

Investigation of Adenosine deamidase (ADA) activity and some biochemical values in lambs with infectious ecthyma symptoms

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[Investigação da atividade da adenosina deamidase (ADA) e alguns valores bioquímicos em cordeiros com sintomas de ectima infeccioso]

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ABSTRACT

Understanding the zoonotic risk posed by the orf virus (ORFV), which causes contagious ecthyma in domestic animals, is crucial for both human and animal health. Contagious ecthyma is a highly contagious, zoonotic, viral skin disease that affects sheep, goats, and certain other domestic and wild animals. In this study, serum malondialdehyde (MDA), nitric oxide (NO), reduced glutathione (GSH) concentrations, and adenosine deaminase (ADA) activity were measured in healthy and naturally infected lambs with ecthyma. Compared to healthy lambs, serum MDA (respectively; 2.69 ± 0.4 vs. 4.73 ± 0.24 $\mu\text{mol/L}$, $P < 0.001$), NO (respectively; 6.12 ± 0.72 vs. 8.32 ± 0.55 $\mu\text{mol/L}$, $P < 0.05$) and ADA (respectively; 7.12 ± 0.60 vs. 21.53 ± 1.82 U/L, $P < 0.001$) concentrations increased, while GSH (respectively; 15.01 ± 1.59 vs. 10.05 ± 1.10 mg/dL, $P < 0.05$) levels decreased in contagious ecthyma lambs. As a result, it was concluded that oxidative stress developed in lambs infected with contagious ecthyma, ADA enzyme activity increased, and this could be useful for the diagnosis of the disease.

Keywords: Adenosine deaminase, ecthyma, lamb, oxidative stress

RESUMO

As zoonoses são definidas pela Organização Mundial da Saúde como doenças e infecções que são naturalmente transmitidas entre animais vertebrados e seres humanos. A compreensão do risco zoonótico representado pelo vírus orf (ORFV), que causa ectima contagioso em animais domésticos, é fundamental para a saúde humana e animal. O ectima contagioso é uma doença de pele viral, zoonótica e altamente contagiosa, que afeta ovelhas, cabras e alguns outros animais domésticos e selvagens. Neste estudo, as concentrações séricas de malondialdeído (MDA), óxido nítrico (NO), glutatona reduzida (GSH) e a atividade da adenosina deaminase (ADA) foram medidas em cordeiros saudáveis e naturalmente infectados com ectima. Em comparação com cordeiros saudáveis, MDA sérico (respectivamente: $4,73 \pm 0,24$ vs. $2,69 \pm 0,44$ $\mu\text{mol/L}$, $P < 0,001$), NO (respectivamente: $6,12 \pm 0,72$ vs. $8,32 \pm 0,55$ $\mu\text{mol/L}$, $P < 0,05$) e ADA (respectivamente: $7,12 \pm 0,60$ vs. $21,53 \pm 1,82$ U/L, $P < 0,001$) aumentaram, enquanto os níveis de GSH (respectivamente: $15,01 \pm 1,59$ vs. $10,05 \pm 1,10$ mg/dL, $P < 0,05$) diminuíram em cordeiros com ectima contagioso. Como resultado, concluiu-se que o estresse oxidativo se desenvolveu em cordeiros infectados com ectima contagioso, a atividade da enzima ADA aumentou, e isso pode ser útil para o diagnóstico da doença.

Palavras-chave: adenosine, deaminase, ectima, cordeiro, estresse oxidativo

INTRODUCTION

Contagious ecthyma is a zoonotic skin infection caused by a parapoxvirus from the family *Poxviridae*, affecting young sheep and goats (Haig and Mercer, 1998; Inoshima *et al.*, 2000; Gökçe *et al.*, 2005). It leads to economic losses in all countries that raise sheep and goats, including Türkiye (Kırmızıgül *et al.*, 2016; Karki *et al.*, 2019; Lawan *et al.*, 2021). In addition to small ruminants, the disease has been reported in other domestic and wild animals as well as in humans who come into contact with infected animals (Gökçe *et al.*, 2005; Teshale and Alemayeh, 2018; Aneed and Alsaad, 2019; Saeidi and Kheradmand, 2023). For this reason, the World Organization for Animal Health (OIE) has recognized contagious ecthyma as a zoonotic disease that can be transmitted from animals to humans (Nadeem *et al.*, 2010; Kumar *et al.*, 2015). The virus can be transmitted through direct contact with infected animals or indirectly through caretakers, contaminated stables, vehicles, and equipment. Occupations such as veterinarians, shepherds, butchers, and animal caretakers are considered at risk.

Ecthyma is also known by other names, including orf, contagious pustular dermatitis, scabby mouth, infectious labial dermatitis, sore mouth, or oral pustules (Hosamani *et al.*, 2009; Wet and Murie, 2011; Spyrou and Valiakos, 2015; Al Saad *et al.*, 2017). It is a highly contagious disease, primarily characterized by distinctive cutaneous lesions that typically appear around the mouth, including the lips, cheeks, and nasal areas, as well as on interdigital regions, teats, and less commonly on the abdominal and inguinal regions (Aneed and Alsaad, 2019; Merhan *et al.*, 2021). The clinical progression includes erythema, vesicle formation, pustule development, and crust formation (Joseph *et al.*, 2015). The clinical diagnosis of the disease can be made based on the presence of characteristic lesions, such as cauliflower-like papillomatous, proliferative, granulomatous, dry crusted, raised, and erosive structures on the nose, mouth, oral mucosa, palate, gums, and skin. Additionally, the diagnosis can be confirmed through histopathological examination of the affected tissues, electron microscopic visualization of the virus from biopsied lesions, and advanced laboratory techniques such as PCR (Inoshima *et al.*, 2001; Gallina *et al.*, 2006; Zheng *et al.*, 2007; López *et al.*, 2019).

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Orf virus (ORFV) can cause recurrent infections in the same animal, and older sheep may carry the virus without displaying lesions, subsequently transmitting the disease to susceptible flocks (Aneed and Alsaad, 2019). The morbidity rate has been reported to range from 20% to 100%, and mortality between 1% and 93%, depending on factors such as the immune system and stress. The prolongation and severity of the infection are associated with secondary bacterial infections (Gökçe *et al.*, 2005; Gallina *et al.*, 2006).

Reactive oxygen species (ROS) are produced during physiological and metabolic processes. An increase in ROS levels above the physiological threshold can lead to oxidative damage to lipids, proteins, and DNA. MDA is one of the final products of lipid peroxidation caused by free radicals and is considered the most important molecule that serves as an indicator of lipid peroxidation (Kohen-Nyska 2002; Nisbet *et al.* 2008; Aslan *et al.* 2017).

Nitric oxide is released from activated macrophages and endothelial cells. NO is a lipophilic, rapidly acting molecule that plays a role in physiological and pathological processes in animals. It is synthesized in amounts exceeding physiological levels during inflammatory diseases, leading to tissue damage (Sharma *et al.*, 2007; Kirbaş *et al.* 2021). NO has been reported to play a significant role in the primary defense against viruses, parasites, and various bacteria (Kontas and Salmanoglu 2006; Nisbet *et al.* 2007; Kandemir *et al.* 2011; Kırmızıgül *et al.* 2016; Atakişi and Merhan 2017; Aslan *et al.* 2017; Bath *et al.* 2021).

There is a physiological balance between free oxygen radicals and antioxidants. Oxidative stress is known as the disruption of this balance due to pathological causes. Antioxidant enzyme systems work to maintain optimal conditions in the presence of oxidative stress, reducing cellular damage and maintaining metabolic stability. One of these systems involves reduced GSH, which participates in reduction reactions in cell membranes and acts as a coenzyme in certain reactions (Güven *et al.*, 2003; Güven and Kısıçam 2020).

Adenosine deaminase is found in all tissues, particularly in lymphoid tissues. ADA plays a crucial role in the development of the immune system, as well as in the differentiation and proliferation of lymphoid cells (Haskó and Cronstein, 2004). ADA activity primarily affects the immune system and is characterized by metabolic deficiencies in conditions where severe lymphopenia, humoral and cellular immune impairment, and immunodeficiency occur. This property of ADA has been utilized in monitoring the treatment of diseases where immunity is compromised (Haskó and Cronstein, 2004; Whitmore and Gaspar 2016). The aim of this study was to evaluate oxidative stress, antioxidant levels, and cellular immunity in diseased animals by examining changes in MDA, GSH, NO, and ADA activities in lambs diagnosed with contagious ecthyma using clinical findings and PCR technique.

MATERIALS AND METHODS

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments of Kafkas University, under the ethical approval code KAÜHADYK/2023-061.

As animal material, a total of 20 Morkaraman lambs aged 30-90 days were used, including 10 lambs infected with contagious ecthyma (6 females, 4 males) and, as a control group, 10 healthy lambs (5 females, 5 males) from a different flock raised under similar conditions. A general clinical examination was performed on healthy lambs to rule out the presence of subclinical infections. Contagious ecthyma was diagnosed in symptomatic lambs based on characteristic clinical signs, such as typical lesion morphology and localization. Tissue samples were collected from the lesion sites of symptomatic animals and subjected to polymerase chain reaction (PCR) testing for disease confirmation. Blood samples were collected from the jugular vein (Vena jugularis) of all animals. The serum obtained from the blood samples was stored at -20°C until analysis.

Malondialdehyde, reduced GSH, and NO concentrations were measured according to the

methods described by by Yoshioka *et al.*, 1979; Beutler *et al.*, 1963 and Miranda *et al.*, 2001 respectively, while ADA activity was measured using a commercial test kit (Elabscience, China) following the manufacturer's procedure with colorimetric analysis (Epoch, Biotek, USA).

The collected tissues were cut into small pieces using sterile scalpels in sterile Petri dishes and then transferred to polystyrene tubes. They were diluted at a 1:10 ratio with phosphate-buffered saline (PBS) and vortexed for 1 minute. The tubes were centrifuged at 5000 rpm for 15 minutes, after which the supernatants were transferred to new tubes. Nucleic acid extraction from the supernatants was performed using the method described by Sambrook and Russell (2001).

The nucleic acid extracts obtained from tissue samples were subjected to PCR using the PP1-PP4 primer set targeting the B2L gene region, following the optimization protocol specified by the researchers (Inoshima *et al.*, 2000). The PCR reaction mix was prepared using a Taq enzyme kit (Hibrogen, Türkiye) according to the manufacturer's datasheet conditions. A parapoxvirus sample obtained from previous studies was used as a positive control. The resulting amplicons were visualized on a 1% agarose gel containing Safe-Red (Safe View™ Cat No: G108-R, Canada) using a transilluminator.

The data obtained were analyzed using SPSS 22.0 software. The normality of the data was tested using the Shapiro-Wilk test. The difference between the healthy and diseased groups was analyzed using a Mann Whitney U test, depending on the non-normality of the data.

RESULTS

Clinical examinations revealed distinct symptoms such as fever, hypersalivation, anorexia, cauliflower-like swellings on the tongue and gums, redness, and the presence of papules, pustules, and nodules (Figures 1). The comparison of serum MDA, NO, GSH levels, and ADA activities between the infected and control groups of lambs is presented in Table 1.



Figure 1: Papillomatosis in lambs with ecthyma. A. Lesion on the gum, B. Lesion on the lips.

Table 1. Levels of Malondialdehyde (MDA), Reduced Glutathione (GSH), Nitric Oxide (NO), and Adenosine Deaminase (ADA) Activity in serums lamb

Parameters	Control	Infected	P value
MDA ($\mu\text{mol/L}$)	2.69 ± 0.44^x	4.73 ± 0.24^y	$P < 0.001$
GSH (mg/dL)	15.01 ± 1.59^a	10.05 ± 1.10^b	$P < 0.05$
NO ($\mu\text{mol/L}$)	6.12 ± 0.72^a	8.32 ± 0.55^b	$P < 0.05$
ADA (U/L)	7.12 ± 0.60^x	21.53 ± 1.82^y	$P < 0.001$

When the amplicons obtained from the PCR were visualized, bands corresponding to the expected product size of 594bp were observed in all 10 samples suspected of ecthyma, confirming

them as positive (Figure 2). The positive and negative controls were also found to have worked correctly.

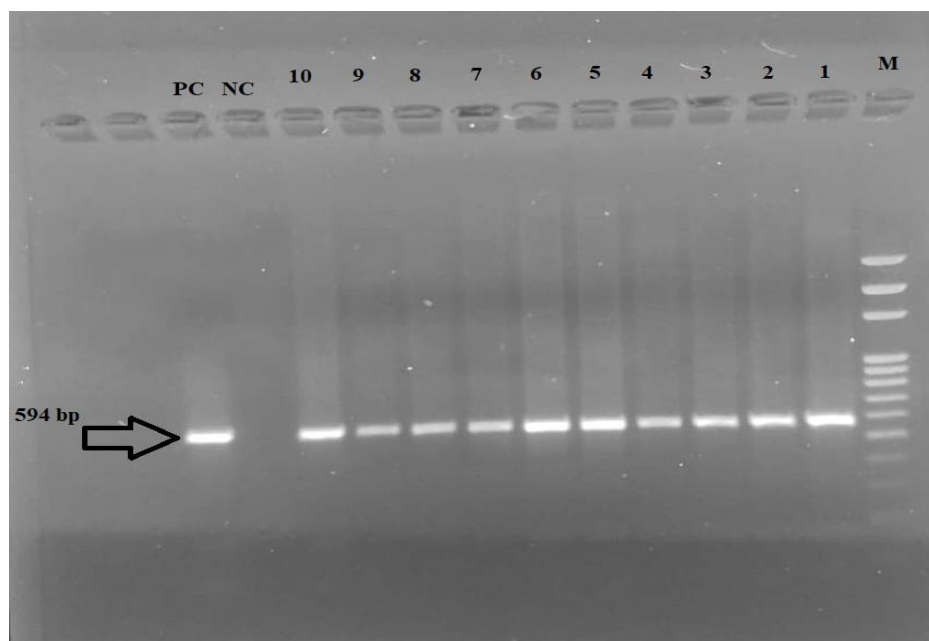


Figure 2. Gel image obtained after the PCR process. M = 100 bp DNA Marker. Samples 1-10. PC = Positive Control, NC = Negative Control.

DISCUSSION

In contagious ecthyma, clinical signs such as anorexia, hypersalivation, cauliflower-like swellings and redness on the tongue and gums, followed by typical lesions like papules, pustules, and nodules, have been reported (Al Saad *et al.*, 2017; Hosamani *et al.*, 2009; Nandi *et al.*, 2011; Joseph *et al.*, 2015). In our study, clinical examination of the animals revealed typical cauliflower-like lesions on the gums in the group of lambs with contagious ecthyma (Figure 1A-B). The findings obtained from clinical examinations were consistent with those reported in previous studies.

Studies have reported that the formation of lipid peroxidation is associated with a decrease in GSH concentration (Güven and Kısacım, 2020). In this study, GSH concentration in the infected group was found to be significantly lower ($P<0.05$) compared to the control group. The decrease in GSH concentration observed in the group of lambs with ecthyma in this study is likely due to the consumption of antioxidants by free radicals, a finding consistent with previous studies on sheep with contagious ecthyma and other infections (Deveci *et al.*, 2017).

In the current study, infected group had higher MDA concentrations than the control group ($P<0.001$). Previous studies on sheep and goats naturally infected with contagious ecthyma have also reported elevated MDA concentrations (Deveci *et al.*, 2017; Kazemi Asl *et al.*, 2018). Additionally, studies conducted on parasitic, viral, and bacterial diseases in sheep, goats, and cattle have shown that serum MDA levels increase because of inflammation (Issi *et al.*, 2008; Nisbet *et al.*, 2008; Ergönül and Konaş Akşar, 2009; Kırmızıgül *et al.*, 2016).

In our study, the NO levels measured in lambs with contagious ecthyma were statistically significantly higher than those in the control group ($P<0.05$). Studies conducted on lambs and sheep with ecthyma have reported elevated NO levels, which have been attributed to an increased release of free radicals due to elevated neutrophil levels (Kandemir *et al.*, 2011; Deveci *et al.*, 2017). The high NO levels observed in lambs with contagious ecthyma in this study are believed to be due to the excessive production of

free radicals caused by the infection, leading to a disruption of physiological balance.

The high NO and MDA levels, along with the low GSH levels observed in this study, are consistent with previous studies that have identified these parameters as important indicators of lipid peroxidation in sheep, goats, and cattle, particularly in the context of poxvirus and other viral, bacterial, and parasitic infections (Deveci and Güven, 2008; Issi *et al.*, 2008; Nisbet *et al.*, 2008; Kırmızıgül *et al.*, 2016; Uzlu *et al.*, 2016; Deveci *et al.*, 2017; Bozukluhan *et al.*, 2018).

In this study, changes in total serum ADA activity in lambs with contagious ecthyma were compared with those in healthy lambs. Serum ADA activity in lambs with contagious ecthyma was significantly higher ($P<0.001$) than in the control group. A reference value study showed ADA activity in sheep to be in the range of 7.57 ± 0.30 IU/L and 7.31 ± 0.47 IU/L (Altuğ *et al.*, 2009). The values measured in healthy lambs in this study fell within this reference range. Elevated serum ADA levels have been reported in bovine leukemia (Yasuda *et al.*, 1996), goats infected with caprine arthritis-encephalitis virus (Souza Rodrigues *et al.*, 2012), sheep with foot-and-mouth disease (Yarım *et al.*, 2006), cows with trichophytosis (Çenesiz *et al.*, 2007), and sheep naturally infected with *Babesia ovis* (Arslan *et al.*, 2018). The high ADA concentrations in lambs with contagious ecthyma compared to the control group in this study are consistent with the literature. This increase is thought to be due to the involvement of ADA in the activation of cell-mediated immunity.

CONCLUSION

In conclusion, it was determined that oxidative stress occurred, and ADA activity increased in lambs with infectious ecthyma disease. Although the changes observed in oxidative stress parameters and ADA activity in lambs with infectious ecthyma disease are a steppingstone to understand the pathophysiology of the disease, further studies are needed to monitor the efficacy of disease treatment and to determine the pathophysiology in detail.

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