a perfect way to escape the protective response of the host (Sacks and Sher, 2002).

This study aimed to determine the presence of oxidative stress in sarcocystosis which is an apicomplexan parasite by investigating antioxidant gene expressions, and the relationship between oxidative stress and apoptosis was examined. The expression levels of the antioxidant enzymes *GPX1, SOD1*and *SOD2* in the tissues with macrocysts caused by sarcocystosis were determined by RT-qPCR. It was determined that the mRNA expression level of the *GPX1* gene coding the glutathione peroxidase 1 enzyme increased approximately by 2.2-fold. No change was observed in the expression of the Sod1 gene which codes another antioxidant enzyme, *SOD1*. However, there was an increase by approximately 1.6-fold in the expression of the *SOD2* gene that transforms mitochondria-related free radical products into diatomic oxygen and hydrogen peroxide.*SOD2* is an enzyme that is especially related to the mitochondrial apoptotic death pathway (Danial and Korsmeyer, 2004).

The neutrophil cytosolic factor 1 (*NCF1*) is a pro-

tein gene. This protein is a subunit of the enzyme complex that is known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that plays an important role in the immune system.This complex is primarily active in immune system cells known as phagocytes. These cells catch and eliminate foreign invaders such as bacteria and fungi ("https://ghr.nlm.nih.gov/gene/NCF1," n.d.).

Nitric oxide (NO) is a free radical with a very short half-life that is formed in mammals by neutrophils and plays a significant role in many biological events such as the defense mechanism. NO is synthesized via the enzyme nitric oxide synthase (NOS). It is an inducible enzyme coded by the *NOS2* gene (Habib and Ali, 2011). An increase in the synthesis of NO may lead to an increase in ROS products. This may lead to more oxidative stress in the cell or tissue, and therefore, molecular damage like DNA damage and lipid peroxidation.It also plays a role in protection of phagocytic leukocytes against their own products and oxygen radicals. The production of these antioxidants is naturally controlled by genes. Increased expression of these genes is highly important in determining the dimensions of oxidative stress in parasitic diseases. Again, in anti-phagocytic effect, an increase in the expressions of the *NCF1* and *NOS2* genes that play a role in especially the secretion of enzymes that are actively found in phagocytes shows that phagocytic activity has increased (Chuenkova et val., 1988; Dede et al., 2002).

Using the RT-qPCR method, the mRNA expression levels of the *NCF1* and *NOS2* genes were checked in the sarcocystosis-infected esophagus tissues. According to the results on the data of the study, in the sarcocystic tissue, the expressions of the oxidative stress markers, the *NCF1* and *NOS2* genes, increased respectively by 2.7- and 2.0-fold.

NADPH oxidase is a multi-component enzyme that is activated to producesuperoxide anions ("https://ghr.nlm.nih.gov/gene/NCF1," n.d.).While low concentrations of NO are beneficial for biological events, at high concentrations, it produces peroxynitrite, which leads to tissue and cellular damage, as a result of its reaction with superoxide (Habib and Ali, 2011; Çekmen et al., 2011). An increase in the mRNA levels of the *NCF1*and*NOS2* genes over the normal expression levels may mean an increase in ROS. In this study, the increase in both of these genes in comparison to the control gene suggested that oxidative stress occurs in the cells of sarcocystosis-infected tissues. Considering not only the inflammation status in the region where the cystic tissue in the esophagus was collected but also the increase in the *NCF1* and *NOS2* gene expressions, it may be concluded that the synthesized NO and O_2^- radicals reached a cytotoxic level, and starting with the cystic region, sarcocystosis may lead to a serious tissue loss. Additionally, the up-regulation of the *GPX1* and *SOD2* enzymes, which are defense mechanisms against oxidative stress, also supported these data.

During their evolution, parasites have developed mechanisms to induce or prevent host cell apoptosis for them to continue and complete their life cycles. There are studies examining apoptosis by various mechanisms in host cells in parasitic diseases. It was reported that *toxoplasma gondii*can control apoptosis, intervene with signal pathways regulating cell survival including caspase 3 activation, PARP-1 or cytochrome C secretion from the mitochondria, induce antiapoptotic gene expression or prevent pro-apoptotic gene expression and lead to DNA fragmentation at the end (Lüder and Gross, 2005). It is stated that*T. Gondii*inhibits host apoptosis through direct and indirect mechanisms, and bcl-2 induced by *T. gondii*is related to increased expression of the antiapoptotic *BCL-2* gene (Contreras-Ochoa e). In *Trypanosoma cruzi* and *Plasmosmodium falciparum* infection, apoptosis was observed in myocardiocytes (De Souza et al., 2003; Wennicke et al., 2008). It isreported that *P. falciparum* induces Ca+2 transition into erythrocytes by increasing the permeability of cation channels, and this leads to the apoptosis of erythrocytes by inducing oxidative stress (Brand et al., 2003).It was also reported that *P. falciparum*-infected erythrocytes adhere to endothelial cells, induce *iNOS* expression in blood-brain barrier endothelial cells and brain cells, and they lead to apoptosis and disruption of the blood-brain barrier by activation of *caspase* 8 and 9 (Taoufiq et al., 2003). There are apoptosis studies in parasitic diseases such as Cryptosporidiosis, Leishmaniosis and Trichinellosis (Shaha, 2006; Piekarska et al., 2010). Philchenkov et al. (2004) stated that host cell deaths could occur with the effects of the products of parasitic diseases and an increase in the antigen-specific T cells. It was reported that sarcocystosis involves not only irregularity of cellular organelles but also muscle cell edema, pathological changes in close tissues and changes that are observed in apoptotic cells in the nuclei and cytoplasm of these cells. These changes suggest the possibility of an internal pathway of apoptosis (Beyer and Radchenko, 2001). In this study, the

gene levels of the pro-apoptotic enzymes of *caspase* 9, 8 and 3 and antiapoptotic enzyme of *BCL-2*were determined by the method of RT-qPCR. According to the results obtained in the study, *caspase 8,* which is effective in the receptor death pathway, showed an approximately 8-fold increase. There was also an approximately 2.5-fold increase in *caspase 3*, which is the effector in the caspase enzyme system in apoptosis. It was determined that *caspase 9,* which is effective in the mitochondrial pathway in the apoptosis process, did not change. Considering these results, it may be concluded sarcocystosis may lead to apoptosis. These results suggest that, as other parasite types, sarcocystosis activates the caspase enzyme system in host cells. At this point, the result of this study was in agreement with the literature (Beyer and Radchenko, 2001; Taoufiqet al., 2003). Despite this, as opposed to different types of parasites reported in the literature, it may be stated that it uses the external pathway, that is, the receptor pathway while carrying the cell to apoptosis instead of the mitochondrial pathway which is internal. This difference may be caused by the type of parasite or the tissue where the parasite settles.

CONCLUSION

Based on the data of this study, it was determined that sarcocystosis led to an up-regulation in the caspase enzyme system with increased oxidant/antioxidant levels. It was found that *Sarcocystis*-type parasites, which may be zoonotic, probably one of the underlying causes of complaints of fibromyalgia and neglected in both veterinary and human medicine, may lead to oxidative stress (Sasani et al., 2018) and apoptosis. Likewise, it has been revealed by several studies including this one that parasitic diseases lead to host cell death via different ways and may lead to tissue damage.

According to the results we obtained in this study, it is recommended to conduct studies that are compatible with the data in the literature (Beyer and Radchenko, 2001; Taoufiq et al., 2003; Habib and Ali, 2011; Çekmen et al., 2011), but further studies and investigation of new parameters are needed to shed light on the exact metabolism. Based on the findings obtained in this study, it may be stated that the expression of these parameters on the molecular level was determined in a more detailed and precise manner in comparison to previous studies.

It is believed that the results of this study may be an example for parasitic disease-related species and shed light on similar studies. Additionally, to clearly demonstrate the obtained results, it is recommended for future studies to determine the protein expressions corresponding to increased mRNA expressions.

There have been many publications showing that oxidative stress and apoptosis are affected by parasitic diseases. However, it is thought that the results obtained in this study on this topic in sarcocystosisin sheep may be considered as important data in providing clinical genetic perspectives. This is because the genes selected here belong to the pathways through which many drugs and preventive agents exert their beneficial effects.In other words, it is thought that determination of the genes and parameters through whichsarcocystosis has negative effects will provide useful information for synthesis, extraction and new therapeutic and preventive substances. Moreover, it is thought that it will be useful to investigate the

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

REFERENCES

Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Demirel HH, Kucukkurt I, Eryavuz A, Kara R, Varol N, Zhu K (2019) Bisphenol-A inducedoxidativestress, inflammatory gene expression, andmetabolicandhistopathologicalchanges in maleWistar albino rats: protective role of boron. Toxicologyresearch *8* (2): 262-269.

Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, Arslan HO, Varol N, Zhu K. (2018) Theameliorativeeffects of boronagainstacrylamide-inducedoxidativestress, inflammatoryresponse, andmetabolicchanges in rats. Foodandchemicaltoxicology 118: 745-752. https://doi.org/10.1016/j.fct.2018.06.029

Akşit H, Bildik A. Apoptosis. Van Veterinary Journal 2008; 19: 55-63

Arslan HO, Keles E, Siuda M, Rostami B, Bollwein H (2021) Effects of the addition of different concentrations of catalase and sodium pyruvate to TRIS egg yolk extender before freezing on quality of frozen-thawed bull sperm. In Reproduction In Domestic Animals 56 (S1):18.

Beyer TV, Radchenko AI (2001) Intracellular parasitism and the problem of sarcocystosis.BiologyBulletin 28 (2): 119-125.

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012) Oxidativestressandantioxidantdefense. World Allergy Organ Journal 5 (1): 9-19

Box ED, Smith JH (1982)The ıntermediate host spectrum in a sarcocystis species of birds. Journal Parasitol 68: 668. https://doi.org/10.2307/3280926

Brand VB, Sandu CD, Duranton C, Tanneur V, Lang KS, Huber SM, Lang F (2003) Dependence of Plasmodium falciparum in vitro growth on the cation permeability of the human host erythrocyte. Cellular Physiology and Biochemistry 2003; 13: 347-356. https://doi.org/10.1159/000075122

Caspari K, Grimm F, Kühn N, Claire Caspari, N, Basso, W, (2011) First report of naturally acquired clinical sarcocystosis in a pig breeding stock. Vet. Parasitol 177:175-178. https://doi.org/10.1 016/j.vetpar.2010.11.019

Chomczynski P, Mackey K (1995) Modification of the TRI Reagent (TM) procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. Biotechniques 19: 942-945.

Chuenkova MV, Sukhareva NN, Sergacheva YY, Sokhanenkova TL, Soprunov FF et al (1998) Effect of vitamin 'E' on the lipid content in red cells of the mice and Plasmodium berghei. Meditsinskaya Parazitologiya i Parazitarnye Bolezni 4:13-15.

Contreras-Ochoa CO, Lagunas-Martínez A, Belkind-Gerson J, Correa D (2012) Toxoplasma gondii invasion and replication in astrocyte primary cultures and astrocytoma cell lines: Systematic review of the literature. Parasitology Research 110: 2089-2094.

Çekmen BM, Turgut M, Türköz Y, Aygün AD, Gözükara ME (2011) Physiological and Pathological Features of Nitric Oxide (NO) and Nitric Oxide Synthase (NOS).Journal of Clinical Pediatric Turkey 10: 226-236.

Danial NN, Korsmeyer SJ (2004) Cell death:critical control points. Cell 116:205-219. https://doi.org/10.1016/S0092-8674 (04)00046-7

De Souza EM, Araújo-Jorge TC, Bailly C, Lansiaux A, Batista, MM Oliveira, GM, Soeiro, MN (2003) Host and parasite apoptosis following Trypanosoma cruzi infection in in vitro and in vivo models. Cell and Tissue Research 314: 223-235. https://doi.org/10.1007/s00441-003-0782-5

Dede S, Değer Y, Kahraman T, Değer S, Alkan M et al (2002). Oxidation products of nitric oxide and the concentrations of antioxidant vitamins in parasitizedgoats. Acta Veterinaria Brno 71, 341-345. https://doi.org/10.2754/avb200271030341

Deger Y, Ertekin A, Deger S, Mert H (2008) Lipidperoxidationandantioxidantpotential of sheepliverinfectednaturallywithdistomatosis. *Türkiye ParazitolDerg 32* (1): 23-26.

Dubey JP, Speer CA, Fayer R (1989) Sarcocystosis of animalsand man. CRC Press, BARC-East, Beltsville, MD, USA: 215 pp.

Ellerby HM, Martin SJ, Ellerby LM, Naiem SS., Rabizadeh S et al (1997) Establishment of a cell-free system of neuronal apoptosis: Comparison of premitochondrial, mitochondrial, and postmitochondrial phases. The Journal of Neuroscience 17: 6165-6178. https://doi.org/10.1523/jneurosci.17-16-06165.1997

Elmore S (2007) Apoptosis: A Review of Programmed Cell Death.Toxicologic Pathology 35: 495-516.

Fayer R (2004) Sarcocystis spp. in human infections. Clinical Microbiology Reviews 17: 894-902. https://doi.org/10.1128/CMR.17.4.894-902.2004

Habib S, Ali A (2011) Biochemistry of nitric oxide. Indian Journal Clinic Biochemstry 26:3-17. https://doi.org/10.1007/s12291-011-0108-4

https://ghr.nlm.nih.gov/gene/NCF1[accessed: 01June2020]

Liles WC (1997) Apoptosis - Role in infection and inflammation. Current Opinion in Infectious Diseases 10: 165-170. https://doi.org/10.1097/00001432-199706000-00002

Lindsay D, Blagburn B, Braund, KG (1995) Sarcocystis spp. and sarcocystosis. Bacteriol. Analytical Methods 5: 249-254.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25 (4):402-408

Lüder CGK, Gross U (2005) Apoptosis and its modulation during infection with Toxoplasma gondii: Molecular mechanisms and role in pathogenesis. Current Topics in Microbiology and Immunology 289: 219-237. https://doi.org/10.1007/3-540-27320-4_10

N.Cruickshanks, L.Booth, Y Tang, P Dent (2013) Apoptosis. In: Brenner's Encyclopedia of Genetics 2nd ed, AcademicPress, USA: pp 166-169

Philchenkov A, Zavelevich M, Kroczak, TJ, Los M (2004) Caspases and cancer: Mechanisms of inactivation and new treatment modalities. Experimental. Oncology 26: 82-97.

Piekarska J, Szczypka M, Michalski A, Obmińska-Mrukowicz B,Gorczykowski M (2010) The effect of immunomodulating drugs on the percentage of apoptotic and necrotic lymphocytes in inflammatory infiltrations in the muscle tissue of mice infected with Trichinella spiralis. Polish Journal of Veterinary Sciences 13: 233-240.

Popova MP, Popov CS (2002) Damage to subcellular structures evoked by lipid peroxidation. Zeitschrift für Naturforschung C Journal Bioscience 57:361-365. https://doi.org/10.1515/znc-2002-3-427

Reiner G, Clemens N, Fischer R, Köhler F, Berge T (2009) Mapping of quantitative trait loci for clinical-chemical traits in swine. Animal Genetics 40: 57-64. https://doi.org/10.1111/j.1365-2052.2008.01804.x

Sacks D, Sher A (2002) Evasion of innate immunity by parasitic protozoa. Nature Immunology 3: 1041-1047. https://doi.org/10.1038/ni1102-1041

Sasani M, Nabavi R, Hajinezhad M, Hasanein, P (2018) Oxidativestressandhepaticinjuryinduced in mice fed a *Sarcocystishirsuta* cystextract. *Journal of veterinaryscience*, *19* (4): 500-504. https://doi.org/10.4142/jvs.2018.19.4.500

Sastry PS andRao KS (2000) Apoptosisandthenervoussystem. Journal of neurochemistry 74 (1): 1-20.

Shaha C (2006) Apoptosis in Leishmania species & its relevance to disease pathogenesis. Indian Journal of Medical Research 123: 233-244.

Smith, NC, Bryant C (1989) Free radical generation during primary infections with Nippostrongylus brasiliensis. Parasite Immunology 11:147-160. https://doi.org/10.1111/j.1365-3024.1989.tb00655.x

Surai PF (2016) Antioxidantsystems in poultrybiology: superoxidedismutase. *Journal of AnimalResearchandNutrition1* (1): 8.

Taoufiq Z, Pino P, Dugas N, Conti M, Tefit M, Mazier D, Vouldoukis I (2003) Plasmodium falciparum P,-Infected erythrocyte adhesion induces caspase activation and apoptosis in human endothelial cells. The Journal of Infectious Diseases 187: 1283-1290. https://doi.org/10.1086/373992

Tenter AM (1995) Current research on sarcocystis species of domestic animals. International Journal for Parasitology 25:1311-1330. https://doi.org/10.1016/0020-7519 (95)00068-D

Valko M, Rhodes CJ, Moncol J, Izakovic M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160: 1-40.

Wang M, Dhingra K, Hittelman WN, Liehr JG., De Andrade M (1996) Lipid peroxidation-induced putative malondialdehyde-DNA adducts

in human breast tissues. Cancer Epidemiology, Biomarkers &Prevention 5:705-710.

Wennicke K, Debierre-Grockiego F, Wichmann D, Brattig NW, Pankuweit S, Maisch B, Schwarz RT, Ruppert V (2008) Glycosylphosphatidylinositol-induced cardiac myocyte death might contribute to the fatal outcome of Plasmodium falciparum malaria. Apoptosis 13: 857-866. https://doi.org/10.1007/s10495-008-0217-6