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CONTENTS

A study of lipid and protein profiles and liver enzyme levels in neonatal diarrheic calves based on clinical severity of the disease

ABSTRACT

The purpose of this study was to investigate the serum lipid and protein profile as well as liver enzyme levels in neonatal calves with diarrhea. The study included 40 calves, 30 with diarrhea and 10 healthy (control). Calves with diarrhea were divided into three groups mild, moderate, and severe based on clinical findings. Blood samples were taken from the *vena jugularis* after routine clinical examinations of the calves to determine the serum lipid and protein profile, liver enzymes and glucose levels. Serum total cholesterol (TC) ($P<0.01$), high-density lipoprotein (HDL) ($P<0.01$), and low-density lipoprotein (LDL) ($P<0.05$) levels were found to be lower in the mild, moderate, and severe groups compared to the control group. No significant difference in total protein (TP) and albumin (ALB) values was found between the groups. Additionally, serum aspartate aminotransferase (AST) ($P<0.01$) and alkaline phosphatase (ALP) ($P<0.05$) levels were higher in calves with diarrhea than in the control group, conversely glucose levels ($P<0.05$) were lower. The current study concluded that there was no change in the protein profile, but the lipid profile was negatively affected, and liver function was impaired in calves with neonatal diarrhea. Furthermore, as the clinical severity of the disease increased the impairment in liver function raised.

Keywords: Calf diarrhea, liver enzymes, lipid profile, protein profile

INTRODUCTION

Neonatal calf diarrhea is a significant problem in cattle breeding that is caused by both infectious and non-infectious causes, is widespread, and causes significant economic loss (Izzo et al., 2011). Bacteria, viruses, and protozoa are among the infectious agents that contribute to the disease's progression (Cho and Yoon, 2014). Clinical findings in neonatal calf diarrhea vary greatly depending on the severity of the diarrhea and the level of inflammation. It progresses from mild watery diarrhea to lethargy and coma in dehydrated and acidotic animals (Grünberk, 2022). Therefore, various clinical scoring methods are used to evaluate the clinical situation (Walker et al., 1998; Sayers et al., 2016).

Lipids serve as a form of energy storage (triglycerides), a component of cell membranes, and the precursor to all steroid hormones (cholesterol) (Arfuso et al., 2017). Therefore, changes in plasma lipid levels affect other organ or tissue functions (Nassaji and Ghorbani, 2012).

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Research Article

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Lipids are insoluble in plasma and are transported bound to carrier proteins called lipoproteins, including high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) (Arfuso et al., 2017). Infections and inflammations are known to interfere with lipid and lipoprotein metabolism, such as lipid oxidation and cholesterol transport, to stimulate the anti-inflammatory response (Tall and Yvan-Charvet, 2015).

Proteins are important components of biological processes; some are involved in the structural support of connective tissues, while others are involved in biochemical reactions (Anderson and Anderson, 2002). Diseases can be caused by any dysfunction or imbalance in their concentration (Pieper et al., 2003). Blood proteins are likely to change during the course of diseases and measuring them is an important part of routine biochemistry and clinical laboratory practice (Tóthová et al., 2016).

The systemic inflammatory response that may develop during infection or endotoxemia, if severe enough, can damage and impair various organ or system functions such as the liver, kidney, respiratory and circulatory system (Ramachandran, 2014).

Digestive system diseases in calves can cause irregularities in the homeostasis of animals by causing changes in blood biochemical parameters including serum lipid and protein levels (Nagyová et al., 2015). The most accurate approach for determining the prognosis of the disease and the treatment protocol in neonatal calf diarrhea is to evaluate the changes in biochemical components in conjunction with the clinical severity of the disease. Few studies have examined the serum lipid and protein profile and liver enzyme levels in calves with diarrhea (Bozukluhan et al., 2017; Al-Alo et al., 2017; Athanasiou et al., 2019). However, no study was found in which the relevant parameters were evaluated according to the clinical severity of the disease. Therefore, the objective of this study was to assess the liver enzyme levels and serum lipid and protein profiles in neonatal calves with diarrhea in accordance with the clinical course of the disease.

MATERIAL and METHOD

Animal Material

The animal material of the study consisted of a total of 40 calves, aged 0-15 days, of different breeds and genders, 30 of them with diarrhea and 10 of which were healthy.

Groups

The study included four groups, one healthy and three diseased groups, each with ten calves. Calves with diarrhea were classified as mild, moderate, or severe based on dehydration and depression criteria (Walker et al., 1998) (Table 1).

Table 1. Clinical scoring in calves with diarrhea (Walker et al, 1998).

Eyeball recession into the orbit	**0: normal position**
	1: mild
	2: severe
Thoracic skin tent duration (s)	0: recovery after 1 seconds
	1: recovery after 1-3 seconds
	2: recovery after >4 seconds
Sucking Reflex	0: strong regular sucking reflex
	1: poor ineffective sucking reflex
	2: no sucking reflex
Fecal consistency	1: watery
	2: pastose
	3: solid

Control group (n=10): This group consisted of healthy calves with no health problems with a score of eyeball appearance: 0, skin tent duration: 0, sucking reflex: 0, and fecal consistency: 3.

Mild group (n=10): This group consisted of calves with mild diarrhea with a score of eyeball recession into the orbit: 0, skin tent duration: 0, sucking reflex: 0, and fecal consistency: 1.

Moderate group (n=10): This group consisted of calves with moderate diarrhea with a score of eyeball recession into the orbit: 1, skin tent duration: 1, sucking reflex: 1, and fecal consistency: 1.

Severe group (n=10): This group consisted of calves with severe diarrhea with a score of eyeball recession into the orbit: 2, skin tent duration: 2, sucking reflex: 2, and fecal consistency: 1.

Blood Sampling

For biochemical analysis, 8 ml blood samples were taken from the *vena jugularis* of all calves into gel serum tubes (Vacutainer®, BD, UK). Blood samples were allowed to clot for 30 minutes at room temperature and centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (Beckman Coulter®, USA). The obtained sera were taken into eppendorf tubes and stored at -80 °C until the day of analysis.

Determination of Lipid Profile

Total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Determination of Protein Profile

Total protein (TP) and albumin (ALB) levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Determination of Liver Enzymes, Direct Bilirubin and Glucose Levels

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), direct bilirubin (DBIL) and glucose levels in serum samples were determined with commercial kits using an autoanalyzer (Mindray BS-300 Chemistry Analyzer®, China).

Statistical Analysis

The data obtained from the study were analysed using the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, IL). Normality of data distribution for each parameter was evaluated using a Kolmogorov–Smirnov test. In order to compare the groups, parametric variables (TC, HDL) were evaluated using one-way ANOVA test, while non-parametric variables (AST, ALP, TG, LDL, TP, ALB, DBIL, Glucose) were evaluated using Kruskal-Wallis test. P values equal or less than 0.05 were considered statistically significant.

RESULTS

Lipid Profile Findings

Table 2 displays the results of the lipid profile analysis. The TC and HDL levels of calves with diarrhea in the mild, moderate, and severe groups were found to be lower than the control group (P=0.000). LDL levels were found to be lower in calves with mild and moderate diarrhea compared to the control group, with the moderate group having the lowest LDL levels (P=0.013). There was no statistically significant difference in the TG levels of the groups.

Table 2. Lipid profile findings in groups.

Parameters	Control (n=10)	Mild (n=10)	Moderate (n=10)	Severe (n=10)	P
TC	71.20±23.98[a]	42.90±15.30[b]	32.50±16.19[b]	42.30±19.92[b]	**0.000**
TG	38.00±16.37	26.60±22.18	40.40±48.56	41.00±49.17	0.326
HDL	44.50±12.87[a]	27.70±9.25[b]	23.40±9.40[b]	26.00±8.95[b]	**0.000**
LDL	36.60±14.80[a]	21.60±7.15[b]	16.20±6.67[c]	23.10±10.12[abc]	**0.013**

TC = total cholesterol, TG = triglyceride, HDL= high-density lipoprotein, LDL = low-density lipoprotein. Data are presented as the mean ± SD, SD: standard deviation. Different letters in the same line are statistically significant (P<0.05)

Protein Profile Findings

Table 3 displays the protein profile findings. The TP levels of the calves with diarrhea in the mild, moderate, and severe groups were found to be higher than the control group, but this difference was not statistically significant. Similarly, ALB levels in calves with diarrhea in the moderate and severe groups were higher than in the control group, but this difference was not statistically significant.

Table 3. Total protein, albumin, liver enzymes, direct bilirubin and glucose levels in the groups.

Parameters	Control (n=10)	Mild (n=10)	Moderate (n=10)	Severe (n=10)	P
TP	6.12±1.03	6.70±1.22	7.02±1.78	7.78±1.82	0.242
ALB	2.66±0.15	2.60±0.26	2.75±0.28	2.96±0.64	0.266
AST	39.70±15.80[a]	48.50±18.52[ab]	63.80±27.04[b]	159.70±164.33[c]	**0.002**
ALP	231.10±79.65[a]	313.10±175.17[ab]	294.40±93.74[ab]	496.90±207.80[b]	**0.017**
DBIL	0.12±0.12	0.15±0.14	0.21±0.20	0.11±0.12	0.515
Glucose	114.20±31.31[a]	83.30±22.17[b]	81.30±27.96[b]	87.60±67.20[b]	**0.040**

TP = total protein, ALB = albumin, AST = aspartate aminotransferase, ALP = alkaline phosphatase, DBIL = direct bilirubin Data are presented as the mean ± SD, SD: standard deviation. Different letters in the same line are statistically significant (P<0.05)

Liver Enzymes, Direct Bilirubin and Glucose Levels

Table 3 displays the levels of liver enzymes, DBIL, and glucose. The AST levels of the calves with diarrhea in the moderate and severe groups were higher than the control group, with the severe group having the highest AST levels (P=0.002). Calves in the severe group had the highest ALP levels (P=0.017). It was determined that there was no statistical difference among the groups' DBIL levels. All diarrheic calves had lower glucose levels than the control group (P=0.040).

DISCUSSION

The purpose of this study was to evaluate the serum lipid and protein profile, as well as liver enzyme levels, in neonatal diarrheic calves based on the clinical course of the disease. When compared to the control group, TC, HDL, LDL, and glucose levels decreased while AST and ALP levels increased in calves with diarrhea. It was found that as the clinical severity of the disease increased, so did the level of liver enzymes.

Calf diarrhea affects not only the gastrointestinal system but also other system or organ functions such as the lung, kidney, and liver (Sobiech et al., 2013). Calf diarrhea, like infectious and inflammatory diseases, can also cause changes in serum lipid and protein profiles (Tall and Yvan-Charvet, 2015; Tóthová et al., 2016). In the present study, a significant decrease was determined in the TC, HDL and LDL levels of the calves with diarrhea compared to the control group. However, no statistical significance was determined in the intergroup comparison of these reductions among calves with diarrhea. There was no

difference in TG levels between the groups. It was thought that these data could be used as an indicator of infection in calves. Lipopolysaccharides have been shown to be neutralized by lipoproteins (Barati et al., 2011; Morin et al., 2015) and thus changes in lipid levels may be an important indicator of acute bacterial infections (Nassaji and Ghorbani 2012). Furthermore, significant changes in plasma lipid and lipoprotein concentration, composition, and function have been observed in humans (Alvarez and Ramos 1986; Wendel et al., 2007; Barati et al., 2011; Cirstea et al., 2017), calves (Civelek et al., 2007; Joshi et al., 2015; Bozukluhan et al., 2017; Aydogdu et al., 2018) and dogs (Yilmaz and Sentürk, 2007) during inflammation and infections, as a decrease in TC, LDL, and HDL levels and an increase in TG levels. The changes in the lipid profile observed in our study were thought to be caused by cytokine release (Hardaróttir et al., 1994; Fraunberger et al., 1999; Khovidhunkit et al., 2000; Murch et al., 2007; Lekkou et al., 2014; Morin et al., 2015; Albayrak and Kabu, 2016). In support of this view, El-Bahr and El-Deep (2013) reported that the cytokine levels in buffalo calves with bronchopneumonia were significantly higher than in healthy calves, while serum TC, HDL and LDL levels were significantly lower. Similarly, in cases of inflammation, it has been reported that while TC levels decrease, cytokine levels such as TNF-α and IL-6 increase (Akgün et al., 1998; Gordon et al., 2001; Lekkou et al., 2014). Sepsis has also been linked to an increase in TG levels in humans and animals, which may be due to the induction of hepatic and adipose tissue lipolysis as well as an increase in VLDL production (Alvarez and Ramos, 1986; Civelek et al., 2007).

Changes in protein profile often occur as secondary manifestations in many diseases. Determining their concentrations can provide important information for differential diagnosis to the clinician (Bartosz and Katarzyna, 2016). High TP levels may indicate higher colostral protein levels in young animals, as a favourable indicator (Marcato et al., 2018), however high TP and ALB levels may indicate dehydration, as an unfavourable indicator (Knowles et al., 1999; Swanson and Morrow-Tesch, 2001). ALB can be used as a prognostic marker or to evaluate the severity of diseases, in addition to measuring dehydration (Humblet et al., 2004; Schneider et al., 2013). Low ALB concentrations in dairy cattle, for example, have been linked to uterine infections (Schneider et al., 2013) and inflammation (Jacobsen et al., 2004). However, it is necessary to investigate whether the increase or decrease in ALB levels in young animals is related to the disease or to future health problems (Marcato et al., 2018). In this study, although there was no significant difference between TP and ALB levels of calves with diarrhea and healthy calves, it was determined that these values were higher in calves with diarrhea and this level was directly proportional to the clinical severity of diarrhea. Dehydration was suspected as the cause of this situation.

A systemic inflammatory response that may develop as a result of infection or endotoxemia can damage and impair the functions of the circulatory system and vital organs. The liver is one of these essential organs (Ramachandran, 2014). On the other hand, it has been reported that new-born calf diarrhea can cause liver function damage, as well as severe necrotic and dystrophic lesions. (Grodzki et al., 1991). Increases in serum creatinine, total bilirubin, DBIL, gamma glutamyl transferase (GGT), AST, and ALT levels have been reported in calves with diarrhea (Irmak and Güzelbekteş, 2003; Russel and Roussel, 2007; Kaneko et al., 2008; Başer and Civelek, 2013; Merhan et al., 2016). In our study, there was no difference between DBIL levels of calves with diarrhea and healthy calves, but AST and ALP levels of calves with diarrhea were found to be significantly higher than those of healthy

calves. It was found that the clinical severity of the condition correlated with an increase in this elevation. In this case, it was concluded that liver damage occurred in calves with new-born diarrhea, and that the severity of liver damage correlated with the severity of the disease. Similarly, Grodzki et al. (1991) reported that there were significant increases in ALP levels in calves aged 1-10 days with diarrhea and liver damage could occur in calves with diarrhea. Bozukluhan et al. (2017) also found significant increases in ALP, total bilirubin and DBIL levels in calves with diarrhea compared to the control group, and they suggested that these findings indicate liver and biliary tract damage in calves with diarrhea. The relationship between hypoglycemia and septicemia/endotoxemia in neonatal calves has been demonstrated in experimental studies (Ballou et al., 2011; Constable et al., 2017). In line with these reports, the current study revealed a significant decrease in glucose levels in calves with diarrhea compared to healthy calves.

CONCLUSION

In the present study, it was concluded that there was no change in the protein profile in calves with neonatal diarrhea, the lipid profile was adversely affected and liver function was impaired, and as the clinical severity of the disease increased, the impairment in liver function also increased.

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Ethical approval:

This study was carried out per Atatürk University's approved ethical rules (protocol no. 2018/50 date: 06/04/2018), and written informed consent was obtained from the owner for each calf.

Conflict of interest: The authors declare no conflict of interest.

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Serum amyloid A, haptoglobin and ceruloplasmin levels before and after treatment in cattle with dermatophytosis

Research Article

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ABSTRACT

The aim of this study is to determine the changes in serum amyloid A (SAA), haptoglobin (Hp) and ceruloplasmin (Cp) levels, before and after treatment in cattle with dermatophytosis. The animal material of the study consisted of a total of 30 cattle, 20 with dermatophytosis and 10 healthy, of different ages and genders. Blood was taken from the sick animals twice, before and after the treatment, and once from the healthy animals, and their serum was separated. The obtained sera were stored at -20 ºC until the Hp, SAA and Cp levels were measured. Sick animals were vaccinated with Trichoben® (Interhas, TR) twice, with an interval of 14 days. When the pre-treatment and post-treatment serum Hp, SAA and Cp values of the sick animals were statistically compared, it was determined that the pre-treatment values were significantly higher ($P<0.001$) than the post-treatment values. Similarly, when the values of pre-treatment and control animals were compared, it was seen that the difference was significant ($P<0.001$). When the serum SAA, Hp and Cp values of the animals in the post-treatment and control groups were compared, it was determined that the difference was statistically insignificant ($P>0.05$). As a result, it was determined that SAA, Hp and Cp values, which are positive acute phase proteins, increased significantly in cattle with dermatophytosis and these values decreased with treatment. According to this result, it was concluded that serum SAA, Hp and Cp values are important biomarkers in the evaluation of the prognosis of the disease in cattle with dermatophytosis.

Keywords: Dermatophytosis, bovine, serum amyloid A, haptoglobin, ceruloplasmin

Introduction

Dermatophytosis (ringworm, trichophytosis) is a group of superficial fungal infections of the skin, hair, feathers and nails rich in keratin, and is an important zoonotic infection limited to inanimate cornified tissues. The disease not only affects the skin but also causes stress in animals, weight loss, decrease in milk yield, growth retardation, and due to these effects, it is economically important in our country (Kırmızıgül et al., 2008, Aslan et al., 2010).

Dermatophytes first affect the inanimate layer of the skin called the stratum corneum and cause inflammatory reactions at the infection site by secreting keratinase enzyme. Infection and inflammatory reactions, which usually start in the head (60%) or neck (30%) region, are mostly in the form of increased local temperature, redness, swelling and first ring-shaped, asbestos-looking alopecia (Aslan et al., 2010, Cafarchia et al., 2010, Lakshmipathy and Kannabiran 2010, Yılmazer et al., 2010, Paksoy et al., 2013, Bhikane et al., 2015).

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In cattle herds, dermatophytosis usually proceeds enzootically. The disease is caused by fungi of the *Trichophyton, Microsporum* and *Epidermophyton* genus, also called dermatophytes in cattle. *Trichophyton* species are mostly responsible for infections in animals, followed by *Epidermophyton* and *Microsporum species* (Kırmızıgül et al., 2008, Aslan et al., 2010, Lakshmipathy and Kannabiran 2010).

Acute phase proteins (APP) are blood proteins synthesized by hepatocytes in acute phase response (APR). The acute phase response, is a non-specific, complex reaction of the organism against to homeostasis that occurs as a result of inflammation, tissue damage, infection, neoplastic growth or immunological disorders (Merhan and Özcan 2010). Acute phase proteins are classified into two groups as positive and negative acute phase proteins. Positive APPs (haptoglobulin, serum amyloid A, ceruloplasmin, alpha 1-acid glycoprotein, C-reactive protein, and fibrinogen) are substances in glycoprotein structure released by the stimulation of inflammatory cytokines from hepatocytes and are proteins with increased serum levels. Negative APPs (albumin, transferrin, retinol binding protein) are structural plasma proteins commonly found in blood (Merhan and Özcan 2010, Sevgisunar and Şahinduran, 2014). An increase in the synthesis of some plasma proteins (C-reactive protein/CRP, Ceruloplasmin/Cp, Haptoglobulin /Hp) is observed during APR. By looking at the plasma or serum levels of these proteins, an important idea that has clinical value in terms of diagnosis and prognosis of the disease can be obtained (Ulutaş and ark. 2007).

Hp is the most important acute phase protein in which two α, two β-polypeptide chains are linked by disulfide bonds and increases approximately 8 hours after inflammatory stimuli. The primary function of Hp is to bind free hemoglobin released from erythrocytes. Hp-hemoglobin compound slows down the growth of bacteria. Hp is one of the important parameters used to determine the severity of the inflammation. Although the functions of serum amyloid A (SAA) are still being investigated, it has been reported that it may have functions such as transport of cholesterol to hepatocytes, suppression of fever, inhibition of oxidative destruction of neutrophil granulocytes, stimulation of calcium mobilization by monocytes, endotoxin detoxification, inhibition of lymphocyte and endothelial cell proliferation. Cp is an important protein involved in the transport of copper in the blood and is one of the acute phase proteins used to understand the severity of inflammation in disease states. Cp prevents further damage to tissues by collecting oxygen radicals (Ulutaş et al., 2007, Karapehlivan et al., 2007; Sevgisunar and Şahinduran, 2014, Tothova et al., 2014).

In this study, it was aimed to determine the changes in some acute phase proteins in cattle with dermatophytosis and to investigate the changes that will occur in these proteins after vaccination against to dermatophytosis.

MATERIALS AND METHODS

All animal material of this study; A total of 30 cattle were brought to the animal hospital of the veterinary faculty from the livestock enterprises in Kars and its villages with the complaint of dermatophytosis or for the purpose of general health check-up. In the dermatophytosis (Group I) group, a total of 20 cattle, 7-24 months old, 7 *Brown Swiss* crosses and 13 *Simmental* crosses, 10 females and 10 males, were included. In the control group (Group II), 10 healthy cattle of the same age range, of different sexes were used. For general examination, blood was drawn from the animals in the first group twice, before and after the treatment. From the healthy animals in the second group, blood was drawn once during the general examination. The blood samples were centrifuged at 3000 rpm for 10 minutes and their serums were separated. These serum samples obtained were stored at -20ºC until the SAA, Hp and Cp values were measured. The animals in the

first group were administered 10 ml of Trichoben® (Interhas, TR) vaccine for therapeutic purposes and the vaccine was repeated for the second time with an interval of 14 days. In addition, animals were checked in weekly periods following drug administration and their recovery processes were observed. Blood samples were taken again from Group I, 28 days after repeat vaccination.

The lesioned areas of cattle in Group I were cleaned with cotton soaked in 70% alcohol before scraping. Then, skin scrapings were taken from the lesioned areas by a sterile scalpel. The samples were first processed with 10% KOH and after the skin samples were prepared, they were examined under the microscope. The appearance of typical spores in the examination was evaluated as positive in terms of dermatophytosis. The scrapings taken from the border areas of the lesioned and healthy skin of the cattle were incubated for 2-6 weeks at 32°C in an aerobic humid environment by sowing with "Sabouraud Dextrose Agara (SDA)" horizontal stub method. The morphologies of the fungal colonies that grew during the incubation period were examined with the naked eye and stereo-microscope.

According to the "k" clause of the 8th paragraph of the 8th article of the relevant regulation, the all-examination materials evaluated in this study were obtained from the samples taken from the animals during the routine examination, with the permission of the animal owners.

Acute Phase Protein Analysis

SAA was determined with an ELISA-based assay, Phase SAA Assay Kit (Tridelta, Development Ltd, Ireland), and Hp was determined Spectrophotometrically Phase Haptoglobulin Assay Kit (Tridelta, Development Ltd, Ireland), Cp concentration was determined by Richterich and Colombo pH 5.6 and at 546 nm. It was measured spectrophotometrically with the "*p-phenylenediamine oxidase activity method*" defined as.

Statistical Analysis

Statistical analyzes were done with SPSS-20.0 windows program. The analysis of variance method was used in repeated measurements to determine the changes in the parameters determined before and after the treatment in animals in the control and Group I. $P<0.05$ and less were considered significant in statistical evaluations.

RESULTS

It was observed that dermatophytosis lesions were located on the head in 11, neck in 5, inguinal region in 2 and dorsal region in 2 of the cattle included in the study. The lesions were "asbestos-looking, scaly, dry and ring-shaped" which classic symptoms for the disease are. As a result of microscopic examinations and cultures made from scrapings taken from the lesioned areas, it was determined that all of the factors were *Trichopyton verrucosum*.

As a result of the study, changes in serum SAA, Hp and Cp values of animals in group I and group II are given in *Table 1*. When the SAA, Hp and Cp values of the animals in Group I were compared (before and after the treatment), the statistical difference between these values was found to be significant ($P<0.001$). In group I, all three parameters were found to be decrease compared to pre-treatment (*Figure 1,2,3*). When the SAA, Hp and Cp values obtained from the cattle in the first group before the treatment were compared with the healthy cattle in the group II, the difference was determined to be statistically significant ($P<0.001$). When the after-treatment values obtained from the cattle in the first group were compared with the values of the healthy animals in group II, the difference between the

results was found to be statistically insignificant (P>0.05).

Tablo 1 SAA, Hp and Cp values of animals in the group I and II.

Parameters	Group I (n=20)		Group II (n=10) (x±Sx) (min-max)	P
	Before Treatment (x±Sx) (min-max)	After Treatment (x±Sx) (min-max)		
SAA (µg/dL)	96.44±174.38 [A]	8.02±20.78 [B]	8.02±19.27 [B]	**0.001**
Hp (µg/dL)	0.21±0.53 [A]	0.06±0.12 [B]	0.07±0.10 [B]	**0.001**
Cp	18.13±34.28 [A]	8.96±16.87 [B]	8.47±14.53 [B]	**0.001**

[A, B] Groups with different letters on the same line are statistically significant.

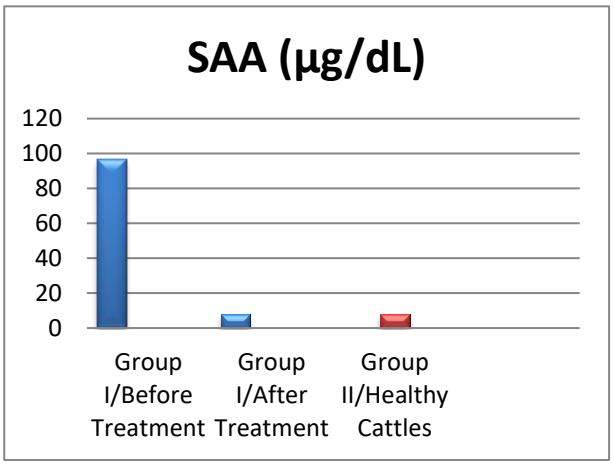

Figure 1. The change in SAA values of Group I cattle "before and after treatment" and Group II healthy cattles.

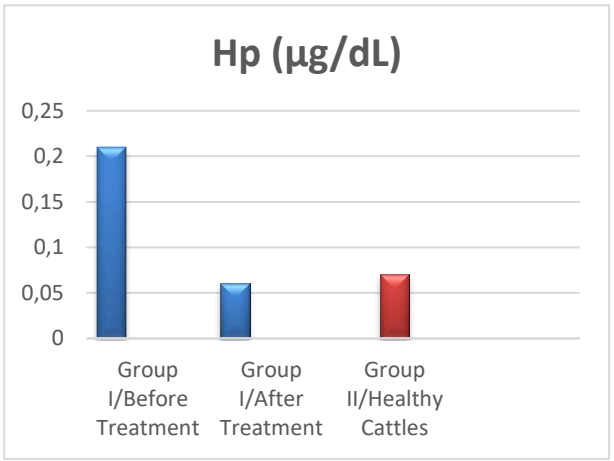

Figure 2. The change in Hp values of Group I cattle "before and after treatment" and Group II healthy cattles.

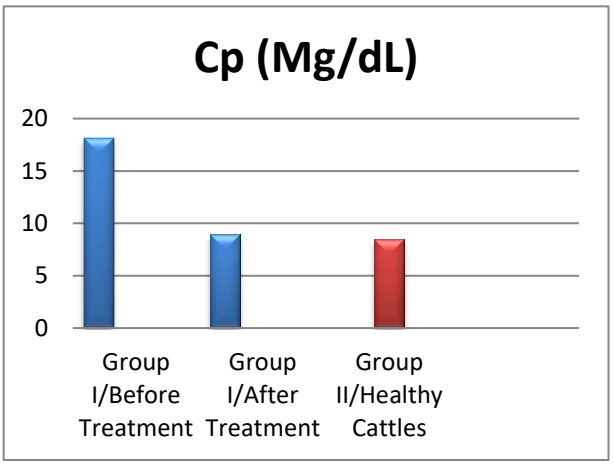

Figure 3. The change in Cp values of Group I cattle "before and after treatment" and Group II healthy cattles.

DISCUSSION AND CONCLUSION

Dermatophytosis is characterized by keratinized thickening of the epithelial layer and shedding of infected hairs in cattle. It is known that dermatophytosis, which is common in the world, causes diseases of varying severity in humans and animals and it is stated to be of zoonotic importance. The disease causes loss of live weight, deterioration of skin quality, growth retardation in animals and it causes serious economic losses as the export of sick animals is prohibited. Dermatophytosis is clinically characterized by raised, ring-shaped alopecia with asbestos-like surface (Aslan et al., 2010, Cafarchia et al., 2010, Lakshmipathy and Kannabiran 2010, Yılmazer et al., 2010, Paksoy et al., 2013, Bhikane et al., 2015). The lesions

determined clinically in this study were similar to those reported by the researchers; it was characterized by ring-shaped, asbestos-like and alopecia.

Significant changes in APP concentrations have been reported by investigators in most inflammatory conditions and diseases diagnosed in animals. However, there are limited studies investigating APPs in dermatological diseases of ruminants (Çitil, 2003, Ulutaş et al., 2007, Kabu and Sayın, 2016).

Serum amyloid A, also called apolipoprotein, although its functions are not fully revealed, in inflammatory events; it is an important AFP with 7 known isoforms in cattle, whose functions such as suppression of fever, inhibition of oxidative destruction of neutrophil granulocytes, inhibition of lymphocyte and endothelial cell proliferation have been demonstrated by researchers (Sevgisunar and Şahinduran 2014, Tothova et al., 2014). Studies examining SAA in dermatological cases in ruminants are very rare. In a study conducted in Anatolian buffaloes, it was reported that SAA increased but it was also determined that the SAA value was not adequately examined in dermatophytosis cases in cattle (Kabu and Sayın, 2016). In the aforementioned study conducted by the researchers, it was reported that the SAA value obtained from buffaloes with dermatophytosis was found to be quite high compared to the control group consisting of healthy buffaloes, while it was determined that the SAA value, we obtained in our study showed statistically similar results with this study ($p<0.001$). These results are explained by many researchers as increasing the SAA value by being affected by inflammatory phenomena or infections (El-Bahr and El-Deeb, 2013, Kabu and Sayın, 2016). In our study, it was thought that the high SAA value we obtained from cattle with dermatophytosis was due to inflammation caused by dermatophytosis, in line with what the researchers reported.

Haptoglobin is one of the most important acute phase proteins and shows a significant increase approximately 8 hours after inflammatory stimuli (Sevgisunar and Şahinduran, 2014; Karaca and Akgül 2016). The primary function of Hp is to bind the free hemoglobin released from erythrocytes and slow down the growth of potential pathogens through this compound. Because of this and many reasons such as its anti-inflammatory properties, Hp is one of the important parameters used to determine the severity and health status of the inflammations (Sevgisunar and Şahinduran 2014, Tothova et al., 2014). There are still not enough studies investigating serum Hp values in cattle in different cases and physiological conditions (Chan et al., 2004, Debski et al., 2016). Studies investigating Hp values in skin problems in different animal species or specifically dermatophytosis cases in ruminants are very limited. Researchers reported that this difference in Hp value, which was determined to be significantly increased in buffaloes with dermatophytosis compared to healthy animals, was also statistically significant ($p<0.001$). In our study, it was determined that the high Hp value we obtained from cattle with dermatophytosis was similar to the Hp values obtained previously from buffaloes with dermatophytosis and dogs with skin problems. Consistent with other researchers who reported that Hp is a very important AFP in understanding the severity of fire, it was concluded that the high Hp value we obtained in group I at the beginning of our study was also caused by severe dermatophytosis (Ulutaş et al., 2007, Kabu and Sayın, 2016). It was determined that the difference between the Hp values we obtained in our study was statistically significant ($P<0.001$).

Ceruloplasmin is a very important AFP that is primarily synthesized in the liver but can also be

produced in extrahepatic areas, is involved in the transport of copper in the blood and can be used for diagnosis in most disease states. Cp is also an AFP that increases to prevent damage by collecting oxygen radicals in tissues damaged by inflammatory events (Ulutaş et al., 2007, Sevgisunar and Şahinduran, 2014, Tothova et al., 2014). In a limited number of studies examining the Cp value in dogs with skin problems, it was reported by researchers that Cp was determined to be higher than in healthy dogs (Charlton et al., 2002, Ulutaş et al., 2007). Arslan et al. (2008), in a study they conducted in cattle exposed to environmental stress, found that the Cp value decreased after in the treatment compared to before the treatment and last Cp levels that did not show any statistical difference with the control group consisting of healthy animals. In our study, it was determined that the after-treatment Cp values obtained in cattle diagnosed with dermatophytosis decreased which was statistically significant ($P< 0.001$). It was determined that the Cp values obtained from Group I after the treatment were very close to the values of the control group and there was no statistical difference between these two values, similar to what the researchers reported (Ulutaş et al., 2007, Arslan et al., 2008). These increases in Cp values obtained from cattle with dermatophytosis were consistent with those reported by the limited number of researchers who evaluated AFPs in animal inflammatory diseases. It was concluded that this increase may be due to intense inflammation caused by dermatophytosis and damage to skin cells and the resulting increased Cp amount.

As a result, some AFPs, which were found to be increased in cattle with dermatophytosis in this study, decreased with the initiation of the successful treatment process following Trichoben® (Interhas, TR) application. This result we obtained was thought to be related to the reduction of stress, cellular healing of the skin, clinical improvement and disappearance of inflammatory symptoms. By this study, the SAA, Hp and Cp values obtained from cattle with dermatophytosis before and on the 14th day after vaccination have not been evaluated and reported by other researchers before. In addition, the present study clearly reveals that SAA, Hp an Cp values are significantly affected in dermatophytosis cases in cattle. It is also concluded that the results obtained from the present study will contribute to future research in inflammatory processes or diseases in which AFP values in cattle or other animal species will be examined, through hypotheses and results.

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Produced from the Master's thesis with the same title in Turkish

Ethical declaration: In accordance with the "Clinical applications for diagnostic and therapeutic purposes" mentioned in Article 1 of the "Regulation on the Working Procedures and Principles of Animal Experimentation Ethics Committees" published in the Official Gazette No. 28914 in 2014, all examination materials evaluated in this study were obtained from the samples taken for diagnostic purposes during routine health examinations from cattle brought to KAU Veterinary Faculty Animal Hospital, Internal Diseases Polyclinic by animal owners at different times, again with the permission of animal owners. During the study period, the animals did not receive any additional medical treatment other than standard medical practices. Conflict of interest: There is no conflict of interest between the authors.

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The relationship between total thiol and pregnancy in hair goats

Research Article

ABSTRACT

Hormonal application have been applied to increase fertility in Hair Goats, but the relationship between reproduction and stress/oxidant values has not been investigated. In this study, the relationship between increase fertility and total thiol values were investigated in Hair Goats. 100 females and 20 male Hair Goats were used in this study. September, 100 females selected and marked, and blood samples were taken from all goats into anticoagulant tubes from vena jugularis 15 days before male introduction to the herd. Blood samples were collected both October and May in all goats. Total thiol values in female were found to be significant in October compared to May. It was determined that there was a difference ($p \leq 0.05$) in the total thiol value in terms of sex and birth type. The total thiol value of twin-bearing hair goats was statistically different from the non-bearing hair goats while the total thiol value in single-bearing hair goats was similar to those that gave birth to twins and those that did not. Goats with low total thiol value had a twin birth and goats with high total thiol value were have no birth. Total thiol value was found to be important for multiple births ($p \leq 0.05$). This study is the first study in the literature to show the relationship between total thiol value and offspring yield in hair goats. As a result, it was concluded that the total thiol value during the breeding season in was related to birth rate and offspring yield in hair goats.

Keywords: Hair goat, total thiol, pregnancy.

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INTRODUCTION

Much research have been carried out in order to increase production in animal husbandry and especially the studies on the breeding of genotype have been emphasized. In some cases, animals do not display their genetic capacity due to insufficient care, nutrition, environmental influences, and oxidative stress. Domestic breed goats, which are procured from enterprises with very good yield performance, cannot show their previous yield performance in the new enterprise. Similar phenomenon is also observed in dairy cattle and management, nutrition and stress factors were thought to be effective. Therefore, the effect of antioxidant substances on the fertility of hair goats should be investigated (Akyüz et al. 2020, Aslankoç et al. 2019, Chianeh et al. 2014, Çamkerten et al. 2019).

During the digestion of foods, some harmful free radicals (ROT) (hydroxyl radicale, superoxide anion, lipid peroxide, nitric oxide, hydrogen peroxide, etc.) are formed (Beaupre and Weiss 2021, Çetinkaya 2020). Damage to the body (especially to proteins, lipids and many biological molecules, especially proteins, lipids and nucleic acids) is called oxidative stress (OSI) (Coşkun et al. 2016, Esra et al. 2012), various enzymes or non-enzymes substances that prevent tissue damage by binding are called antioxidants (TAS) (Güntürk 2021, Çetinkaya 2020, Başkol and Köse 2004).

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In reducing oxidative stress, i.e. repairing damage to tissue, the organic matter that allows antioxidants to bind to damaged tissue is called Thiol (Öktem et al. 2021, Chianeh and Prabhu 2014). Thiol is an organic compound containing a sulfidril (-SH) group that is important in preventing oxidative stress in cells (Beaupre and Weiss 2021, Sen and Packer 20005). The amount of cells damaged by free radicals and the total amount of Thiol are directly proportional (Haydar et al. 2020). The first target of free radicals is thiol groups. With free radicals, the thiol groups in the environment are oxidized and transformed into reversible disulfide bonds. This conversion is the earliest sign of radical-mediated protein oxidation (Öktem et al. 2021, Haydar et al. 2020). Accordingly, the decrease in the amount of Total Thiol in the blood is associated with the damage to the tissues, and the increase in the value of antioxidants for tissue repair and the decrease in the value of thiol are formed. Oxidative stress is defined as the imbalance between oxidants and antioxidants at the cell value (Beaupre and Weiss 2021, Haydar et al. 2020). Antioxidant enzymes are capable of neutralizing harmful free radicals and preventing oxidative stress (tissue damage). Thiol groups provide this operation (Beaupre and Weiss 2021, Büyükoğlu 2018). Thiol groups also have critical roles in antioxidant defense, apoptosis, immune response, inflammation and intracellular signaling mechanisms. Oxidative products such as reactive oxygen species formed in the organism are reduced by transferring their excess electrons to compounds containing thiol (Beaupre and Weiss 2021, Gümüşyayla et al. 2016), while thiol groups are oxidized. The oxidation of thiol groups causes the formation of disulfide bonds. However, this is a reversible reaction, and the disulfide bonds formed can be reduced back to thiol groups. Some of the antioxidants that reduce/eliminate OSI by Thiol are ascorbic acid (Vit. C), α-tocopherol (Vit E) and Ceruloplasmin (Dimri et al. 2010). In addition, zinc, copper, iron and selenium are essential components of certain substances such as hormones and enzymes and endogenous antioxidants (Aslankoç et al. 2019). It has been reported that oxidative stress creates pathological conditions for the cell and impairs the comfort of farm animals, thus affecting production (Lykkesfeldt and Svendsen 2007). Çamkerten et al. (2019) found that the disulfide balance in infested sheep is affected by the pendulum scabies factor and they suggest that antioxidant molecules that will provide this balance should be used in the treatment. Total Disulfide Hemostasis has been shown to suggest that imbalance triggers disease through oxidative stress and tissue inflammation (Üstüner 2018). Haçarız and Baykal (2014) state that antioxidant substances (in order to ensure the regeneration of the damage caused) are more active in the infested liver tissue with Fasciola Hepatica to stimulate the immune system. Thiol groups interact with antioxidants and are neutralized. Total concentrations of thiol and natural thiol, a less toxic product called disulfide, have been shown to decrease (Erel and Neşelioğlu 2014).

MATERIAL and METHOD

Animal Material

The material of the study consisted of 100 heads of females with a live weight (LW) of 45-50 kg and 20 heads of male goats 60-70 kg LW aged 2-4 years in a family business located in Aksaray province. In September, the animals in the enterprise were fed with an average of 100 g of wheat hay and 80-100 g of barley in addition to pasture, while in May, they were fed entirely based on pasture. The water needs of the animals were ad libitum. In October, 15 days before the male introduction, of all goats included in the stady blood samples were taken. Blood was taken from the same animals again before the start of lactation in May. Blood samples were taken from anticoagulant tubes from vena jugularis. The samples were

transported to the laboratory in accordance with the cold chain rule. The tubes containing blood samples were centrifuged at +4°C, 3000 RPM for 10 minutes and the sera were removed. Each resulting serum was transferred to two Eppendorf tubes and stored at -20°C until further analysis. Total thiol values were determined by the colorimetric method revealed by Erel and Neşelioğlu.

Statistical Analysis

Descriptive statistics for continuous variables are given as Mean, Standard Deviation, Minimum and Maximum values, while for Categorical variables it is expressed as number and percentage. One-way analysis of variance was performed to compare group averages in terms of continuous variables. Following the analysis of variance, Duncan multiple comparison test was used to identify different groups. Pearson correlation coefficients were calculated to determine the relationship between these variables. In determining the relationship between groups and categorical variables, Chi-square test was performed. The statistical significance value was taken as 5% in the calculations and SPSS (ver.21) statistical package program was used for the calculations.

RESULTS

Lipid Profile Findings

Total thiol values varied depending on sex and season (low in both sexes compared to May in October) (Table 1) and the importance of the effect of these values on fertility were determined by correlation (Table 3). It was determined that there was a statistical difference ($p \leq 0.05$) in total thiol value in terms of sex and birth Type (Table 2). It was found that the total thiol value of twin-bearing hair goats was similar to single-bearing hair goats but statistically different from infertile hair goats (Table 2). It was found that single-bearing and infertile goats were statistically similar (Table 2). Twin births were higher in hair goats with low total thiol value and no birth was found in hair goats with high total thiol value (Table 2). Total thiol value was found to be important for multiple births ($p \leq 0.05$, $r \geq 0.05$) (Table 2, Table 3). Total thiol values in single-bearing hair goats were similar to those of twin-bearing and non-twin-bearing hair goats (Table 2). From the data obtained in this study, it was seen that there was a correlation between Total thiol and antioxidants (r=0.549) (Table 3).

Table 1. Descriptive statistics and comparison results by season and gender

	Gender	Oktober			May		
		Mean	SD	SEM	Mean	SD	SEM
TotalThiol (µmolL)	M	◊ 298,15 #	66,72	14,92	◊622,95	136,08	30,43
	F	451,84 #	73,11	7,31	518,35	125,49	12,55

Statistically different from May.◊ Statistically different from females

Table 2. Descriptive statistical values and number of animals for total thiol value in October (October Addition) for sterile, single and twin births.

		Mean	SD	Min.	Max.	P
Total Thiol (µmol/L)	Single (n=73)	AB 462,42	62,688	352	568	0,003
	Twin (n=23)	B 414,65	95,197	332	553	
	Infertile (n=4)	A 472,50	41,485	414	502	
	Total	481,00	126,441	101	784	

A, B,C↓: The category that takes different uppercase letters in the same column is statistically significant

Table 3. Correlations between Total Tiol value and TAS, TOS, OSI in October

	Total Thiol (µmol/L)	TAS (mmol/L)	TOS (µmol/L)	OSI
Total Thiol (µmol/L)	1			
TAS (mmol/L)	0,549**	1		
TOS (µmol/L)	0,167**	0,094	1	
OSI	0,047	0,124	0,973**	1

** p< 0.01 *: p<0.05

DISCUSSION

Small ruminants are the animals that make the best use of the land that is not suitable for agriculture and create employment and economy with products such as meat, milk leaf/mohair. Although it is difficult to manage compared to sheep, hair goats make better use of rugged and unproductive pastures. Although the birth rate is high in hair goats, twinning rate is quite low (Boztepe et al. 2014, Erduran 2010). Since hair goats are grazed in pastures with poor vegetation and low grass yield which cause oxidative stress due to the lack of balanced feeding, and therefore it is stated that the fertility is low (Boztepe et al. 2014, Gökdal 20 20). As a matter of fact, in the study presented, it is seen that the birth rate is high in hair goats with low Total Thiol values, which provide neutralization of oxidative stress factors (environment for antioxidants to function) during the breeding season (October) (Table 2).

In the literature review, there were no studies on the subject. Studies aimed at increasing the yield of offspring in goats have been mostly associated with hormone values and studies have been carried out in this direction. This study, which identified a situation that it is possible to increase fertility by reducing oxidative stress, revealed that there is a relationship between Total Thiol value and fertility (Table 3). Ghavipanje et al. (2021), who have studied a similar subject, state that there is not enough data on the evaluation of yield in dairy goats related to TAS. There has been no study revealing the relationship between Total Thiol value and offspring yield in hair goats or any animals. For this reason, it is believed that this study is the first research to evaluate the total thiol value and the fertility of hair goats. Because OSI, which occurs because of diseases and unbalanced feeding in animals, is expressed by many scientists to have a significant effect on comfort, fertility and ovarian functions (Çoşkun et al. 2016, Klir et al. 2019).

In a study conducted at Ankara Numune Training and Research Hospital, the rate of Thiol was found to be significantly higher in Hyper Blood Pressure (HT) patients than in healthy patients. Because cell damage (apopotosis) is higher in sick individuals, to eliminate cell damage binding thiols bind via sulfidril bonds (–SH) to prepare antioxidants to prevent this damage. In other words, thiols will be used, and cell damage will be tried to be eliminated. In healthy ones, if there is existing damage using Thiols, it is reduced by using it in regeneration (Ates et al. 2016). In the current study, antioxidants function by using Thiols (by decreasing the value of Total Thiol), oxidative stress was reduced, and twin (majority) births occurred (Table 2).

It isreported that there are non-significant decreases and increases in total thiol values in calves that have been discharged by applying sedative, local anesthetic, and anti-inflammatory drugs in the dehorning surgery until the 120th minute after application (Erdoğan et al. 2019). In the present study, it is found that there are differences in total Thiol values between the seasons (October/May) and it is like Erdoğan et al. (2019).

Total thiol values are expected to be low in individuals with low Oxidative stress since the

enzyme that allows antioxidant substances to bind to the oxidant substances (Gumusyayla et al. 2016). Since a reference value has not yet been established with the value of Total Thiol in hair goats, it is seen that the total thiol ratio is quite high compared to other species (human, cattle) in the presented study. The total Thiol values obtained in this study are also thought to constitute a reference value. The twin birth rate (23%) increased in hair goats whose total thiol value was below the average Thiol value (451.84 µmol/L, Table 1) (Table 2). Most births were quite high in the enterprise where the study was conducted.

Total thiol values were found to be significantly lower in people with chronic renal failure (CKD) compared to healthy people. In the presented study, the low total thiol rate in hair goats with multiple births compared to infertile (sick) hair goats coincides with the reports of Coşkun et al. (2016). Üstüner (218) states that there is a significant difference in total thiol values in patients with vitiligo compared to healthy people. In the presented study, it is similar to the high value of total thiol in infertile hair goats (health problems). It is stated that the total thiol value decreases with recovery as the total thiol value is high at the beginning of the infection in infants with pneumonia (Öktem et al. 2021). Başgöz et al. (2021) reported that 66 newborns with sepsis had high total thiol values before treatment. Due to severe infection or oxidative stress, the total thiol value is highest in those who do not give birth and lowest in those who give birth to twins, coincides with some reports (Öktem et al. 2021, Başgöz et al. 2021).

In the case of oxidative stress due to infection, thiol values have been reported to decrease (Esen et al. 2015). It has been demonstrated by many scientists that oxidative stress adversely affects fertility. It was determined that there was a significant correlation between oxidative stress and total thiol values (Table 3) ($r \geq 0.5$). In this case, it will be possible that since there is a correlation with total thiol and oxidative stress, there is also an important correlation between total thiol and fertility (Table 3).

CONCLUSION

This study is the first study in the literature to show the relationship between total thiol value and offspring yield in hair goats. We report that there is a need to investigate the effect of oxidative stress and the measure to alleviate its effect on fertility in hair goats and other farm animals.

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This study was presented as an oral presentation with the title "Effect of Thiol Disulfite on Pregnancy Rate In Hair Goat" at the 9th national KOP Regional Development Symposium on 24-26 October 2022.

Ethical approval:

The study was carried out by the approval of Selcuk University Faculty of Veterinary Medicine, Experimental Animals Production and Research Center Ethics Committee (09.12.2020 and 2020/116).

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İshalli köpeklerde *Cryptosporidium parvum*'un varlığının araştırılması

Research Article

Investigation of the presence of *Cryptosporidium parvum* in dogs with diarrhea

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ÖZET

Sunulan çalışmada Türkiye'nin çesitli bölgelerine köpek yetiştiren Jandarma At ve Köpek Eğitim Merkezi Komutanlığı'ndaki yavru köpeklerde karşılaşılan ishallerde *Cryptosporidium parvum* varlığının belirlenmesi amaçlandı. Hayvan materyalini 100 adet farklı ırk (Pointer, Alman çoban köpeği, Belçika Malinois, Çatalburun, Labrador ve Golden Retriever), yaş (28 gün-9 aylık) ve cinsiyette ishalli köpek oluşturdu. Köpeklerden alınan dışkı örneklerinde natif, flotasyon ve karbol fuksin boyama yöntemi ile parazitolojik inceleme yapıldı. Ayrıca immunokromotografik hızlı test kitleri kullanılarak *C. parvum*'un varlığı araştırıldı. Çalışmaya dahil edilen köpeklerden 18'inde *Toxocara canis* (%18), 3'ünde *Toxoscaris leonine* (%3), 8'inde *Giardia* spp. (%8), 25'inde ise *Cystoisospora* spp. (%25) belirlenirken *Cryptosporidium parvum* tespit edilemedi. *Cystoisospora* spp. yüzdesi bakımından yaş ve ırk arasında anlamlı bir ilişki bulunurken ($P<0.05$), diğer parazit türleri yüzdeleri bakımından yaş-ırk, ırk-cinsiyet ve yaş-cinsiyet degişkenleri arasında anlamlı bir ilişki bulunmadı ($P>0.05$). Sonuç olarak; *Cryptosporidium parvum* için test edilen 100 köpeğin dışkı örnekleri, nativ, flotasyon ve karbol fuksin boyama yöntemleri ve immunokromatografik hızlı test kiti muayeneleriyle negatif bulundu.

Anahtar Kelimeler: *Cryptosporidium parvum;* ishal; karbol fuksin; köpek; kriptosporidiozis.

ABSTRACT

In the presented study, it was aimed to determine the presence of Cryptosporidium parvum in the diarrhea encountered in dogs in the Gendarmerie Horse and Dog Training Center Command, which breeds dogs in various regions of Turkey. The animal material consisted of 100 dogs with diarrhea of different breeds (Pointer, German shepherd, Belgian Malinois, Çatalburun, Labrador and Golden Retriever), age (28 days-9 months) and gender. The parasitological examination was performed on faecal samples taken from dogs by native, flotation and carbol fuchsin staining methods. In addition, the presence of *C. parvum* was investigated by using immunochromatographic rapid test kits. As a result, while *Toxocara canis* was determined in 18 (18%), *Toxoscaris leonina* in 3 (3%), *Giardia* spp. in 8 (8%), *Cystoisospora* spp. in 25 (25%) of the dogs included in the study, *Cryptosporidium parvum* could not be detected. Only in terms of the percentage of *Cystoisospora* spp., a significant correlation was found between age and race ($P<0.05$), when there was no significant relationship among age-breed, breed-gender and age-gender variables of other parasite species ($P>0.05$). As a result, faecal samples of 100 dogs tested for *Cryptosporidium parvum* were found negative with native, flotation and carbol fuchsin staining methods and immunochromatographic rapid test kits.

Keywords: Carbol fuchsin, cryptosporidiosis, *Cryptosporidium parvum;* diarrhea; dog.

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GİRİŞ

Köpeklerde görülen ishallere 0-1 yaşa kadar genelikle viral olarak Parvovirüs, Coronavirüs, Enterovirus vb, bakteriyel olarak *E.coli*, stafilokoklar, streptekoklar vb, paraziter olarak *Gidardia* spp., *Cystoispospora canis*, *Toxocara canis*, *Toxascaris leonina* ve kriptosporidiozis gibi etkenler neden olmaktadır. Köpekler, giardiozis, trichomoniazis, entamoebiazis, neosporozis, hammondiozis ve isosporozis gibi önemli zoonotik protozoon parazitlerin ara konakçılığını yapmaktadır (Bridger ve Whitney, 2009). Kriptosporidiozis, *Cryptosporidium* soyuna bağlı protozoonlarca meydana getirilen, özellikle genç ve bağışıklık sistemi düşük olan hayvan ve insanlarda hastalığa sebep olan ve tüm dünyada yaygın olarak bulunan bir enfeksiyondur. Hayvan ve insanların sindirim sistemi epitel hücrelerine yerleşerek sağlığı olumsuz etkilemektedir (Miller ve ark., 2003). Kriptosporidiumlara ilk olarak farelerin mide mukozalarında (Tyzzer, 1907; 1910), ince bağırsaklarda (Tyzzer, 1912) ve tavukların Bursa fabrisyus'unda (Angus, 1983; Fayer ve Xiao, 2008) tespit edilmiştir. Bu türler ve genotipleri konaklarda klinik veya subklinik enfeksiyonlara neden olabilmektedir. Köpeklerde yapılan araştırmalar neticesinde *Criptosporidium spp.* ile enfekte olma olasılığının %45'lere kadar çıktığı görülmüştür (Lindsay ve Zajac, 2004). *C. parvum* ile enfekte köpeklerde ilk olarak tespit edilen uzun süren ishalle karakterize bir tablodur. İshalli köpeklerde her ne kadar sağaltım yapılabilse de ispatlanmış bir tedavi protokolü belirlenememiştir (Lucio-Forster ve ark., 2010).

Bu açıdan bakıldığında bu hastalığa hayvan sağlığında özellikle de kedi ve köpek hekimliğinde gereken önemin verilmediği görülmektedir. Köpek ve kedilerin evde bakılmaya başlanması ile birlikte zoonoz hastalıkların yaygınlaşması gibi birçok riski de beraberinde getirmiştir. Veteriner hekimlik sahasında bu ve benzeri hastalıkların teşhiş ve tedavi yöntemlerindeki ve *C. parvum* ile ilgili bilgi yetersizlikleri dikkate alındığında hastalık belirtisi gösteren hayvanların bir an önce kesin teşhisinin yapılarak sağaltımın yapılması önem arz etmektedir.

Bu çalışmada Türkiye'nin çeşitli bölgelerine köpek yetiştiren Jandarma At ve Köpek Eğitim Merkezi Komutanlığı'ndaki (JAKEM) yavru köpeklerde karşılaşılan ishallerde *Cryptosporidium parvum* hastalığının varlığının belirlenmesi amaçlanmıştır. Nevşehir Jandarma At ve Köpek Eğitim Merkezi Komutanlığı'nda köpeklerde ishal vakalarında ilk olarak akla parvoviral enterit, *Toxocara canis*, *Giardia* spp., *Isospora* spp. gibi çeşitli hastalıklar gelmekte bunlarla ilgili teşhis ve tedaviler yapılmaktadır. Fakat immun sistemi zayıf hayvanlarda ölümcül olabilen, zoonotik karakterde olan, insan ve hayvan sağlığı için tehdit olabilecek *Cryptosporidium parvum* ilk etapta düşünülmemektedir. Veteriner hekimlikte bu hastalıkların çeşitliliği, tanı yöntemlerinin sınırlılığı ve *Cryptopsoridium* spp. ile ilgili bilgilerin yetersizliği göz önünde bulundurulduğunda, hastalık bulguları görülen köpeklerde zaman kaybetmeksizin kesin tanının konulması, tedavisinin yapılması ve hastanın izlenmesi çok önemlidir.

MATERYAL VE METHOD

Bu çalışma, Jandarma At ve Köpek Eğitim Merkezi Komutanlığı At/Köpek Muayene ve Tedavi Merkezi'nde Mayıs 2021- Mayıs 2022 döneminde yapılmıştır. Çalışmaya, JAKEM Komutanlığı bünyesinde bulunan, 28 gün-9 aylık yaş aralığında olan ve ishal semptomu gösteren 100 köpek dahil edilmiştir (Tablo 1.).

Tablo 1. Çalışmaya dâhil edilen köpeklerin yaş, cinsiyet ve ırkları

Yaş (0-1) (n=100)	Cinsiyet	Irk
28 günlük (n=5)	Dişi	Pointer
1 aylık (n=26)	Dişi	Alman Çoban Köpeği
1 aylık (n=18)	Dişi	Belçika Malinois
1,5 aylık (n=11)	Erkek	Belçika Malinois
1,5 aylık (n=2)	Erkek	Çatalburun
2 aylık (n=3)	Erkek	Pointer
2 aylık (n=9)	Erkek	Belçika Malinois
2 aylık (n=5)	Dişi	Labrador
3 aylık (n=5)	Dişi	Belçika Malinois
4 aylık (n=7)	Erkek	Alman Çoban Köpeği
5 aylık (n=2)	Erkek	Golden Retriever
7 aylık (n=4)	Erkek	Alman Çoban Köpeği
9 aylık (n=3)	Dişi	Labrador

Söz konusu köpeklerde ilk antiparaziter uygulama doğumdan sonraki 28. günde, 2. uygulama ise 40. günde yapıldı. Daha sonra 3 aylık periyotlar halinde antiparaziter uygulamaya devam edildi (Tablo 2).

Tablo 2. Çalışmaya dâhil edilen köpeklere uygulanan aşı ve ilaçlama protokolü

AŞI VE İLAÇLAMA PROTOKOLÜ		
YAPILDIĞI GÜN	**AŞI/İLAÇ ADI**	**UYGULANAN DOZ**
Doğumdan sonra 28.gün	Kontil (pirantel pamoat)	5-10 mg/kg PO
40.gün	İç-dış parazit (Endopet-Fiprovet sprey)	Endopet: 10 kg canlı ağırlığa 1 tablet PO
45.gün	Nobivac Puppy Dp	
60.gün	Karma-1 (DHPP-L)	
74.gün	Karma-2 (DHPP-L)	
81.gün	Bronchine-1	
88.gün	Corona	
90.gün	Dış parazit (Fiprovet)	
95.gün	Bronchine-2	
105.gün	Kuduz	
120.gün	İç-dış parazit (Endopet-Fiprovet sprey)	Endopet: 10 kg canlı ağırlığa 1 tablet PO

Dışkı numunelerinin alımı

Çalışmaya dahil edilen köpeklerden ağzı kapaklı gaita toplama kaplarına dışkı örnekleri svap yardımıyla ortalama 5 gr kadar alınarak incelemek için bekletilmeden laboratuvara alındı.

Laboratuvar muayeneleri

Toplanan dışkı örneklerinde nativ ve flotasyon yöntemi ile direkt mikroskobik inceleme yapıldı. Daha sonra alınan örnekler immunokromatografik hızlı test kiti (FASTest CRYPTO Strip ad us. vet., MegaCor Diagnostik) ve karbol-fuksin yöntemi ile boyanarak *C. parvum* ookistleri yönünden 100'lük büyütme altında mikroskopta incelendi. Ayrıca ishale neden olabilecek diğer parazit ve protozoonlar belirlendi.

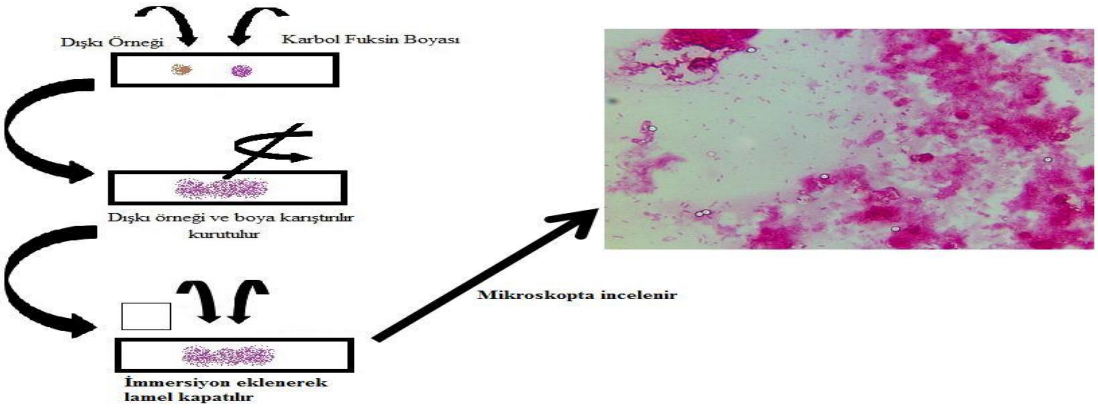
İmmunokromatografik hızlı test kiti

Kapaklı gaita kabı içerisine örnekler alındı. Gaita örnekleri tampon çözeltili örnek tüpü içerisine konuldu ve homojen bir karışım elde edilene kadar karıştırıldı. Dışkı partiküllerinin sedimentasyonu için örnek tüpü düz bir yüzeye yatay bir şekilde 1-5dk bekletildi. Hızlı test kiti dikey bir şekilde üzerindeki oklar aşağı bakacak şekilde tüpün içerisine konularak 1 dk beklendi. Test kiti üzerindeki kontrol çizgisi belirene kadar beklendi ve çıkarılarak düz bir yüzey

üzerine yatay şekilde bırakıldı. Mavi test çizgisiyle beraber kırmızı test çizgisi oluşması halinde pozitif sadece mavi test çizgisi oluşması halinde ise negatif kabul edildi.

Karbol-fuksin boyama yöntemi

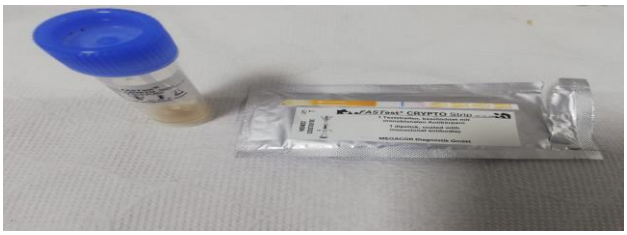
Steril plastik kaplara alınarak laboratuvara getirilen dışkı örnekleri hiç bekletilmeden, ookist varlığını ortaya koymak amacıyla karbol-fuksin boyama yöntemi kullanılarak incelendi (Pasmans ve ark., 2008) (Şekil 2.). Buna göre, eter-alkol karışımında temizlenerek yağı giderilmiş lam üzerine, iyice karıştırılarak homejenize edilen dışkı örneklerinden pipet yardımı ile 50 µL alındı ve aynı miktarda karbol-fuksin filtre kağıdından süzüldükten sonra eklenerek 5-10 sn boyamaya bırakıldı. Kuruyan preparatlar üzerine immersiyon yağı damlatılarak x100'lük büyütme ile *Cryptosporidium* oocystleri yönünden incelendi. İki nitel değişen arasındaki parazit yüzdeleri arasındaki farkın önem kontrollerinde Fisher's exact testi kullanıldı. Veriler sayı (yüzde) tanımlayıcı istatistikleri ile gösterildi. Verilerin istatistiksel analizleri R 4.2.0 yazılımı ile yapıldı. İstatistiksel anlamlılık seviyesi $P<0.05$ olarak kabul edildi.



Şekil 1. Karbol-fuksin boyama yönteminin yapılışı (Pasmans ve ark., 2008)

BULGULAR

Çalışmaya dahil edilen ishalli köpeklerin 77' sinin 28 günlük-2 aylık, 14' ünün 3-5 aylık ve 7' sinin 7-9 aylık yaşlarda olduğu belirlendi. İncelemeye tabi tutulan 100 dışkı numunesi üzerinden yapılan araştırmada *Cryptosporidium parvum* ookistleriyle karşılaşılmamış olup (Şekil 2.) dışkı muayeneleri sırasında belirlenen diğer parazit ve/veya protozoonlar Tablo 3'de gösterildi. İshalli köpeklerde parazit ve/veya protozoon olarak 28 günlük-2 aylık olanlarda *Toxocara canis* (n=16), *Giardia* spp. (n=7) ve *Cystoisospora* spp (n=21) belirlenirken, 3-5 aylık köpeklerde *Toxoscaris leonina* (n=3) ve *Cystoisospora* spp. (n=4) ve 7-9 aylık köpeklerde *Toxocara canis* (n=2) ve *Giardia* spp (n=1) belirlendi. Sadece *Cystoisospora* spp. yüzdesi bakımından yaş ve ırk arasında anlamlı bir ilişki bulundu ($P<0.05$). Diğer parazit türleri yüzdeleri bakımından yaş-ırk, ırk-cinsiyet ve yaş-cinsiyet değişkenleri arasında anlamlı bir ilişki bulunmadı ($P>0.05$).



Şekil 2. FASTest CRYPTO Strip ad us. vet. Immunokromotografik hızlı test kitinin negatif sonucu

Tablo 3. İshalli köpeklerden alınan dışkı örneklerinde tespit edilen diğer parazit ve/veya protozon sayıları

Yaş (0-1)	Cinsiyet	Irki	Dışkı Numune Adeti	Nativ ve/veya Flotasyonda Tespit Edilen Parazit/Protozoonlar			
				Toxocara canis	Toxoscaris leonina	Giardia spp.	Cystoisospora spp.
28 günlük	Dişi	Pointer	5	2			2
1 aylık	Dişi	Belçika Malinois	18	3		5	3
1 aylık	Dişi	Alman Çoban Köpeği	26	2			6
1,5 aylık	Erkek	Belçika Malinois	11	1			8
2 aylık	Erkek	Belçika Malinois	9	5		2	
2 aylık	Dişi	Labrador	5	3			
2 aylık	Erkek	Pointer	3				2
3 aylık	Dişi	Belçika Malinois	5		2		
4 aylık	Erkek	Alman Çoban Köpeği	7				4
5 aylık	Erkek	Golden Retriever	2		1		
7 aylık	Erkek	Alman Çoban Köpeği	4	2			
9 aylık	Dişi	Labrador	3			1	

TARTIŞMA

Köpeklerde görülen ishallere 0-1 yaşa kadar genelikle, paraziter olarak *Gidardia* spp., *Cystoispospora canis, Toxocara canis, Toxascaris leonina* ve kriptosporidiozis gibi etkenler neden olmaktadır. Köpekler, giardiozis, trichomoniazis, entamoebiazis, neosporozis, hammondiozis ve isosporozis gibi önemli zoonotik protozoon parazitlerin ara konakçılığını yapmaktadır (Bridger ve Whitney, 2009). Kriptosporidiozis, *Cryptosporidium* soyuna bağlı protozoonlarca meydana getirilen, özellikle genç ve bağışıklık sistemi düşük olan hayvan ve insanlarda hastalığa sebep olan ve tüm dünyada yaygın olarak bulunan bir enfeksiyondur. Sunulan çalışmada ise çalışmaya dahil edilen köpeklerden 18'inde *Toxocara canis,* 3'ünde *Toxoscaris leonina,* 8'inde *Giardia spp.* 25'inde ise *Cystoisospora spp.* belirlenmiştir.

Dışarıdan hayvan giriş çıkışının kontrollü olarak yapılmasına rağmen bulaş riski olabileceği, kullanılan antiparaziter ilaçlara karşı oluşabilecek direnç gelişimi, doğan yavru sayısının fazla oluşundan ve bir arada bulunmasından dolayı bir bulaş söz konusu olduğunda yayılım oranının fazla olmasından kaynaklı parazitlerin görülme olasılığı artmaktadır. *Cryptosporidium spp.* insan başta olmak üzere kanatlı ve diğer memelilerde mide bağırsak sisteminde yerleşen ve bu bölgelerde hastalıklara yol açan bir parazittir (Mundim ve ark., 2007**)**. Söz konusu parazit bağışıklık sistemi baskılanmış bireylerde hastalığa neden olabilmektedir. Bu hastalıkla ilgili olarak konak sayısındaki artış, ookist saçılımının fazla oluşu, tek bir ookist dahi olsa enfektif ediciliğinin yüksek oluşu gibi nedenlerden dolayı hayvan ve insan sağlığını olumsuz yönde etkileyeceğinden büyük önem arz etmektedir (Scorza ve Lappin, 2012). Köpeklerde ince bağırsağa yerleşen *C. canis, C. parvum* ve *C. meleagridis* ve mideye yerleşen *C. muris* olmak üzere 4 farklı türden bahsedilebilir (Cuia ve ark., 2018). *C. parvum*'un tespiti için birçok yöntem kullanılmaktadır. Farklı boyama teknikleriyle direkt mikroskobik inceleme, immunofloresan yöntemi, polimeraz zincir reaksiyonu (PCR) ve enzime bağlı immünosorbent testi (ELISA) gibi serolojik ve moleküler düzeyde tanı yapılabilmektedir. Aynı zamanda birden fazla tanı tekniğinin kullanılması da sonuçların doğruluğunu teyit edebilmektedir (Bennett ve ark., 1985). Yapılan bu çalışmada ishalli

köpeklerde *C. parvum* belirlenmesi amacıyla dışkı örnekleri laboratuarda mikroskobik olarak karbol fuksin boyama yöntemi ve immunokromotografik hızlı test kitleriyle incelendi. Kriptosporidiozisin daha çok yavru veya gençlerde görüldüğü bilinmesine karşın yapılan epidemiyolojik araştırmalarda söz konusu enfeksiyonun yaş ile ilişkisinin bulunmadığı (Moreira ve ark., 2018), bazı araştırmacılar tarafından ise yavru veya gençlerde daha fazla görüldüğü (Olabanji ve ark., 2016; Pivoto ve ark., 2013), yine bazı araştırmacılarda bu hastalığın yetişkinlerde daha fazla hastalık oluşturduğunu ileri sürmüşlerdir (Bresciani ve ark., 2008). Bunun üzerine yapılan bir araştırmada ookist yüzdesi bir yaşın altındakilerde %25 iken bir yaşın üstündekilerde %23 olarak tespit edilmiştir (Pivoto ve ark., 2013). Her ne kadar yaş ile enfeksiyon arasında bir bağlantı olmadığı söylense de 3-6 aylık köpeklerde enfeksiyonun görülme sıklığı daha fazla olduğu bildirilmiştir. Bu araştırmayı destekler nitelikte olan bazı araştırmacılar; Thompson ve ark., (2005), yetişkin köpeklerde enfeksiyonun nüks ettiği, fakat yavru veya genç köpeklerde enfeksiyon sıklığının daha fazla olduğunu bildirmiştir. Oğlak veya genç keçilerde daha fazla prevalans değerine sahip olduğunun bildirilmesi de bu veriyi destekler niteliktedir (Noordeen ve ark., 2001; Bajer ve ark., 2012). Olabanji ve ark., (2016) da yaptıkları araştırmada 7 aylık ile 2 yaş aralığındaki bireylerde enfeksiyonun görülme olasılığının daha fazla olduğunu bildirmiştir. Türkiye'de köpeklerde *Cryptosporidium* spp. ile ilgili yapılan bir çalışmada pozitif görülme oranının; 0-6 aylık yaş aralığındaki köpeklerde %11.3 oranında, 7-24 ay arası köpeklerde %24.1 oranında, 25 ay ve üzeri köpeklerde ise %14.3 oranında olduğu bildirilmektedir (Öner, 2019). Sunulan araştırmada, çalışmaya 28 günlük-9 aylık köpeklerde karbol fuksin boyama ve immunokromotografik yöntemleriyle *C. parvum* etkenine rastlanmamıştır. *Cryptosporidium* spp. ile enfekte bireylerde bir de cinsiyet ile ilişkisine bakılmış olup yapılan araştırma neticesinde cinsiyetin hastalığın prevalansına etki etmediği görülse de (Mundim ve ark., 2007), dişi köpeklerde daha fazla olabileceği bildirilmiştir. Dişilerde prevalansın daha yüksek oluşunu fizyolojik olarak bazı dönemlerde bağışıklık sistemindeki yetersizliğe bağlamıştır. Bazı araştırmacılar ise erkeklerin dişilerden daha fazla prevalansa sahip olduğunu söylemişlerdir (Zelalem ve ark., 2012). Türkiye'de Ege bölgesindeki köpeklerde yapılan çalışmada cinsiyete göre *Cryptosporidium* spp. pozitif görülme oranının erkek köpeklerde % 16.4 ve dişi köpeklerde ise %14.6 olduğu bildirilmiştir (Öner, 2019). Sunulan çalışmada farklı ırk ve yaştan 38 erkek köpek ile yine farklı ırk ve yaştan 62 dişi köpek çalışmaya dâhil edilmiş olup, araştırma sonucunda erkek ve dişi köpeklerde *Cryptosporidium parvum* etkenine rastlanmamıştır.

Kriptosporidiozisin prevalansının melez olmayan, saf ırklarda daha fazla görüldüğü bildirilmiştir (Mundim ve ark., 2007**)**. Bu çalışmada kullanılan köpek ırklarını saf ırklar oluşturmaktadır. Ancak söz konusu köpeklerde bu etkenle karşılaşılmamıştır. Dünya genelinde yapılmış birçok araştırmada köpeklerde bu enfeksiyonun görülme olasılığı %0 ile %44 arasında olduğu bildirilmiştir (Lindsay ve Zajac, 2004). Yurt dışında yapılan bazı araştırmalarda bu parazitin görülme olasılığının Amerika'da %2- %17, İspanya'da %6 ve Avusturalya'da %0-%11 arasında olduğu görülmüştür (Scorza ve Tangtrongsup, 2010). Kanada'da yapılan araştırmada bu hastalığın görülme olasılığı kliniklerde %10 iken barınak ortamında bulunan köpeklerde %8 olarak tespit edilmiştir (Uehlinger ve ark., 2013). ELISA ile yapılan araştırmalarda prevalans yüzdeleri Kanada'da %7.4, İtalya'da % 1.7 ve

Almanya'da %23 iken (Cirak ve Bauer, 2004; Shukla ve ark., 2006), bu oran Norveçte % 44 olarak tespit edilmiştir (Hamnes ve ark., 2006). Bu farklılığın kullanılan kit ile ilgili olduğu varsayılmaktadır (Titilincu ve ark., 2010). Türkiye'de daha önce Ege bölgesindeki köpeklerde modifiye Ziehl-Neelsen tekniği kullanılarak kriptosporidiozisin prevalans çalışması yapılmış ve %15.5 olarak bulunmuştur. *Cryptosporidium parvum*'un görülme olasılığı köpeklerin bakım, beslenme ve ortam şartlarına, hijyen ve dezenfeksiyon işlemlerine, coğrafi konum ve bu parazitin tanısında kullanılan yöntemlere bağlı olarak değişmektedir. Sunulan çalışmada köpeklerde kriptosporidiozis görülmemesi JAKEM'de yetiştirilen köpeklerin sağlık, bakım-besleme ve ilaçlama protokollerine uyulması, dışarıdan hayvan giriş çıkışının kontrollü olarak yapılması olarak yorumlanabilir.

SONUÇ

Köpek, kedi vb evcil hayvanların evde beslenmeye başlamasıyla birlikte hayvanlardan insanlara geçen hastalıkların önemi de iyice arttırmıştır. Veteriner hekimlikte zoonoz hastalıklar önemli bir yere sahiptir ve sık olarak karşılaşılmaktadır. Kriptosporidiozis, *Cryptosporidium* soyuna bağlı protozoonlarca meydana getirilen ve tüm dünyada yaygın olarak bulunan, hayvan ve insanların sindirim sistemi epitel hücrelerine yerleşen bu protozoonlar özellikle genç ve bağışıklık sistemi düşük olan hayvanlarda hastalığa sebep olarak insan ve hayvan sağlığını olumsuz etkilemektedir. Ülkemizde buzağılarda kriptosporidiozis ile ilgili yapılmış çalışmalar olmakla birlikte, köpeklerdeki varlığının belirlenmesine dair çok az çalışma mevcuttur. Bu kapsamda ishalli köpeklerde *Cryptosporidium parvum* varlığının ve görülme oranının belirlenmesi gerekmektedir. Sunulan çalışmada; ishalli köpeklerde *Cryptosporidium parvum*'un varlığının karbol fuksin ve immunokromotografik olarak belirlenmesi amaçlanmış ve sonuçta JAKEM Komutanlığında bulunan köpeklerde *Cryptosporidium parvum*'un görülme sıklığı %0 olarak belirlenmiştir. Sunulan çalışmanın gelecekte köpeklerde kriptosporidiozis ile ilgili yapılacak çalışmalara bir referans olabileceği düşünülmektedir.

AÇIKLAMALAR

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Bu çalışma "İshalli Köpeklerde *Cryptosporidium Parvum*'un Varlığının Araştırılması" başlık tezden özetlenmiştir**.**

Etik beyan: Bu tez çalışmasında; 15.02.2014 tarih ve 28914 sayılı resmî gazetede yayımlanan Hayvan deneyi etik kurulu çalışma usul ve esaslarına dair yönetmeliğin ikinci maddesinin (b) bendinde "deneysel olmayan klinik veteriner hekimliği uygulamalarında etik kurul onayına gerek olmadığı" bildirimi doğrultusunda, ERÜ HADYEK'in 21/106 sayılı ve 05.05.2021 tarihli yazısı kapsamında çalışma yürütülmüştür.

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Veteriner cerrahide lokal kök hücre uygulamaları

In Veterinary surgery local stem cell applications

Research Article

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ÖZET

Kök hücreler; organizmada organların ve dokuların yapısını oluşturan ana hücrelerdir. Bu sebeple ihtiyaç halinde hasar gören tüm doku ve organlara dönüşüm sağlayarak bunların onarılmasında ciddi rol almaktadır. İnsan hekimliğinde son 10 yılda yaygın bir kullanım alanı bulan kök hücre tedavisi, günümüzde veteriner tıbbında da uygulanmaktadır. Mezenkimal kök hücreler hayvanlarda pek çok hastalığın tedavisinde kullanılmaktadır. Steril ortamlarda kemik iliği, kordon kanı, ve yağ dokudan elde edildikten sonra sıvı azot tanklarında dondurularak saklanmaktadır. Hastaya uygulama öncesinde enjeksiyona hazır hale getirilerek taze olarak verilmektedir. Kök hücreler, hayvanlara lokal enjeksiyon ya da intra venöz yolla uygulanmaktadır. Viral ve enfeksiyöz hastalıklarda canlıda immun sistemin güçlendirilmesi amacıyla intravenöz yolla mezenkimal kök hücreler kullanılmaktadır. Çeşitli ortopedik, nörolojik ve oftalmolojik hastalıkların tedavisinde ise kök hücreler lokal olarak uygulanmaktadır. Sunulan bu çalışmada, 8 köpek, 6 kedi üzerinde toplam 14 vakaya ait veriler yer almaktadır. 7 ortopedik, 5 nörolojik, 2 oftalmolojik vakada lokal yolla uygulanan mezenkimal kök hücre tedavisi ve elde edilen sonuçlar değerlendirilmiştir. Ortopedik ve nörolojik vakalarda büyük oranda iyileşme sağlandığı, oftalmolojik olgularda da belirgin düzelmeler olduğu saptanmıştır.

Anahtar Kelimeler: Keratit, kornea ülseri, kök hücre, nonunion, nöropati, osteoatritis

ABSTRACT

Stem cells; they are the main cells that form the structure of organs and tissues in the organism. For this reason, it takes a serious role in repairing all tissues and organs that are damaged when needed. Stem cell therapy, which has found widespread use in human medicine in the last 10 years, is also applied in veterinary medicine today. Mesenchymal stem cells are used in the treatment of many diseases in animals. It is obtained from bone marrow, cord blood, and adipose tissue in sterile environments and stored in liquid nitrogen tanks by freezing. It is given to the patient freshly by making it ready for injection before administration. Stem cells are administered to animals by local injection or intravenous route. In order to strengthen the immune system in vivo in viral and infectious diseases, intravenous mesenchymal stem cells are used. Stem cells are applied locally in the treatment of various orthopedic, neurological and ophthalmological diseases. In this presented study, data of 14 cases on 8 dogs and 6 cats are included. Locally applied mesenchymal stem cell therapy in 7 orthopedic, 5 neurological and 2 ophthalmologic cases and the results obtained were evaluated. It was determined that a great improvement was achieved in orthopedic and neurological cases, and significant improvements were also observed in ophthalmological cases.

Keywords: Corneal ulcer, keratitis, neuropathy, nonunion, osteoarthritis, stem cells

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GİRİŞ

Mezenkimal kök hücreler ilk olarak 1951 yılında ortopedi alanında kullanılmaya başlamıştır. Özellikle kırık onarımı ve metabolik kemik hastalıklarında elde edilen başarılı sonuçlar bilinmektedir (Undale et al., 2011). Kemik iliği mezenkimal kök hücreler için ana kaynak olsa da kemik, diş pulpası, karaciğer, kordon kanı, plasenta, amniyon sıvısı, sinovial sıvı hatta periferik kan, yağ dokusu gibi diğer bazı doku ve organlardan da mezenkimal kök hücreler elde edilmektedir (Ding et al., 2011).

Elde edilmelerinde minimal invaziv yöntemler kullanılarak, özellikle kemik iliği ve yağ dokusundan toplanan mezenkimal kök hücrelerin (MKH'ler), Osteoartritisisin (OA) sağaltımında kullanımı gün geçtikçe artmaktadır (Moroni ve Fornasari 2013). Kök hücre tedavisi oldukça güvenli ve herhangi bir yan etkisi yoktur (Borakati et al., 2017). Kök hücrelerin intraartiküler uygulanması, özellikle osteoartritisli eklemlerde geniş kıkırdak hasar varlığında oldukça yararlıdır.

Kök hücre transplantasyonunun nöral dokularda fonksiyonel iyileşmeyi desteklediğini gösteren pek çok çalışma bulunmaktadır. Kemik iliği mezenkimal kök hücreleri, nöral kök hücreleri ve glia hücreleri dahil olmak üzere çeşitli kök veya progenitör (öncül) hücre tiplerinin, hasarlı omuriliğe naklini takiben ilgili bölgede fonksiyonel iyileşme sağladığı belirlenmiştir (McDonald et al., 1999; Teng et al., 2002; Hofstetter et al., 2002; Ogawa et al., 2002; Cao et al, 2005; Cummings et al., 2005; Keirstead et al., 2005; Iwanami et al., 2005; Karimi-Abdolrezaee et al., 2006; Xu et al., 2006).

Kornea rejenerasyonunu hızlandırmak için uygulanan değişik biyomühendislik implantlar farklı çalışmalarda denenmiştir (Griffith et al., 2016). Bu amaçla kornea stromasından elde edilen ve laboratuvar koşullarında üretilen kök hücreler, hasarlı kornea bölgelerine trasplante edilerek oldukça iyi bir korneal iyileşme sağlanmıştır. Aynı zamanda mezenkimal kök hücre transplantasyonu, stromal tüm yara ve defektleri kapatmakta, eksik kollajen fibril organizasyonunu replase etmektedir (Du et al., 2009; Syed-Picard et al., 2016).

Sunulan bu çalışmada, kedi ve köpeklerde ortopedik, nörolojik ve oftalmolojik vakalarda bilinen konvansiyonel yöntemlerin dışında, mezenkimal kök hücreler lokal olarak uygulanmış ve elde edilen sonuçlar değerlendirilmiştir.

MATERYAL VE METHOD

Bu Araştırma İstanbul Vetmemorial Veteriner Kliniği'nde gerçekleştirilmiştir. Araştırmada yer alan tüm hayvanlar için hasta onam izinleri alınmıştır. Araştırmada 8 köpek ve 6 kedi olmak üzere toplam 14 vaka yer almıştır. Bu hastalara ait anamnez, kök hücre dozları, bulgular ve sonuçlar ayrı ayrı değerlendirilmiştir. Vakalara ilişkin bilgiler tablo 1'de belirtilmiştir

Sunulan bu araştırmada kullanılan mezenkimal kök hücreler medyumu, Dulbecco'nun Sodyum Piruvat ve L-Glutamin İçermeyen Modifiye Kartal Ortamı (DMEM) (Biological Industry, İsrail) idi. Kullanılan DMEM'de 1 g/I D-Glikoz (düşük glikoz) ve 1,1 g/I sodyum piruvat bulunmaktadır. Yağ dokusu, mezenkimal kök hücre kaynağı olarak kullanılmıştır. Bu doku -78 derecede sıvı nitrojen içinde saklanmıştır. Kural olarak kök hücreler, randevu tarihinde vakalara soğuk zincir korunarak, kök hücre firması tarafından ulaştırılmış ve steril koşullarda maksimum 3 saat içerinde ilgili bölgelere enjekte edilerek, hücrelerin canlılığının korunması sağlanmıştır.

Tablo 1. Vakalara ait bilgiler ve uygulanan kök hücre dozları

Vaka Sıra No	Tür	Irk	Cinsiyet	Yaş	Klinik Tanı	Kök Hücre Uygulama Yolu	Kök Hücre Dozu	Kök Hücre Enjeksiyon Sayısı	Sonuç
1	Kedi	Melez	Dişi	11 yaş	C4-5-6 dejenerasyon, nöropati	Paravertebral	1 milyon/ünite	3	Olumlu
2	Köpek	Yorkshire Terrier	Dişi	10 yaş	Bilateral patella lukzasyonu, osteoartritisis	İntraartiküler	1 milyon/ünite	2	Olumlu
3	Kedi	Melez	Dişi	15 yaş	C2-3-4 disk dejenerasyonu, protrüzyon	Paravertebral	1 milyon/ünite	2	Olumsuz
4	Köpek	Kaniş	Erkek	3 yaş	Kaput femoris nonunion	Non-union bölgesine lokal enj.	2 milyon/ ünite	2	Olumlu
5	Köpek	Pug	Dişi	10 yaş	T12-13 disk protrüzyonu	Paravertebral	2 milyon/ünite	1	Olumlu
6	Kedi	Melez	Dişi	2 yaş	Lumbosakral travma	Paravertebral	1 milyon/ünite	2	Olumlu
7	Kedi	Melez	Erkek	1 yaş	Lumbosakral travma	Paravertebral	2 milyon/ünite	2	Olumlu
8	Köpek	Beagle	Erkek	10 yaş	Dirsek eklemi nonunion	Non-union bölgesine lokal enj.	2 milyon/ünite	2	Olumlu
9	Köpek	Cane corsa	Erkek	7 yaş	Bilateral osteoartritis	İntraartiküler	2 milyon/ünite	2	Olumlu
10	Köpek	Chow chow	Erkek	7 yaş	Bilateral osteartrit	İntraartiküler	2 milyon/ünite	1	Olumlu
11	Kedi	Melez	Dişi	1 yaş	Keratitis pannosa euzinofilika	Subkonjunktival	1 milyon/ünite	1	Olumlu
12	Kedi	Melez	Erkek	5 yaş	Kornea ülseri	Subkonjunktival	1 milyon/ünite	1	Olumlu
13	Köpek	Kaniş	Erkek	2 yaş	Çapraz bağ rupturu, Patella lukzasyonu	İntraartiküler	1milyon/ünite	1	% 50 Olumlu
14	Köpek	Yorkshire Terrier	Dişi	10 yaş	Coxafemoral eklemde osteoartritis	İntraartiküler	2 milyon/ünite	1	Olumlu

BULGULAR

1. Vaka: Kedi, melez, dişi, 11 yaş.

C4-5-6 omurlar arasındaki kalsifikasyona bağlı oluşan nöropati nedeniyle servikal bölgeye paravertebral 3 hafta ara ile 3 defa 1milyon/ünite kök hücre enjeksiyonu yapılmıştır. İlk enjeksiyondan 3 hafta sonra etki oluşmaya başlamıştır. İkinci enjeksiyondan sonra hasta daha iyi hale gelmiştir. Adımlayarak yürümeye başlamıştır. Bu olumlu gelişme üzerine 3. enjeksiyon da yapılmıştır.

2. Vaka: Köpek, York Shire Terrier, dişi, 10 yaş.

Bilateral patella lukzasyonu ve hafif osteoartritis tanısı konulmuştur. Hasta yaşlı ve özellikle trakeal bölgede daralma olduğu için hasta sahibi genel anestezi altında bir operasyon uygulanmasını istememiştir. Bu sebeple diz eklemindeki eklem sıvısının artışını sağlayarak, oluşan patella lukzasyonunun, kondiluslara sürtünerek tahribatının önüne geçilmesi amaçlanmıştır. Bu sebeple diz eklemine 2 defa 1 milyon/ünite kök hücre uygulanmıştır. Gayet olumlu sonuç alınmıştır. Patellanın takılmadığı,

ağrının olmadığı, rahat bir yürümenin elde edildiği görülmüştür.

Aynı köpekte 1 yıl sonra, sağ kalça ekleminde osteoartritis geliştiğini görülmüştür. Bu art. Coxae'ya intra artiküler 1 doz 1 milyon/ünite kök hücre enjeksiyonu yapılmıştır. Hasta 2 hafta sonra bacağını gayet rahat kullanmaya başlamıştır.

3. Vaka: Kedi, melez, dişi, 15 yaş.

C2-3-4 bölgede disk dejenerasyonu ve hafif protrüzyonlar magnetik rezonans (MR) çekim tekniği ile tespit edilmiştir. Hastada ön bacaklarını kullanamama, inkoordinasyon, devrilip yıkılma gibi belirtiler göstermiştir. Bu hastaya 2 defa 1 milyon/ünite servikal paravertebral olarak kök hücre uygulanmıştır. Ancak bu vakada istenilen sonuç elde edilememiştir.

4. Vaka: Köpek, Kaniş, erkek, 3 yaş.

Travmaya bağlı, caput femoris'te non-union oluştuğu tespit edilmiştir. Bu vakaya intraartiküler caput femoris hizasına iki defa 2 milyon /ünite kök hücre enjeksiyonu yapılmıştır. Başarılı sonuç elde edilmiştir.

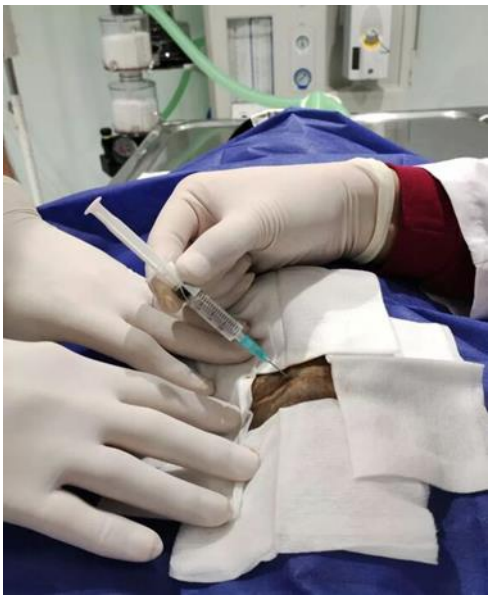
5. Vaka: Köpek, Pug, dişi, 10 yaş.

Torakal 12-13 ve Lumbal 1'de disk protrüzyonu MR muayenesinde tespit edilmiştir. Sağ arka bacakta propiyosepsiyon refleksinde azalma görülmüştür. Bir defa 2 milyon / ünite kök hücre enjeksiyonu paravertebral uygulanmıştır (Resim 1). 1 ay sonra kontrole getirildiğinde eskisine oranla daha iyi bastığı gözlemlenmiştir. Ek bir enjeksiyon daha yapılması önerilmiş ancak hasta sahibi ekonomik sebeplerle ilave enjeksiyonu yaptıramamıştır.

6. Vaka: Kedi, melez, dişi, 2 yaş.

Hastanın pencere pervazında asılı kalarak sıkıştığı bildirilmiştir. MR sonucunda lumbo-sakral bölgede medulla spinalis ezilmesine bağlı dejenerasyon varlığı tespit edilmiştir. Bu hastaya lumbo-sakral bölgeye paravertebral olarak 3 hafta ara ile 2 defa 1 milyon /ünite kök hücre enjeksiyonu yapılmıştır (Resim 2). Birinci uygulamadan 3 hafta sonra yavaş yavaş yürümeye başlamıştır. İkinci uygulamadan sonra ise tamamen iyileştiği görülmüştür.

Resim 1. Paravertebral enjeksiyon noktaları



Resim 2. Paravertebral kök hücre enjeksiyonun yapılışı.

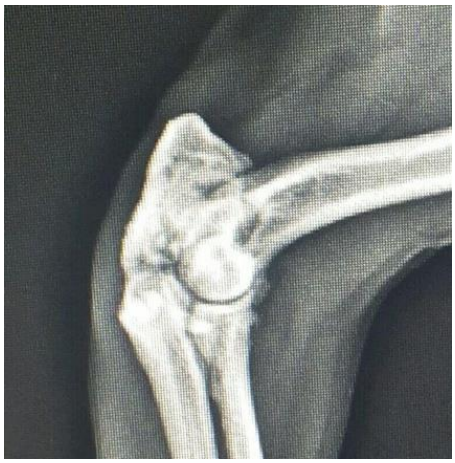
7. Vaka: Kedi, melez, erkek, 1 yaş.

Hastanın pencere pervazına sıkıştığı bildirilmiştir. Tam paraplejik bir durumda

olduğu tespit edilmiştir. MR sonucunda, lumbosakral bölgede yoğun kanama, ödem, infilamasyon görülmüştür. Bu hastaya lumbosakral bölgeye paravertebral olarak iki hafta ara ile 2 doz 1 milyon/ünite kök hücre yapılmıştır. Uygulamalardan iki hafta sonra yavaş yavaş yürümeye başlamıştır. İkinci dozdan sonra ise tamamen iyileştiği görülmüştür.

8. Vaka: Köpek, Beagle, erkek, 10 yaş.

Olecranon'un hemen altında, daha önce fark edilmeyen, eski bir non-union kırık varlığı tespit edilmiştir (Resim 3). İlgili ekstremitede hafif topallık olduğu görülmüştür. Hastaya nonunion bölgesine 2 defa 2 milyon /ünite kök hücre enjeksiyonu yapılmıştır. Kontrol neticesinde osteogenezisin başladığı görülmüştür.



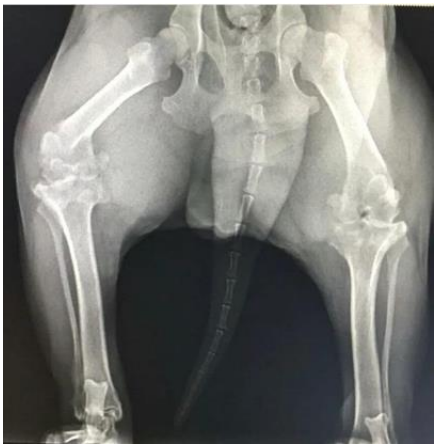
Resim 3. Olecranon distalinde nonunion fraktür tespiti.

9. Vaka: Köpek, Cane Corsa, erkek, 7yaş

Her iki diz ekleminde OA tespit edilmiştir. Diz eklemine 2 defa 2 milyon /ünite kök hücre enjeksiyonu yapılmıştır. Hastanın diz eklemlerinde ağrı azalmış ve eklem kısıtlığının rahatladığı gözlenmiştir.

10. Olgu: Köpek, Chow chow, erkek, 7 yaş.

Diz eklemlerinde bilateral OA, eklem sıvısında azalma, ağrı tespit edilmiştir (Resim 4). Hastaya intraartiküler yolla 2 milyon/ünite kök hücre enjeksiyonu yapılmıştır. Bir ay sonra kontrole getirildiğinde daha iyi olduğu görülmüştür. Eklem sıvısında artış sayesinde eklem aralığının arttığı yapılan radyolojik muayenede tespit edilmiştir. İkinci dozun da uygulanması önerilmiş ancak hasta sahibi ekonomik sebeplerle bunu kabul etmemiştir.



Resim 4. Diz eklemlerinde bilateral osteoartritis, eklem aralıklarındaki daralma.

11. Vaka: Kedi, melez, dişi, 7 yaş.

Hastada keratitis pannosa tespit edilmiştir. Pannus operasyonla uzaklaştırılmıştır. Pürüzlü korneanın yapısını iyileştirmek için 1 milyon /ünite subkonjonktival yolla kök hücre enjeksiyonu yapılmıştır. 21 gün sonraki kontrolde korneadaki iyileşmenin sağlandığı tespit edilmiştir (Resim 5).

Resim 5. Kök hücre enjeksiyonundan sonra kornea yapısındaki pürüzlenmenin azalması.

12. Vaka: Kedi, melez, erkek, 1 yaş.

Hastada kornea ülseri tespit edilmiştir. Ülser üzerinde siyah bir tabakanın varlığı görülmüştür.

Genel anestezi altında, opratif müdahale ile oluşan kabuk uzaklaştırılmıştır. Daha sonra

subkonjunktival yolla 1 milyon / ünite kök hücre enjeksiyonu yapılmıştır. Olgudan olumlu sonuç alınmıştır.

13. Vaka: Köpek, Kaniş, 2 yaş, erkek.

Hastada eski çapraz bağ kopuğu ve patella çıkığı tespit edilmiştir. Operatif müdahale esnasında kıkırdakta tahribat ve menisküslerde hasar görülmüştür. Çapraz bağ kopuğu balıkçı misinası kullanılarak ekstrakapsüler (ekstraartiküler) stabilizasyon yöntemi ile onarılmıştır. Eklemi kapatırken 1 milyon/ünite kök hücre enjeksiyonu yapılmıştır. Ancak bu hastada yaklaşık %50 başarılı sonuç elde edilmiştir.

14. Vaka: Köpek, Yorkshire Terrier, dişi, 10 yaş.

Sağ arka bacakta topallık şikâyeti ile getirilmiştir. Sağ coxafemoral eklemde OA tanısı konmuştur. İntraartiküler yolla bir defa 2 milyon/ünite kök hücre uygulanmıştır. Olumlu sonuç alınmış ve topallık ortadan kalkmıştır.

TARTIŞMA

Veteriner tıpta yeni yeni uygulama alanı bulan mezenkimal kök hücre tedavisi, bu çalışmanın da konusu olmuştur. Sistemik, dahili ve viral hastalıklarda intravenöz yolla uygulanabilen mezenkimal kök hücreler, bu çalışmada ise ortopedik, nörolojik ve oftalmolojik vakalarda lokal enjeksiyon şeklinde kullanılmıştır.

Çalışmamızda yer alan 7 ortopedik vakanın 5 tanesinde gelişen OA nedeniyle kök hücreler intraartiküler olarak uygulanmış; 2 non-union vakasında ise ilgili kemik bölgesine lokal olarak enjekte edilmiştir. OA olgularında enjeksiyondan sonraki süreçte ilgili hasarlı kıkırdak dokuda kök hücreler, kondrsositlere dönüşerek eklemin rejenerasyonunu sağlamış, inflamasyonu önlemiş ve eklem sıvısı artışı sağlamıştır. Böylelikle tedavi öncesindeki ağrı, topallık, eklem hareketlerinin kısıtlanması gibi belirtiler ortadan kalkmıştır. Bu bulgularımız, OA ile ilgili benzer çalışmalar yapan araştırıcıların bulguları ile uyumludur (Undale et al., 2011; Moroni ve Fornasari 2013). Bunun yanı sıra OA'in klasik sağaltımında kullanılan kortikosteroidlerin eklem kıkırdağında kondrosit yıkımına sebep olduğu; yine bu amaçla kullanılan NSAİ'lerin gastrointestinal sistem üzerindeki yan etkileri ile kıyaslandığında, intraartiküler kök hücre enjeksiyonlarının üstünlüğü aşikardır (Anandacoomarasamy ve March, 2010; da Costa et al., 2017).

Ortopedik olgular içerisinde yer alan non-union vakasında ise osteogenezisi teşvik amacıyla ilgili kırık bölgelerine lokal kök kök hücre enjeksiyonu yapılmıştır. Kök hücreler, ilgili bölgede çoğalıp, osteositlere dönüşerek, fragmentler arasında yeni bir kemik doku oluşumunu teşvik etmiştir.

Nörolojik orijinli problemlerde kök hücre tedavileri gerek klinik gerek laboratuvar çalışmalarında başarı ile uygulanmıştır. Bu çalışmalarda, kök hücrelerin lokal uygulamalarının sinir rejenerasyonuna katkıları vurgulanmıştır (Tuszynski et al., 1994; Xu et al., 1995; Li et al., 1997; Liu et al., 1999; Teng et al., 2002). Bu araştırmada karşılaştığımız 5 nörolojik vakanın 4'ünde başarılı sonuçlar elde edilmiş, nöropati nedeniyle ekstremite fonksiyonlarının sekteye uğradığı vakalar, yeniden belirgin fonksiyon kazanmıştır. Bir vakada ise gerek hastanın yaşı gerekse sinirin geçtiği ilgili bölgedeki belirgin kalsifikasyon nedeniyle lokal uygulanan kök hücrelerin sinir dokuya ulaşamaması düşünüldüğünden başarısız olunmuştur.

Kornea ülseri ve keratitis pannosa vakalarımızda ise kök hücreler, subkonjunktival yolla enjekte edilmiştir. Diğer araştırmacıların (Du et al., 2009; Griffith et al., 2016; Syed-Picard et al., 2016) yaptığı korneaya ilişkin çalışmalarla benzer şekilde lokal kök hücre tedavileri gerek kornea ülserinde gerek kerattis

pannosa olgusunda korneada yeterli rejenerasyon sağlamıştır.

Hastaya uygulanan hücre sayısı milyon ünite olarak ifade edilir. Bir hayvana kaç milyon lokal kök hücre uygulanacağı hayvanın türü, yaşı, kilosu, uygulanacak bölgeye bağlı olarak değişkenlik gösterir. Kural olarak minimum 1 milyon üniteden başlayıp, ihtiyaca göre 2-3-4 milyon/ünite kök hücreye kadar lokal uygulamalar yapılabilir. Uygulamalar, 2-3 hafta aralıklarla 2 ya da 3 kez yapılır (Perk, 2022).

Sunulan bu çalışmada araştırmacılar olarak gözlemlediğimiz önemli bir konu; kök hücre enjeksiyonlarının ikincil, üçüncül uygulamalarının çok daha olumlu sonuçlar verdiğidir. Ayrıca tüm vakalarda fonksiyonel iyileşmeye ilişkin bulguların, yaklaşık 3. haftada ortaya çıkmış olduğudur. Bunun nedeninin ilgili bölgelere lokal olarak uygulanan kök hücrelerin çoğalması, tutunması ve ilgili dokunun orijinal hücrelerine dönüşmesi için belli bir süreç gerekmesinden olduğu düşünülmektedir. Sonuç olarak çalışmamızda 14 vakada lokal olarak uyguladığımız kök hücre enjeksiyonlarının oldukça yararlı sonuçlar verdiği görülmüştür.

SONUÇ

Gerçekleştirilen bu çalışmada 7 ortopedik, 5 nörolojik ve 2 oftalmolojik vakada lokal kök hücre uygulamaları değerlendirilmiştir. Sonuç olarak ortopedik 7 vakanın 6'sında tedavi öncesine oranla yüzde yüze varan fonksiyonel iyileşme elde edilirken, bir olguda başarı oranı yüzde elli olarak gerçekleşmiştir. Nörolojik 5 vakada paravertebral olarak kök hücre enjeksiyonlarında bir vaka dışında diğer tüm vakalarda olumlu sonuçlar alınmıştır. Oftalmolojik 2 olguda subkonjunktival yolla uygulanan kök hücre tedavisi sonucunda olumlu sonuçlar elde edilmiştir.

AÇIKLAMALAR

Bu araştırmadaki tüm destekleri ile bilime katkılarından dolayı İstanbul Vetmemorial Veteriner Kliniği'ne teşekkür ederiz.

Etik beyan:

Hem klinik hem de hasta sahiplerinin bilgisi ve izni ile yapıldığından ek bir etik izin gerekmemektedir.

Çıkar çatışması:

Çıkar çatışması yoktur.

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Evaluation of anaesthesia with xylazine-ketamine and xylazine-fentanyl-ketamine in rabbits:A comparative study

ABSTRACT

Clinical and serum biochemical markers were utilized to assess the clinical efficacy of routinely used preanaesthetics and induction agents in rabbits. Eight healthy rabbits (3.0-3.5kg) of either sex were randomly assigned to one of two groups: XK (Xylazine-ketamine) or XFK (Xylazine–fentanyl–ketamine). Intramuscular injections of xylazine (5 mg/kg), ketamine (35 mg/kg), and fentanyl (0.02 mg/kg) were given to rabbits. Clinical parameters (rectal temperature, heart rate, and respiratory rate), as well as reflexes (righting reflex, palpebral reflex, and pedal reflex), were measured before and after anaesthetic injection at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 minutes. Blood samples were also taken before anaesthesia and 30 minutes following induction. An autoanalyzer was used to examine serum biochemical parameters. In the XFK group, we observed that rectal temperature increased considerably ($P<0.05$) at 20 and 30 minutes after induction and then gradually fell to preanaesthetic control values. During the anaesthetic phase, both groups' heart rates and respiration rates reduced significantly. In XK-injected rabbits, the return of righting reflexes was delayed. Surgical anaesthesia lasted much longer in the animals of XK groups. During surgical anaesthesia, the values of albumin, cholesterol, phosphorus, HDL, and LDL were significantly increased ($P<0.05$) after administration of XK, whereas the values of total protein, globulin, cholesterol, triglyceride, creatinine, HDL, potassium, and chloride were significantly decreased ($P<0.05$). The XK combination provided sufficient anaesthesia for rabbits, as evidenced by a prolonged anaesthetic period, and good cardiovascular and other clinical indices.

Keywords: Anaesthesia, clinical chemistry, fentanyl, ketamine, rabbit, xylazine

INTRODUCTION

Rabbits are frequently employed as animal models in a variety of medical and veterinary procedures, including experimental surgery and biomedical research (Kihç, 2004). Because the difference between surgical anaesthesia and respiratory arrest is so small, they are the most difficult laboratory animals to anesthetize (Kamal et al., 2019). The susceptibility of the rabbit respiratory center to the depressive effects of anaesthetic regimens has been blamed for the high rate of death during rabbit anaesthesia (Kaya et al., 2002). Anesthesia is used for a range of surgical operations, including sedation for blood collection (since rabbits become nervous while being handled), intravenous cannula installation, and other operating measures like neutering, gastrotomy, cystotomy, and fracture fixation (Brodbelt, 2009). When all safety precautions are performed, such as using the correct anesthetics and following the specified dosing schedule, administering anesthetics to rabbits is deemed safe.

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Research Article

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In rabbit anaesthesia, xylazine and ketamine are commonly used anaesthetics (Oguntoye and Oke, 2014). When ketamine is employed as the sole anesthetic agent, hypertonus, inadequate muscular relaxation, persistent pain reflex responses, and violent recovery from anaesthesia occur (Chen et al., 2015), necessitating the inclusion of preanesthetic drugs. Pre-anesthetics are also used in conjunction with general anesthetics; in most species, xylazine hydrochloride is used to reduce stress, relax the animal, and minimize the overall dose of general anesthetics. Fentanyl citrate is an opioid agonist with high potency (Kaya et al., 2002). Although it can produce marked respiratory depression, it only causes minor alterations in circulatory variables. The respiratory depressive effect of fentanyl, like that of longer-lasting opioid analgesics, may last longer than the analgesic effect (Dupras et al., 2001). Injectable anaesthetics are extensively used in rabbits because they are simple to administer. They can be administered intravenously, intramuscularly, intraperitoneally, or subcutaneously. Based on the foregoing information, we set out to conduct this study with the following goals: to compare the clinical effects of the tested anaesthetic combinations (xylazine-ketamine and xylazine-fentanyl-ketamine); to assess reflex responses and anaesthetic indices, and to investigate changes in serum biochemical parameters during anaesthesia.

MATERIAL and METHOD

This study compared the effects of two different anesthetic combinations in New Zealand White Rabbits. Animal Welfare and Experimentation Ethics Committee (AWEEC) of the Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh offered recommendations for the study.

Experimental Animals

Eight clinically healthy White New Zealand rabbits of either sex, weighing roughly 3.0 to 3.5 kg and aged between 8 and 10 months, were used in this investigation and were obtained from a local market in Mymensingh. The rabbits were given a 7-day acclimatization period in the Department of Medicine, Faculty of Veterinary Science, BAU's experimental shed. Individual wooden-wire cages with controlled environmental conditions were employed to house the rabbits (temperature, relative humidity, air changes, and light). Seasonal fresh grass, fresh vegetables, commercial rabbit feed, and ad libitum water were supplied to the rabbits. Food, but not drink, was put on hold for 12 hours before the trial began.

Experimental Design

The rabbits in the experiment were randomly allocated into two groups, each with four rabbits. The anaesthetics were given out in the following order: Group XK: Xylazine-Ketamine and Group XFK: Xylazine-Fentanyl-Ketamine.

Group XK

For anaesthesia, the animals in group XK were injected with xylazine hydrochloride (Xyla®, Interchmie Pharmaceuticals, Holland) and ketamine hydrochloride (Ketalar®, Popular Pharmaceuticals, Tongi, Bangladesh). Xylazine hydrochloride was given intramuscularly at a dose rate of 5 mg/kg. After 15 minutes, ketamine hydrochloride was delivered intramuscularly at a dose rate of 35 mg/kg.

Group XFK

Xylazine (Xyla®, Interchmie Pharmaceuticals, Holland), Fentanyl (Fentanyl Citrate®, Martindale Pharmaceuticals, Romford, UK), and Ketamine (Ketalar®, Popular Pharmaceuticals, Tongi, Bangladesh) were used to anesthetize this group of animals. Xylazine hydrochloride was delivered intramuscularly at a dose rate of 5 mg/kg, fentanyl was injected intramuscularly at a dose rate of 0.02 mg/kg BW, and ketamine hydrochloride was injected intramuscularly at a dose rate of 35 mg/kg BW after 15 minutes of fentanyl administration.

Anesthetic Procedure

The animal was placed on the surgical table in a dorsal posture before anaesthesia. The anaesthetic drugs were given intramuscularly using 1 ml and 3 ml disposable plastic syringes, where the animals were gripped by an assistant. Puncture of the needle and observation of distinct reflexes results verified induction.

Clinical Evaluation

Before the injection (0 minutes) and at 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 minutes following the injection of the anesthetic agent, heart rate, respiration rate, and body temperature were measured. Following the injection, the depth of anaesthesia was measured using the righting reflex, palpebral reflex, and pedal reflexes at 10-minute intervals until the anaesthesia was terminated in all groups. The time between the injection of the induction agent and the disappearance of the righting reflex was used to calculate the induction time. The capacity of the animal to reestablish the righting reflex was used to assess recovery following anaesthetic.

Clinical Examination of Temperature, Respiratory Rate, and Heart Rate

A stethoscope was placed on the lower left lateral thoracic wall to assess the heart rate. The body temperature was recorded using a thermometer and the respiratory rate was determined using a stethoscope by measuring the chest movement of the thoraco-abdomen.

Clinical Examination of Reflexes

The rabbit's righting reflex was assessed by timing how long it took it to move from dorsal to sternal recumbency. When no response was elicited by stroking the dorsal eyelid with a cotton-tip applicator, the palpebral reflex was reported as missing. The pedal reflexes were checked by pinching the fore limb and hind limb with a needle (right and left).

Collection of Blood Sample

Each experimental animal had three ml of blood drawn through jugular venipuncture with a 3 ml disposable syringe, which was immediately transferred to a vacutainer (clot activator tube) for serum separation and biochemical analysis.

Biochemical Examinations

Blood samples were centrifuged for 15 minutes at 3000 rpm after one hour. For biochemical analysis, the supernatant serum was collected in an Eppendorf tube using a micropipette. Total Protein (TP), Albumin, Globulin, Cholesterol, Triglyceride (TG), Calcium (Ca), Phosphorus (P), High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Creatinine, Sodium, Potassium, and Chloride were all measured in the serum samples. The serum biochemistry was measured using a photometric approach utilizing a T80 UV/VIS Spectrometer (USA) at the Mohammad Hossain Central Laboratory, BAU, Mymensingh.

Statistical Analysis

All the data was presented as Mean ± SEM (Standard Error of Mean). Statistical Package for the Social Sciences (SPSS) version 20.0 was used to compare data within and across groups using one-way ANOVA (Analysis of Variance). Probability $P<0.05$ or less was considered as statistically significant.

RESULTS

Effect of Different Anaesthetic Combinations on Clinical Parameters in Rabbit Effect on Rectal Temperature (RT)

We found no significant changes in rectal temperature in the animals of group XK at different time intervals in this investigation. Rectal temperature in the animals of group XFK, on the other hand, was considerably higher at 20 and 30 minutes compared to the preanaesthetic control value (Figure 1).

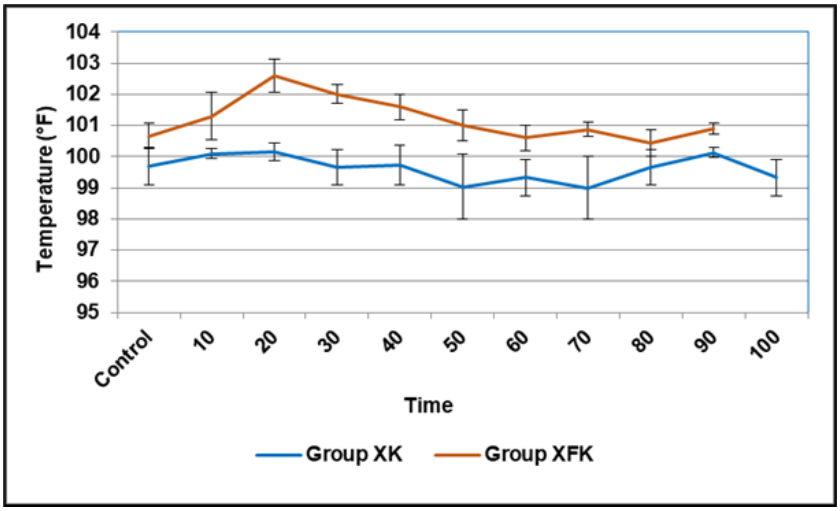

Figure 1. Effects of different anaesthetic combinations on body temperature**.**

Effect on Heart Rate (HR)

We found significant (P<0.05) variations in heart rate in group XK animals at different time intervals during the anaesthetic period when compared to the preanaesthetic control value. In the animals of group XFK, the heart rate declined considerably (P<0.05) at 40 and 50 minutes, then increased significantly (P<0.05) at 60 minutes, then decreased significantly (P<0.05) as compared to the preanaesthetic control value (Figure 2).

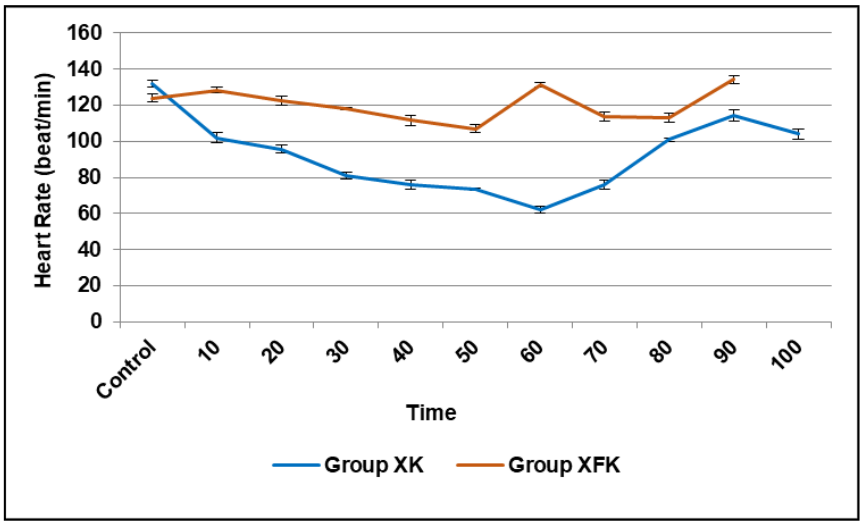

Figure 2. Effects of different anaesthetic combinations on heart rate.

Effect on Respiratory Rate (RR)

The respiratory rate in the animals of groups XK and XFK was significantly (P<0.05) altered throughout the study period when compared to the preanaesthetic control values (Figure 3).

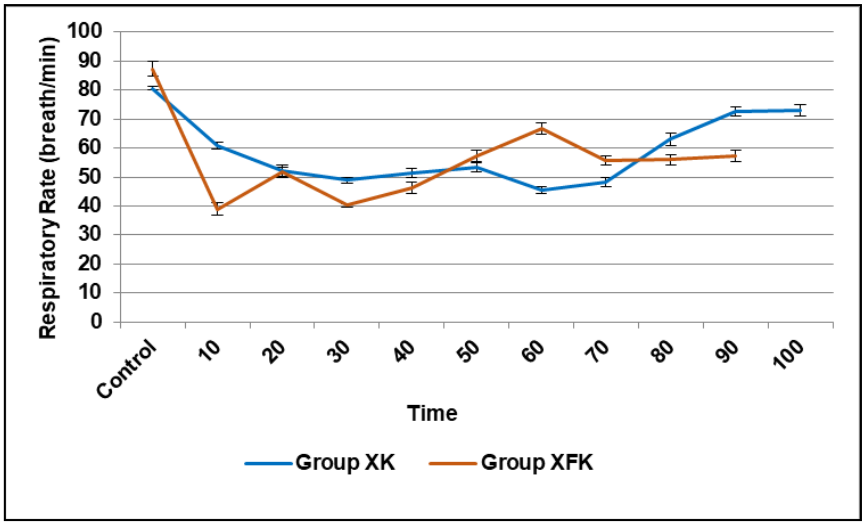

Figure 3. Effects of different anaesthetic combinations on respiratory rate.

Comparison of Different Anaesthetic Regimens on the Reflex Responses in Rabbits

Table 1 shows the effect of various anaesthetic combinations on reflex responses following anaesthesia. Within 1 minute, all of the animals in groups XK and XFK had lost their righting reflex. The animals in group XFK had the quickest loss of righting reflex, and the animals in group XK had the longest loss of palpebral reflex. In the animals of group XK, the pedal reflexes were never fully lost. The animals in group XK had the longest length of righting reflex recovery. The highest withdrawal time of palpebral reflex was found in the animal of group XFK as compared to group XK.

Table 1. Effect of different anaesthetic combinations on the reflex renposes following anaesthesia in rabbits

Group	Loss of Righting Reflex (sec)	Return of Righting Reflex(min)	Loss of Palpebral Reflex (sec)	Return of Palpebral Reflex (min)	Loss of Pedal Reflex(min)	Return of Pedal Reflex (min)
XK	60.5± 0.015	113.5±0.707	60.5±0.015	59± 2.828	-	-
XFK	20.5± 2.121	91.5±3.535	22± 1.414	71± 2.828	12.5± 2.121	37.677±2.081

Comparison of Different Anaesthetic Regimens on the Onset of Induction and Duration of Anaesthesia in Rabbits

The longest induction period as well as the highest duration of anaesthesia was found in the animals of group XK (Table 2).

Table 2. Effect of different anaesthetic combinations in rabbits on the onset of induction and duration of anaesthesia

Anaesthetic Combinations	Onset of Induction Time (sec)	Duration of Anaesthesia (hr)
XK	60.5± 0.015	1.75±0.353
XFK	20.5± 2.121	1.425±0.035

Effect of Different Anaesthetic Combinations on Biochemical Parameters in Rabbit

Effects of anaesthetic combinations on some biochemical parameters (Total protein, albumin, globulin, creatinine, cholesterol, triglyceride, HDL, LDL, sodium, potassium, calcium, phosphorus, chloride) in rabbits are shown in Table 3.

At 30 minutes, we observed no significant change in the total protein value in the animals in group XK. When compared to the preanaesthetic control value, the value of TP in group XFK was considerably (P<0.05) lower at 30 minutes during anaesthesia.

At 30 minutes after induction, the blood albumin level in the animals of group XK was substantially (P<0.05) greater than the preanaesthetic control values. At 30 minutes following induction of anaesthesia, we found no significant changes in serum albumin levels in the animals in group XFK.

When compared to preanaesthetic control values, the value of creatinine in the animals of group XFK was considerably (P<0.05) lower at 30 minutes. At 30 minutes following induction of anaesthesia, we found no significant changes in the levels of creatinine in the animals in group XK.

At 30 minutes, the serum cholesterol level in group XK animals was significantly (P<0.05) higher. Whereas at 30 minutes after induction, the cholesterol level in group XFK animals was considerably (P<0.05) lower than the preanaesthetic control values.

When compared to preanesthetic control values, the value of triglyceride in the animals of groups XK and XFK was considerably (P<0.05) lower at 30 minutes.

When compared to preanaesthetic control values, the HDL and LDL levels in group XK animals were significantly (P<0.05) higher at 30 minutes. At 30 minutes after induction, the value of HDL in the animals of group XFK was considerably (P<0.05) lower.

The value of sodium in the animals of group XK was found to be considerably (P<0.05) lower at 30 minutes after induction in this investigation. At 30 minutes after induction, the sodium and potassium levels in the animals in group XFK were significantly (P<0.05) higher. At 30 minutes after induction, we found no significant changes in potassium in the animals of group XK.

At 30 minutes, the calcium value in the animals of group XK was considerably (P<0.05) decreased, while the calcium value in the animals of group XFK was significantly (P<0.05) increased. At 30 minutes, the calcium and phosphorus values in the animals of group XFK were substantially (P<0.05) greater than the preanaesthetic control value.

When compared to preanaesthetic control values, the value of chloride in the animals of groups XK and XFK was considerably (P<0.05) lower 30 minutes after induction.

Table 3. Effects of different anaesthetic combinations on some serum biochemical parameters in rabbits

Parameter	Group	Preanaesthetic Control	30 min after induction
1. Total protein (gm/dl)	XK	6.357± 0.04[a]	6.287± 0.025 [a]
	XFK	6.492± 0.33 [a]	5.467± 0.028[b]
2. Albumin (gm/dl)	XK	3.324± 0.015 [a]	3.48± 0.01 [b]
	XFK	3.38±0.31[a]	2.87± 0.02 [a]
3. Creatinine (mg/dl)	XK	1.434±0.063 [a]	1.34± 0.037 [a]
	XFK	1.48±0.05 [a]	1.26± 0.037 [b]
4. Cholesterol (mg/dl)	XK	94.443± 0.025 [a]	106.06± 0.142 [b]
	XFK	94.60± 1.0 [a]	80.77 ±0.497 [b]
5. Triglyceride (mg/dl)	XK	83.734 ± 0.352 [a]	82.947± 0.160 [b]
	XFK	82.7± 1.91 [a]	69.64± 0.056 [b]

6. HDL (mg/dl)	XK	44.443± 0.030 [a]	52.034 ± 0.153 [b]
	XFK	44.15±0.26 [a]	28.68± 0.05 [b]
7. LDL (mg/dl)	XK	33.517± 0.256 [a]	37.484± 0.041 [b]
	XFK	33.25±0.52 [a]	35.2± 0.05 [b]
8. Sodium (mmol/l)	XK	155.463± 0.508 [a]	147.8± 0.556 [b]
	XFK	154.28±1.00 [a]	158.967± 0.929 [b]
9. Potassium (mmol/l)	XK	4.72± 0.056 [a]	4.3± 0.2 [a]
	XFK	4.58±0.161 [a]	3.59± 0.04 [b]
10. Calcium (mg/dl)	XK	9.72± 0.135 [a]	8.713± 0.036 [b]
	XFK	9.33± 0.55 [a]	10.75± 0.087 [b]
11. Phosphorus (mg/dl)	XK	2.7967± 0.085 [a]	3.914± 0.045 [b]
	XFK	2.59±0.41 [a]	3.66± 0.053 [b]
12. Chloride (mmol/l)	XK	107.234 ± 0.352 [a]	106.134 ± 0.153 [b]
	XFK	107.18±0.28 [a]	105.467 ± 0.252 [b]

DISCUSSION

Throughout the anaesthetic period, there were no significant changes in rectal temperature in group XK in this investigation. Similar findings have been reported by others (Oguntoye and Oke, 2014). In group XFK, however, the rectal temperature was considerably higher at 20 and 30 minutes compared to preanaesthetic control values. The increase in body temperature could be due to anesthetics (xylazine, fentanyl, ketamine) eliciting the thermoregulatory center and causing animals to become hyperthermic (Afshar et al., 2005).

Heart rate reduced significantly from 10 to 60 minutes (group XK) and 40 to 80 minutes (group XFK) compared to preanaesthetic control values, then increased and returned to baseline values in group XK and group XFK animals at 100 minutes. This conclusion was in line with the findings of (Afshar et al.,2005). When xylazine is given, it causes peripheral vasoconstriction, which causes an increase in arterial blood pressure and a drop-in heart rate (Kaya et al., 2002).

In this investigation, the RR was first reduced from its preanaesthetic control values, then fluctuated up to recovery in all groups of animals. According to (Kamal et al., 2019) and Murrell (2007), xylazine's respiratory effects are normally clinically negligible, but it can cause respiratory depression, with a decrease in tidal volume and respiratory rate, when used in combination with other medicines.

Because no surgery was conducted in this investigation, the surgical anaesthetic duration was measured using the righting reflex, palpebral reflex, and pedal withdrawal reflex, as described in the literature. In adult rabbits, Karasu et al. (2018) and Bienert et al. (2014) found that reflex loss and return periods varied according to the dose of anaesthetic regimens, with stronger doses providing longer sedation durations and longer pedal withdrawal reflex return times.

In this investigation, we found that after 30 minutes, the values of TP and albumin in all groups of animals were lower than their preanaesthetized control values. Gil et al. (2004) found that TP and albumin levels in rabbits

decreased under intramuscular anaesthesia, which is consistent with this finding. The decrease in TP, albumin, and globulin in this study could be related to anaesthetic drug haemodilution and haemodynamic alterations in cell membrane permeability. When compared to preanaesthetic control values, serum creatinine levels in group XFK were lower at 30 minutes. In contrast to our findings, Gil et al. (2004) observed an increase in serum creatinine levels 30 minutes following the treatment of Xylazine-ketamine to rabbits. However, in this investigation, lower creatinine concentrations could be linked to the anesthetics' short-term effects on renal function.

The liver and the stress response have a direct impact on serum cholesterol levels (Akter et al., 2020). At 30 minutes after induction, blood cholesterol levels were considerably lower in group XK and significantly higher in group XFK in this investigation. Similar findings have been reported by others (Gil et al., 2004). Lipolysis caused an increase in serum cholesterol levels. The most frequent kind of lipid storage is triglycerides, which are an important source of energy. The triglyceride value in the animals of groups XK and XFK was considerably lower at 30 minutes. This result is reliable to the findings of (Gil et al., 2004).

In all groups, the HDL value was considerably lower at 30 minutes compared to preanaesthetic control values. In all groups, the LDL value was considerably higher at 30 minutes compared to preanaesthetic control values. Similar findings have been reported by others (Perumal et al., 2007). Because ketamine promotes sympathetic nerve activity, ketamine anesthesia decreased serum HDL levels while increasing serum LDL levels (Akter et al., 2020).

The value of sodium was decreased in group XK and raised in group XFK in this investigation, which corresponded with the findings of the previous study (Gil et al., 2004). As a result, the elevated serum sodium concentrations are probably linked to a decrease in renal blood flow. Potassium levels in all groups of animals were lower than preanaesthetic control levels. Rahman et al. (2021) found a considerable increase in serum potassium levels after ketamine–xylazine injection compared to control levels; this increase was likely due to the impact of xylazine, an alpha 2-adrenoceptor agonist, which contradicted this conclusion.

The calcium value in the animal group XK declined at 30 minutes from the pre-anaesthetized control value, which is similar to the findings of another study (Khalaf et al., 2014). However, when compared to preanaesthetic control values, the calcium level in group XFK animals was higher at 30 minutes. Similar findings have been reported by others (Kamal et al., 2019). Rapid mobilization of calcium following the xylazine-ketamine-related influence on renal function could explain the significant increase in serum calcium levels. All groups' serum phosphorus concentrations were higher at 30 minutes than preanaesthetic control values, which is consistent to the findings of (Karasu et al., 2018). In comparison to preanaesthetic control values, serum chloride concentrations in groups XK and XFK dropped.

CONCLUSION

Our findings suggest that xylazine-ketamine induced sufficient depth and duration of anaesthesia compared to xylazine-fentanyl-ketamine. For surgical treatments, the longer anaesthetic duration may be advantageous. When planning an invasive rabbit study, researchers should take into account the alterations in serum biochemical parameters caused by this combination.

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Ethical approval:

This animal work was carried out in accordance with the guidelines and approval of the Animal Welfare, Experimentation and Ethics Committee (AWEEC) of the Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh [Permission Number: AWEEC/BAU/2021 (45)].

Conflict of interest:

No conflict of interest.

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Effects of hoof trimming on feed consumption, milk yield, oxidant and antioxidant system in dairy cows with hoof deformities

ABSTRACT

In this study is objected to reveal the importance of hoof trimming (HT) in cows by determining the changes in feed consumption, milk yield, oxidant, and antioxidant parameters in the days before and after HT in cows with hoof deformities. This research was conducted on 12 female Brown Swiss dairy cows late lactation period that had healthy hooves showing symptoms lameness due to hoof deformities. Daily feed consumption and milk yield findings before and after HT were recorded. Total oxidant capacity (TOC) and total antioxidant capacity (TAC) tests for determination of oxidative stress index in serum in blood samples, for the evaluation of antioxidant potential; glutathione peroxidase (GSH-Px), glutathione (GSH), superoxide dismutase (SOD), Vitamin E, A, and C levels were measured. According to the findings of this study, after HT increased feed consumption and milk yield in dairy cows ($P<0.05$). After HT, TOC decreased ($P<0.05$), TAC ($P<0.05$), GSH-Px ($P<0.05$, $P<0.001$), GSH ($P<0.05$, $P<0.001$), SOD ($P<0.05$, $P<0.001$), Vitamin E ($P<0.05$, $P<0.001$) and Vitamin C ($P<0.05$) levels increased significantly, Vitamin A ($P>0.05$) levels did not change significantly. The results of this study showed that the oxidant system was suppressed and the antioxidant system was supported in lactating cows, which was done to prevent lameness due to deformations in the keratin tissue of the hoof, but without lesions in the soft tissue of the hoof.

Keywords: Hoof Trimming, Oxidant and Antioxidant System, Animal Welfare, Cow

INTRODUCTION

Hoof deformities and lameness in dairy cows are an increasingly problem in modern dairy farms (Bicalho and Oikonomou, 2013; Flower et al., 2006). Various factors such as sheltering environment, high productivity, high herd density, and individual sensitivity make cows prone to claw disorders (Bielfeldt et al., 2005; Demirkan et al., 2000; Faye and Lescourret, 1989; Manske et al., 2002; Somers et al., 2003). Lameness causes significant economic losses in dairy cattle farms and the most important reason of this economic loss is the decrease in milk production (Charfeddine and Perez-Cabal, 2017; Demir et al., 2013; Entig et al., 1997; Onyiro et al., 2008; Reader et al., 2011; Sogstad et al., 2007). After the appearance of lameness, the decrease in milk yield, the cow does not want to go to the feeder due to pain, and when it does, barely standing for feed consumption, and therefore feed consumption is reduced. In conclusion, milk yield decreases due to the decrease in feed consumption (Arican et al., 2018; González et al., 2008; Green et al., 2002; Hassall et al., 1993; Warnick et al., 2001). Additionally, to economic losses foot and hoof deformities also adversely influence the cow's welfare (Alvergnas et al., 2019; Stoddard and Cramer, 2017; Yakan and Duzguner, 2019). Therefore, there is a raising awareness of the importance of HT in cattle in terms of animal health and welfare.

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Research Article

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Hoof trimming is done to prevent lesions on the claws and to improve gait by correcting and maintaining foot symmetry and shape, which ensures the correct distribution of weight. Lameness caused by hoof deformities can be treated with correct HT. The correct claw shape helps restore weight-bearing balance and supports recovery from hoof deformities Neveux et al., 2006; Pesenhofer et al., 2006; Shearer and Van Amstel, 2001; Van der Tol et al., 2004; Yakan and Duzguner, 2019). However, the discomfort caused by only deformed hoof structures in the animal and the effect of the correct hoof shape on animal welfare has not been reported. In this study, it was objectived to compare the changes in feed consumption, milk yield, oxidant and antioxidant parameters before and after HT. In addition to previous studies, also with the findings of this study effects of HT on the economy and animal welfare will be revealed.

MATERIAL and METHOD

Animals

This research was conducted on 12 female Brown Swiss dairy cows aged 3- 6 years with a mean bodyweight of 550 ± 600 kg that was in the late lactation period 4- 6 months pregnant and showing symptoms of lameness due to only hoof deformities (without lesion in the living tissue of the foot) at the Ağrı İbrahim Çeçen University Eleşkirt Celal Oruç Academy of Livestock Production, Education, Research and Application Farm, Ağrı, TR. Data were collected for each cow from the farm registration system: age, the first calving age of cows, two birth intervals, the mean number of calving, days in lactation, number of lactations, lactation stage, daily milk yield, stay or removal age in the herd.

Feeding and Determination of Daily Feed Consumption

Cows were fed the same ration during the research. Concentrate (8 kg milk feed) [Cattle Dairy Feed 18%, Birlik Feed Erzurum, Turkey] and roughage (20 kg corn silage, 4 kg clover dry grass, and 3 kg meadow dry grass) were given immediately after morning and evening milking divided into two in a day fed *ad libitum*. Daily energy needs were computed from the mean weight and daily milk yield to the National Research Council (NRC) requirements (NRC, 1989). To determine the daily feed consumption of each cow during the study, the animals in the study group were kept in separate compartments on the farm. They were subjected to individual feeding. Daily total feed consumption, morning and evening before feeding was determined by weighing the increased feed in front of the animals (Jadever JWQ-30 Digital precision scale). Daily feed consumption findings on days the 1,7 (before HT), 13 (on the day of HT), 19, 25, and 31 (after HT) of the study were recorded. The mean feed composition is given in Table 1. Clean drinking water was always kept in front of the animals.

Table 1. Composition of concentrated and roughage mixes used in the study.

Ingredient	Daily quantity-kg/cow
Concentrated feed	8
Corn silage	20
Clover dry grass	4
Meadow dry grass	3
Salt	0.004
Vitamin-mineral premix*	0.003

*Provided per 1 kg of premix: Vit. A 15000000 IU, Vit D3 3000000 IU, Vit E 30000 mg, Mn 50000 mg, Zn 50000 mg, Fe 50000 mg, Cu 10000 mg, I 800 mg, Co 150 mg, Se 150 mg.

Hoof Trimming

Twelve cows that had healthy hooves showing symptoms lameness due to claw disorder were selected for the study. The health status of the cows was determined by rectal temperature, heart rand respiration rate 12 hours before HT. Cows showing the values of these 3 parameters outside the physiological range were excluded from the study. On days the 13th of the study, it was taken to the travail that provided the standing fixation of the animal for HT. HT was started from the medial hoof in the hind legs and lateral hoof in the anterior legs. Following the method, the horn hoof was trimmed and corrected by the investigator (SY) and this procedure was completed in approximately 15 min. Claws of the cows were trimmed on different days and checked for hoof disease. The hooves of cows were trimmed following a 1-year interval since the previous trim.

Daily Milk Yield

Cows that were in the late lactation stage were chosen for the research. The average days in lactation for the cows were 230.4 (inter 124-289 days) and the average number of calvings was 2.06 times (inter 1-4 times) before HT. The milk yield of 12 cows was recorded twice a day (at 5:00 a.m and 5:00 p.m) on days 1, 7, 13, 19, 25, 31st of the study using an automatic milking system (milkline® milking).

Biochemical Analysis

Blood samples taken on days 1, 7, 13, 19, 25, and 31st of the study were brought to the laboratory as soon as possible and centrifuged at 3000 rpm for 10 minutes at room temperature, and stored at -80 ^{0}C until testing. Total oxidant capacity (TOC) [Bovine (TOC) ELISA kit] and total antioxidant capacity (TAC) [Bovine (TAC) ELISA kit] tests for determination of oxidative stress index in serum samples, from antioxidant enzymes for the evaluation of antioxidant potential; Glutathione Peroxidase (GSH-Px) [Glutathione Peroxidase Assay kit], Glutathione (GSH) [Glutathione Assay kit], Superoxide Dismutase (SOD) [peroxide Dismutase Assay kit] enzyme levels were measured by ELISA using a commercial kit. Vitamin E and A levels, which are antioxidant vitamins, were determined with the help of commercial test kits in accordance with the technique on the HPLC device at Ağrı İbrahim Çeçen University Central Laboratory. Vitamin C levels were determined colorimetrically by the appropriate technique using the phosphotungunstic acid method (Kyaw, 1978) at Ağrı İbrahim Çeçen University Central Laboratory.

Statistical Analysis

The one-way analysis of variance (ANOVA) and post hoc Duncan tests were applied to the data to examine the differences among times using the SPSS statistical software package. The findings are showed as average ± SE. A value of $P<0.05$ was accepted significantly.

RESULTS

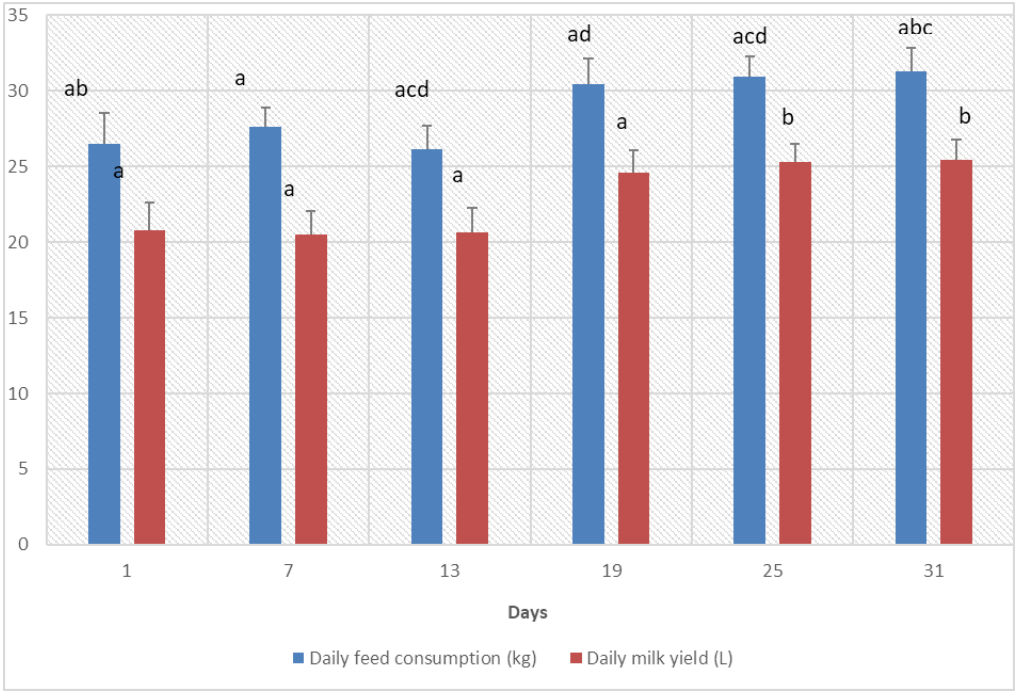
Daily Feed Consumption (kg)

In the findings of daily feed consumption on days 1,7,13,19,25 and 31st, in the comparisons between with the day 1st, days 7,13,19,25 and 31st, no statistically significant difference was found between with the day 1st, days 7 and 13th ($P>0.05$), a statistically significant result was found between with the day 1st, days 19, 25 and 31st ($P<0.05$) and feed consumption increased on days 19, 25, 31st according to the day 1st. In comparison between the with the days 7th, days 13, 19, 25 and 31st, no statistically significant difference was found between days 7 and 13th ($P>0.05$), a

statistically significant result was found in the between with the days 7th, days 19, 25 and 31st (P<0.05) and feed consumption increased on days 19, 25, 31st according to days 7. In comparison between with the days 13th, days 19,25, and 31st, statistically significant results were found (P<0.05) and feed consumption increased on days 19, 25, 31st according to days 13th. In the comparisons between with the days 19th, days 25 and 31st, no statistically significant difference was found (P>0.05). No statistically significant difference was found in the comparison between the days 25 and 31st (P>0.05), (Graph 1).

Daily Milk Yield (L)

In the findings of daily milk yield on days 1,7,13,19,25 and 31st, in the comparisons between with the day 1st, days 7,13,19,25 and 31st, there was no statistically significant difference between with the day 1st, days 7,13 and 19th (P >0.05), a statistically significant difference was found between with the day 1st, days 25 and 31st (P<0.05), milk yield increased on the days 25 and 31st according to the day 1st. In comparisons between with days 7th, days 13, 19, 25 and 31st, there was no statistically significant difference between the with days 7th, days 13 and 19th (P>0.05), the measurement results between with the days 7th, days 25 and 31st were statistically significant (P<0.05), milk yield increased on the 25th and 31st days according to the 7th days. In the comparison between with the days 13th, days 19,25 and 31st, there was no statistically significant difference between days 13 and 19th (P>0.05), a statistically significant difference was found between with the days 13th, days 25 and 31st (P<0.05), and the milk yield increased on the days 25 and 31st according to the days 13th. Found statistically significant difference in the comparison between with the days 19th, days 25 and 31st (P<0.05), and the milk yield increased on the days 25 and 31st according to the days 19th. There was no statistically significant difference in comparison between the days 25 and 31st (P>0.05), (Graph 1).



Graph 1. Daily feed consumption and milk yield findings on days 1, 7, 13, 19, 25 and 31of study. A statistically significant difference was found between values with different letters in the daily feed consumption column (P<0.05). A statistically significant difference was found between the values with different letters in the daily milk yield column (P<0.05).

Oxidant and Antioxidant Parameters

In Total Oxidant Capacity (TOC) measurement results on days 1,7,13,19,25 and 31st, in the comparisons between with the day 1st, days 7,13,19,25 and 31st, no statistically significant difference was found between with the day 1st, days 7 and 13th (P>0.05), a statistically significant difference was found in the comparisons between with the day 1st, days 19, 25 and 31st (P<0.05), and the TOC decreased on days 19, 25 and 31st according to the day1st. In comparisons between with days 7th, days 13, 19, 25, 31st, there was no statistically significant difference between days 7 and 13 (P>0.05), a statistically significant difference was found between with the days 7th, days 19, 25 and 31st (P<0.05), and TOC decreased on days 19, 25 and 31st according to days 7th. In the comparison between with the days 13th, days 19,25, and 31st, a statistically significant difference was found between the days 13th, days 19, 25, 31st (P<0.05), and TOC decreased on days 19, 25 and 31st according to the days 13th. In the comparisons between with the days 19th, days 25 and 31st, no statistically significant difference was found between the 19th, days 25 and 31st (P>0.05). There was no statistically significant difference in comparison between days the 25 and 31st (P>0.05).

In Total Antioxidant Capacity (TAC) measurement results on days 1,7,13,19,25 and 31st, in the comparisons between with the day 1st, days 7,13,19,25 and 31st, no statistically significant difference was found between days the 1 and 7th (P>0.05), a statistically significant difference was found between the days 1 and13th (P<0.05), and TAC decreased on days 13th, no statistically significant difference was found between with day the 1st, days 19, 25, 31st (P> 0.05). In the comparisons between with the days 7th, days 13,19,25 and 31st, statistically significant difference was found between the days 7 and 13th (P<0.05), and TAC decreased on the days 13th, no statistically significant difference was found between with the days 7th, days 19, 25, 31st (P>0.05). In the comparisons between with the days 13th, days 19,25, and 31st, statistically significant difference was found between the days13th, days 19, 25, and 31st (P < 0.05), and TAC increased on days 19, 25, 31th. No statistically significant difference was found in the comparisons between the days 19th, days 25 and 31st (P>0.05). No statistically significant difference was found between the days 25 and 31st comparisons (P>0.05).

In Glutathione Peroxidase (GSH-Px) measurement results on days 1,7,13,19,25 and 31st, in the comparisons between with the day 1st, days 7,13,19,25 and 31st, there was no statistically significant difference between with the day 1st, days 7 and 13th (P>0.05), a statistically significant difference was found between the with day 1st, days 19 (P<0.05), 25 (P<0.05) and 31st (P<0.001), GSH-Px activity increased on days 19, 25 and 31st. In comparisons between with days 7th, days 13, 19, 25 and 31st, there was no statistically significant difference between days 7 and 13th (P > 0.05), a statistically significant difference was found between with the days 7th, days 19 (P < 0.05), 25 (P<0.05) and 31st (P<0.001), and GSH-Px activity increased on days 19, 25 and 31. In the comparisons between with days the 13th, days 19,25 and 31st, a statistically significant difference was found between with the days 13th, days 19 (P<0.05), 25 (P<0.05) and 31st (P<0.001), GSH-Px activity increased on days 19, 25 and 31st. In the comparison between with the days 19th, days 25 and 31st, there was no statistically significant difference between days the 19th, days 25 and 31st (P>0.05).

There was no statistically significant difference in comparison between days the 25 and 31st (P>0.05).

In Glutathione (GSH) measurement results on days 1,7,13,19,25 and 31st, in the comparison between with day 1st, days 7,13,19,25 and 31st, a statistically significant difference was found between with the 1st, days 7 and 13st (P<0.05), GSH levels decreased on days 7 and 13th, there was no statistically significant difference between days 1 and 19th (P>0.05), a statistically significant difference was found between with day 1th days 25 and 31st (P<0.001), GSH levels increased on days 25 and 31st. In the comparison between with the days7th, days 13,19, 25 and 31st, there was no statistically significant difference between days 7 and 13th (P> 0.05), a statistically significant difference was found between with days 7th, days 19 (P< 0.05), 25 (P<0.001) and 31st (P<0.001), and GSH level increased on days 19, 25 and 31st. In the comparisons between with the days 13th, days 19, 25 and 31st, there was a statistically significant difference between with 13th, days 19 (P<0.05), 25 (P<0.001) and 31st (P<0.001), and GSH level increased on days 19, 25 and 31st. A statistically significant difference was found in the comparisons between days 19th, days 25 and 31st (P<0.001), and GSH level increased on days 25 and 31st. A statistically significant difference was found in the comparison between the days 25 and 31st (P<0.05), and GSH level increased on days 31st according to 25 days.

In Superoxide dismutase (SOD) measurement results on days 1,7,13,19,25 and 31st, in the comparison between with the day 1st days 7,13,19,25 and 31st, there was no statistically significant difference between with the day 1st, days 7 and 13th (P>0.05), there were statistically significant between with the day 1st, days 19 (P<0.05), 25 (P<0.001) and 31st (P<0.001), and SOD activity increased on days 19, 25 and 31st. In comparisons between with days 7th, days 13, 19, 25 and 31st, there was no statistically significant difference between the measurement results between days 7 and 13th (P>0.05), a statistically significant difference was found between with the days 7th, days 19 (P<0.05), 25 (P<0.001) and 31st (P<0.001), and SOD activity increased on days 19, 25 and 31st. A statistically significant difference was found in the comparison between with days 13th, days 19, 25, and 31st (P<0.001), and the SOD activity increased on days 19, 25, and 31st. A statistically significant difference was found between with the days 19th, days 25 and 31st (P<0.05), and SOD activity increased on days 25 and 31st. There was no statistically significant difference in comparison between the days 25 and 31st (P>0.05).

In Vit C measurement results on days 1,7,13,19,25 and 31st, in the comparison between with the day 1st, days 7,13,19,25 and 31st, there was no statistically significant difference between with the day 1st, days 7 and 13th (P>0.05), there was a statistically significant difference between with the day 1st, the 19th, 25th and 31st days (P<0.05), Vit C level increased on the 19th, 25th and 31st days according to the day 1st. In the comparison between with the day 7th, days 13,19,25 and 31st, while there was no statistically significant difference between the 7th and 13th days, there was a statistically significant difference between the days 7th, 19th, 25th and 31st days (P<0.05). In the comparison between with the day 13th, days 19,25 and 31st, a statistically significant difference was found between the days13th, days 19th, 25th and 31st (P<0.05). In the comparison between with the day 19th, days 25 and 31st, there was

no statistically significant difference in comparisons between the 19th, 25th and 31st days ($P>0.05$). There was no statistically significant difference in comparison between the days 25 and 31st ($P>0.05$). Vit C level increased after HT.

In Vit E measurement results, in the comparison between with the day 1st, days 7,13,19,25 and 31st, no statistically significant difference was found between with the day 1st, days 7,13,19th ($P>0.05$), there was a statistically significant difference between days 25th ($P<0.05$) and 31st ($P<0.001$). Vit E level increased on the 25th and 31st days compared to the day 1st. In the comparison between with the day 7th, days 13,19,25 and 31st, there was no statistically significant difference between with the days 7th, days 13th and 19th ($P>0.05$), while there was a statistically significant difference between days 25th ($P<0.05$) and 31st ($P<0.001$). In the comparison between with the day 13th, days 19,25 and 31st, there was no significant difference in the comparisons between the 13th and 19th days ($P>0.05$), there was a statistically significant difference in the comparisons between days 25th ($P<0.05$) and 31st ($P<0.001$). There was a statistically significant difference in the comparisons between with the days 19th, 25th and 31st days ($P<0.05$). There was a statistically significant difference in the comparisons between the 25th and 31st days ($P<0.05$). Vit E level increased after HT.

In Vit A measurement results, there was no statistically significant difference in comparisons between all time ($P>0.05$).

Measurement results of oxidant and antioxidant parameters on days 1,7,13,19,25,31st of the study are given in Table 2.

Table 2. Measurement results of oxidant and antioxidant parameters on days 1,7,13,19,25,31st of study.

Time (days)	TOC (µmol/L)	TAC (U/mL)	GSH-Px (mmol/L)	GSH (mmol/L)	SOD (U/mL)	Vit C (mg/L)	Vit E (mg/L)	Vit A (mg/L)
1	64.29 ± 5.71^{a}	4.27 ± 0.22^{a}	4.51 ± 0.43^{a}	15.08 ± 0.45^{a}	8.05 ± 0.25^{a}	5.84 ± 0.94^{a}	5.11 ± 0.28^{a}	0.51 ± 0.05^{a}
7	60.00 ± 5.34^{a}	4.07 ± 0.26^{a}	4.46 ± 0.33^{a}	12.43 ± 0.62^{ab}	7.80 ± 0.29^{a}	5.69 ± 0.71^{a}	5.44 ± 1.13^{a}	0.58 ± 0.51^{a}
13	70.00 ± 7.87^{a}	3.87 ± 0.28^{ab}	4.43 ± 0.26^{a}	13.50 ± 0.48^{ab}	7.71 ± 0.20^{a}	5.01 ± 0.21^{a}	5.28 ± 2.11^{a}	0.46 ± 0.45^{a}
19	45.71 ± 4.81^{ab}	4.44 ± 0.12^{a}	6.00 ± 0.38^{ab}	16.06 ± 0.67^{a}	9.67 ± 0.49^{ab}	6.99 ± 078^{b}	5.49 ± 3.12^{a}	0.44 ± 0.43^{a}
25	41.43 ± 4.04^{ab}	4.51 ± 0.10^{a}	5.90 ± 0.24^{ab}	22.97 ± 1.11^{abc}	10.61 ± 0.36^{abc}	7.39 ± 0.76^{b}	7.11 ± 0.22^{b}	0.51 ± 0.21^{a}
31	42.86 ± 7.14^{ab}	4.43±0.11^{a}	6.50 ± 0.14^{ab}	26.83 ± 1.34^{abcd}	10.64 ± 0.24^{abc}	7.45 ± 0.55^{b}	8.13 ± 2.66^{ab}	0.43 ± 0.11^{a}

TOC: Total Oxidant Capacity; TAC: Total Antioxidant Capacity; GSH-Px: Glutathione Peroxidase; GSH: Glutathione; SOD: Superoxide Dismutase; Vitamin E, A, and C. Statistically significant difference was found between values with different letters in the same line ($P<0.05$, $P<0.001$).

DISCUSSION

Many factors genetics, season, shelter type, herd size, exercise, lying surface, age, pregnancy and lactation, and feeding conditions are effective on foot health. One or more of these factors come together to determine the herd's foot health (Cramer et al., 2009; Dippel et al., 2009; Frankena et al., 2009; Van der Waaij et al., 2005). Regular hoof trimming is the only way to minimize the effects of these factors (Van Hertem et al., 2014). Functional HT, disrupts the

vicious circle of excessive mechanical load on the claw, ensuring that the weight is evenly distributed on both claws. Healthy claw tissue production is stimulated as the pressure on keratogenic cells will increase with routine HT in thin bottomed animals (Bryan et al., 2012; Erol et al., 2019; Kummer et al., 2009; Van der Tol et al., 2004).

In research by Aoki *et al*. (2006), walking behavior, limb angles, back posture and vertical movement of the back while walking were measured after HT. Walking speed, stride length, and stride speed were found to increase significantly after HT. Phillips *et al*. (2000) have shown that HT body weight is distributed evenly on the foot and therefore on the claws and suggested a corresponding influence on posture. Nishimori *et al*. (2006) reported a possibility that a change in weight-bearing and posture may affect dry matter intake. They showed that by measuring different blood measurement parameters, cows started eating more roughage after HT. In the present study, each cow included in the study were subjected to individual feeding separate compartment to determine the effect on feed consumption of HT and daily feed consumption was measured on days 1,7,13,19,25 and 31st of the study. Cows' claws were trimmed on days the 13th of the study. Although the day of the HT was not statistically significant, the feed consumption of cows decreased temporarily compared to the 1st and 7th days of the study. The slightly reduced in feed consumption on the day of trimming can be attributed to the stress occurring in the animal due to HT, interruption of the daily routine and the fact that it does not yet take the normal shape of the claw and does not press the ground completely and does not adapt to the new claw of the animal, on the days following HT in cows had a decrease in activity for all these reasons feed consumption decreased. In the measurements made on the 19th, 25th, and 31st days after HT feed consumption was found to increase statistically significantly. Feed consumption started to increase on days the 6th (on days the 19th of study) after HT may be attributed to the normal shape of the claw and the animal can comfortably step on the ground. Lastly, the increase in feed consumption after HT can be attributed to getting healthy claws of the animals. It was also revealed by the findings of this study that HT increased feed consumption in cows.

Claw health has a pronounced effect on milk production (Charfeddine and Perez-Cabal., 2017; Coulon et al., 1996; Demirkan et al., 2000; Entig et al., 1997; Flower et al., 2006; Reader et al., 2011). Cows with painful hoof disorders eat less food, are less willing to move, and as a result, they can yield less milk than cows without claw disorders. Likely, the decrease in milk production associated with foot and claw lesions is due to raised energy requirement due to pain, which may also be current without a decrease in feed consumption or a noticeable lameness (Bielfeldt et al., 2005; Flower et al., 2006; Kyaw, 1978; Reader et al., 2011). Several studies showed the expected decrease in milk production in cows with claw and limb disorders. A study by Sogstad *et al*. (2007) reported that cows yielded more milk then HT than they did before HT. In another study by Kibar and Caglayan (2016), they determined that HT of a one-time hoof increased milk production in dairy cattle with hoof disorders in commercial dairy farms. But in some studies, higher milk yield was not detected after HT. A study investigated the effects on milk yield of one-time HT by Nishimori *et al*. (2006) demonstrated that milk yield did not change after HT. Taguchi *et al*. (2001) have reported a similar experiment, but no differences in milk production and composition were showed in their research. As many researchers have reported (Bielfeldt et al., 2005; Demir et al., 2013; Dippel et al., 2009; Van der Tool et al., 2004;) milk yield in the lactation stage is effected by herd factors such as management and nutrition and individual factors as genetics, parity, and disease. Differences in the literature about the influence of lameness and

hoof disorders on milk production are comparatively the conclusion of these complex effects. In the current study, daily milk yield findings were recorded on days 1,7,13,19,25, and 31st of the research to determine the effect of HT on milk yield in healthy cows. No diversity was showed in milk production in the measurements made on days 1,7 (before HT), 13 (on the day of HT). On the day of HT, the daily milk yield showed not change according to days 1 and 7th, and also the time needed for the complete HT procedure with a mean of 15 minutes was very short. On the 6th day after the HT (19th days of the study), milk yield was increased, though not statistically significant. On the 25 and 31st days of the study (after HT), a statistically significant difference was observed in the increase of milk yield. On days 10th after HT on the daily milk yield had recovered to its original value (on days 25th of study) and final measurements were made on the 31st day of the study. Accordingly, milk yield after HT was higher than before HT. Subsequently, milk yield was increased after HT. Results of the current study concurred with the findings of some researchers (Bryan et al., 2012; Kibar and Caglayan, 2016; Sogstad et al., 2007) increased in the milk yield following HT. Increased milk production after HT can be increased feed consumption as a conclusion of having healthier hooves and walking more comfortably and standing because of smooth hoof figure then HT. In the current study, a 0.9 positive relation between feed consumption and daily milk yield was also shown.

Claw disorders can be responsible for the deterioration of animal welfare by causing pain and stress in cattle (Bustamante et al., 2015; O'Callaghan et al., 2003; Shearer et al., 2013; Stock et al., 2015). Oxidant and antioxidant parameters are often used to assess pain and stress in animals (Erol et al., 2019). However, in the literature searches, no publication investigating the effect on the oxidant and antioxidant system of HT to evaluate the pain and stress caused by claw disorder in cattle. For this reason, this study also aimed to determine the effect on oxidant and antioxidant systems of HT in cows.

Under normal conditions, oxidants and antioxidants are in balance in the organism. However, in situations such as inflammation, infection, pain, and stress, this balance is disrupted in favor of oxidants, and free radicals occur, which can cause damage to cells or tissues (Halliwell and Gutteridge, 1989).

It is recommended to measure TOC and TAC to determine the oxidant and antioxidant status in the organism and their balance. In this study, a significant decrease in TOC values was observed in the measurement results after the HT. In TAC measurements, the day of HT decreased significantly and showed a significant increase in measurement days after HT. GSH-Px is the most effective of antioxidant enzymes. It is responsible for the destruction of intracellular hydroperoxides. By converting H_2O_2 to water, it prevents the formation of methemoglobin and protects the membrane lipids against peroxide anion and protects the integrity of the cell membrane. GSH-Px values were significantly lower before and on the days of HT, there was a significant increase in the measurements made after HT. GSH is an important intracellular nonenzymatic antioxidant. Its oxidized form is involved in inhibition of free radicals, stabilization of reduced sulfhydryl groups, and regeneration of tocopherol and ascorbate. It also acts as the cofactor of GSH-Px. GSH values were significantly lower before HT and on the days of HT, there was a significant increase in the measurements made after HT. SOD is the first enzyme to act in the anti-oxidative system, which is found in the mitochondria matrix of hepatocytes, erythrocytes, and brain cells. It has a stable structure. It catalyzes the reaction that converts O_2- to H_2O_2. SOD values were significantly lower before HT and on the days of HT, there was a significant increase in the measurements made after HT. The low levels of GSH-Px, GSH, SOD before HT are thought to be

due to their use in order to neutralize the radicals that occur due to oxidative stress developing during lameness.

The synergism between Vitamin C and E in preventing lipid peroxidation is well known. While Vitamin C increases the antioxidant effect of Vitamin E, it also reduces its consumption. Under normal conditions, Vitamin C is synthesized by the liver of adult cattle and this synthesis is sufficient for physiological needs. However, ruminants are susceptible to deficiencies due to the destruction of Vitamin C by the rumen microflora. Vitamin C deficiency also reduces the body's defense power against infections. In the present study, it is noteworthy that after HT, Vitamin E and C levels were significantly increased in cattle compared to before. It is thought that the reason for this situation is the decrease in feed consumption due to lameness and the increasing use due to developing oxidative stress. On the other hand, in Vitamin A levels, no significant change was observed in concentrations at study through. This may be related to the use of vitamins such as E and C primarily during oxidative stress.

This study showed that after HT, the oxidant system was suppressed and the antioxidant system was supported in dairy cows. In this study, it has been shown with the findings of the antioxidant defense system that HT increases animal welfare in cattle.

CONCLUSION

In this study, an increase in feed consumption and milk yield was observed after HT. At the same time, in this study where the effects of HT on the oxidant and antioxidant system were investigated, it was observed that the oxidant system was suppressed and the antioxidant system was supported after the HT. In light of all this information, the hypothesis that HT the necessity of regular claw trimming to ensure healthy claws and prevent lameness is clear, and it is, therefore, an integral part of improving the welfare of cattle was confirmed.

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Ethical approval:

This animal work was carried out in accordance with the guidelines and approval of the Animal Welfare, Experimentation and Ethics Committee (AWEEC) of the Çukurova University, [Permission Number: 058 / 06.07.2017].

Conflict of interest:

No conflict of interest.

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Determination of the relationship between ultrasonographic examination of hepatic lipidosis and obesity assessment parameters in cats of different body conditions

Research Article

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ABSTRACT

This study aimed to determine the relationship between ultrasonographic examination of hepatic lipidosis and obesity assessment parameters in cats of different body conditions. For this purpose, 27 cats in different body conditions which have not any systemic health problems were evaluated. Body condition score and body fat index were examined by inspection and palpation; subcutaneous adipose tissue thickness, hepatorenal index and hepatic lipidosis grade by ultrasonography. The relationship between parameters on the basis of all individuals was evaluated. Also, the cats were divided into three groups according to their body conditions, and whether there was any difference between these groups in terms of subcutaneous adipose tissue thickness, hepatorenal index and hepatic lipidosis grade were investigated. There was a significant positive correlation between body condition score and body fat index, subcutaneous adipose tissue thickness, hepatic lipidosis grade and hepatorenal index, respectively. Different degrees of hepatic lipidosis were observed in at least some individuals in all body condition groups. There was a significant difference between the different body condition groups in terms of subcutaneous adipose tissue thickness, hepatorenal index and hepatic lipidosis grade. This study showed that mild hepatic lipidosis can be seen even in cats with normal body condition; increased body condition and body fat cause an increased risk of hepatic lipidosis in cats; subcutaneous adipose tissue thickness measurement during clinical evaluation and hepatorenal index during ultrasonographic examination can be a use practical and reliable option for prediction and grading of hepatic lipidosis.

Keywords: Feline, Body condition score, Body fat index, Hepatorenal index, Hepatic lipidosis grade

INTRODUCTION

Obesity is defined as the accumulation of fat in the body that causes health problems. The ratio of body fat to body weight between 15% and 20% in cats and dogs is considered as normal and above these rates is important in terms of obesity risk. (Armstrong&Lusby, 2011). Obesity is often associated with insulin resistance, diabetes, cancer, cardiovascular, orthopedic, reproductive, dermatological and urological problems (Loftus & Wakshlag, 2015). In addition, an increase in the serum fatty acids (triglyceride) concentrations in obese individuals can cause damage to the liver, which is the metabolic center of the body (Fujiwara et al., 2015). Triglycerides accumulated in the liver in obese individuals causes hepatic lipidosis, which is characterized by stress, calorie restriction and long-term fasting, with vomiting, anorexia, jaundice, blindness and coma (Pazak et al., 1998).

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There are a number of methods that can be used to assess body composition in humans in terms of obesity risk. Body condition score, body fat index, measuring with tape measure, body mass index, bioelectrical impedance analysis, isotope dilution methods, dual energy x-ray absorptiometry, ultrasound, magnetic resonance and computed tomography are the most commonly used methods (Santarossa et al., 2017).

In the literature review, a very limited number of studies on hepatic steosis were found in pets, especially in cats which obesity is common because they generally lead a sedentary life. With this study, it is aimed to reveal the sensitivity of the evaluation by comparing the subcutaneous fat layer measurement and hepatorenal index, which is a practical and objective evaluation method that can be easily applied in routine examinations, with other methods.

MATERIAL and METHOD

Animal Studied

This study was carried out on 27 cats of different breeds, between 3.5-8.4 kg and 1-6 years old, who were brought to our clinics and did not have any systemic health problems. The cats were divided into three groups according to their body condition scores as ideal (BCS 4 and 5, n:9), above-ideal (BCS 6, n:9) and overweight/obese (BCS 7, 8 and 9, n:9).

The ethical approval of the study was provided by the University's Institutional Animal Care and Use Committee (approval number: 64583101/2021/021). In this study, a signed consent form was obtained from the owners for the study.

Methods

In the study, firstly, the relationship between body condition score and body fat index, subcutaneous adipose tissue thickness, hepatic steatosis and hepatorenal index were examined in all cats (n:27). Then, whether there was a significant difference between the groups in terms of subcutaneous adipose tissue thickness, liver fat level and hepatorenal index in ideal, above-ideal and overweight/obese cats was examined.

For body condition scoring, 9-point scoring system used by Laflamme et al. (1997), and for body fat index scoring, the system, giving score from 20 to 70 in 10-point increments described by Witzel et al. (2014), was preferred.

Subcutaneous adipose tissue thickness measurements were made by measuring the hypodermis thickness between the dermis and the muscle layer in the region behind the last rib from the right side in the left lateral lying position, as defined by Iwazaki et al. (2018). An 8 MHz frequency microconvex probe ultrasound device (Mylab 30-Esaote, Genova, Italy) was used for all ultrasonographic measurements.

In order to determine the degree of hepatic lipidosis Hamaguchi's hepatic steatosis scoring system was used and images were taken from the right hepatorenal window in the left lateral recumbency and from the diaphragmatic window in the dorsal recumbency for this purpose. To calculate the Hamaguchi score the scores for a, b, and c are added together (if A ≥1) and hepatic lipidosis diagnosed if the score is ≥ 1. A; Bright liver and hepato-renal contrast (0: both absent; 1= hepato-renal contrast or bright liver present; 2: mild bright liver, hepato-renal contrast present; 3: severe bright liver, hepato-renal contrast present), B; Deep attenuation (0: absent; 1: impaired visualization of diaphragm; 2: no visualization of the diaphragm), C; Vessel blurring (0: absent; 1: present).

Images taken from the right hepatorenal window were analyzed to calculate the hepatorenal index using the gray scale measurement feature on the ImageJ.v1.31 program (developed by National Institutes of Health and available as open source). Hepatorenal index was obtained by dividing the gray scale mean of 3 regions selected from the caudate lobe of the right liver by the gray scale mean of 3 regions selected from the cranial cortex of the right kidney, as defined by Yabuki et al. (2008) (Figure 1).

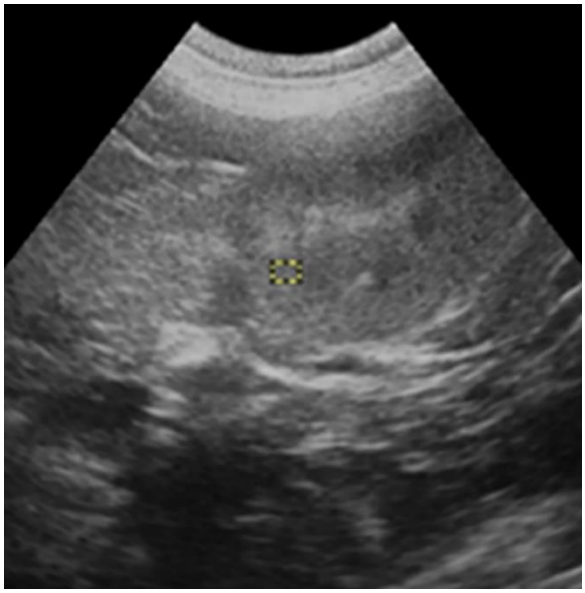

Figure 1. Selection area for kidney echogenicity

Statistical Analysis

Pearson and Spearman correlation analyzes were used to determine the relationship between parameters such as body condition score, body fat index, subcutaneous adipose tissue thickness, hepatorenal index and the degree of liver fattening measured by ultrasonography, in accordance with the type of data. Group averages were compared with one-way analysis of variance (One Way Anova) in subcutaneous adipose tissue and hepatorenal index measurements, and Tukey test as an advanced test. Due to the sequential data type of liver fattening, the Dwass-Steel-Critchlow-Fligner advanced stage test was used too, after examining with the Kruskal-Wallis test. The all tests were carried out with the IBM® SPSS Statistics V22.0 (New York, USA) package program, and $p<0.05$ was taken as the significance criterion in all tests.

RESULTS

A positive significant correlation was found between body condition score and body fat index, subcutaneous adipose tissue thickness, hepatic lipidosis grade and between hepatorenal index and hepatic lipidosis grade parameters obtained from all cats included in the study. (Table 1).

Table 1. Correlation between body condition score and body fat index, subcutaneous adipose tissue thickness, hepatic lipidosis grade and between hepatorenal index and hepatic lipidosis grade (n:27)

PARAMETERS	Body Condition Score	Hepatorenal Index	Body Fat Index	Subcutaneous Adipose Tissue Thickness (mm)	Hepatic Lipidosis Grade
Body Condition Score	-	0.915***	0.979***	0.828***	0.935***
Hepatorenal Index	-	-	-	0.749***	0.960***
***p<0.001					

Significant differences were found between ideal, above-ideal and overweight/obese groups in terms of subcutaneous fat thickness, hepatorenal index and hepatic lipidosis grade ($p<0.001$) (Table 2).

Table 2. The differences between the body condition groups in terms of subcutaneous fat thickness, hepatorenal index and hepatic lipidosis grade

Parameter Body Condition Score	Subcutaneous Adipose Tissue Thickness (mm) $\bar{X} \pm S_{\bar{x}}$	Hepatorenal Index $\bar{X} \pm S_{\bar{x}}$	Hepatic Lipidosis Grade $\bar{X} \pm S_{\bar{x}}$
İdeal	2.058±0,20[c]	1.065±0,13[c]	0.333±0,50[c]
Above-ideal	2.521±0,07[b]	1.325±0,11[b]	1.778±0,44[b]
Overweight/obese	3.184±0,85[a]	1.536±0,11[a]	2.667±0,50[a]
a, b, c: Differences between the mean indicated by different letters in the same column are significant ($p<0.001$).			

From a different angle, mild hepatic lipidosis was detected in 33.3% of the cats with ideal body condition. While 22.2% of the cats with above-ideal body condition had mild hepatic lipidosis, 77.7% of them had moderate hepatic lipidosis. In the overweight/obese group, 33.3% had moderate hepatic lipidosis and 66.6% had severe hepatic lipidosis (Figure 2).

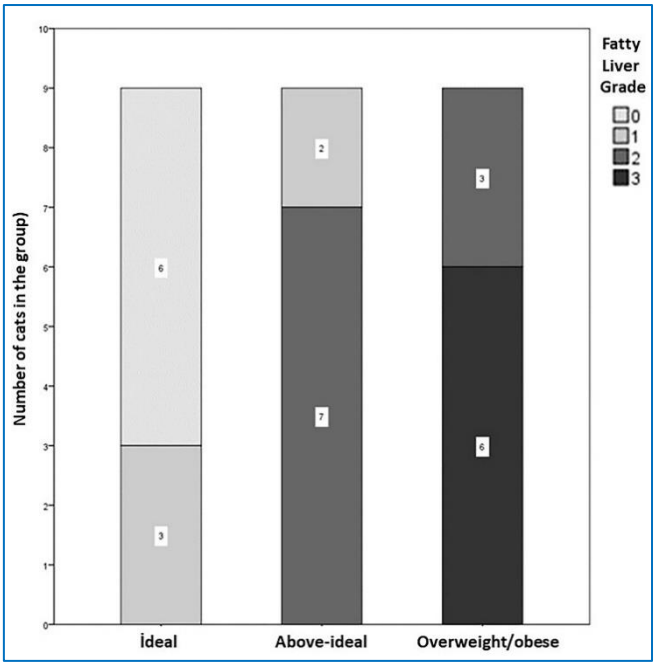

Figure 2. Distribution of hepatic lipidosis grades by group

In addition, hepatorenal index was measured as 0.981±0.018 in cats without hepatic lipidosis, 1.208±0.048 in mild hepatic lipidosis, 1.382±0.074 in moderate hepatic lipidosis, and 1.595±0.055 in severe hepatic lipidosis. (Figure 3).

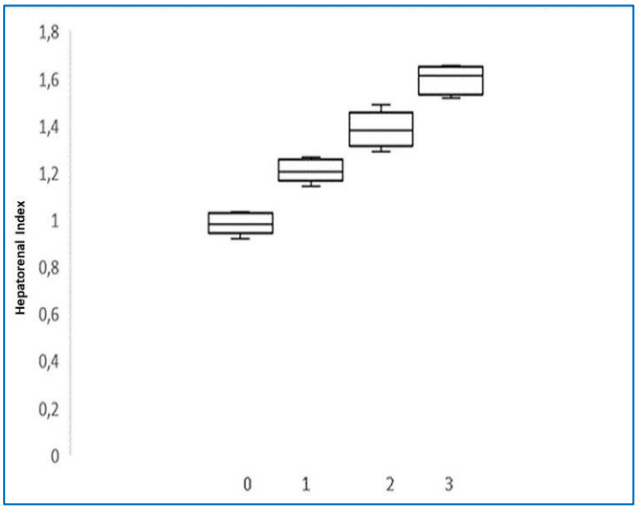

Figure 3. Hepatorenal index values of cats with different degrees of liver fattening

DISCUSSION

Various body composition analysis methods are used in the evaluation of obesity in cats and dogs. Body condition score and body fat index are frequently preferred in veterinary clinics. Both methods have their own advantages. In studies with different animal groups, it has been shown that body condition score can be determined more easily, and body fat index better represents body fat ratio (Mattiello et al., 2009; Witzel et al., 2014). In a study conducted with cats and dogs based on the evaluation of animal owners, a significant positive correlation was found between these two evaluation methods. Supporting this study, there are other studies showing that body condition score and body fat index decrease linearly in both cats and dogs in weight loss (Christmann et al., 2015; Christmann et al., 2016). In the present study, a positive significant correlation was found between body condition score and body fat index, which supports previous studies ($P<0,001$, $r = 0.979$). Since the relevant methods were applied by a single veterinarian in the study, it was thought that the correlation rate was higher than previous studies. From this point of view, it is revealed that the results obtained will be more consistent if the relevant evaluations are made by a single experienced person. In addition, it is thought that both methods can be used interchangeably due to the positive significant correlation between the two parameters.

Measurement of subcutaneous adipose tissue thickness is another method that can be used to evaluate body composition. A variable correlation between the subcutaneous fat layer in different body regions and the body condition score was shown in dogs, previously (Payan-Carreira et al., 2016). Similarly, in another study conducted in cats, a positive significant correlation was found between both parameters and also declared that the mean subcutaneous fat layer thickness to be 0.22 ± 0.01 cm in cats up to 10 years of age. (Iwazaki et al., 2018). In the present study, although a positive significant correlation was obtained between these two parameters ($P<0.001$); The average values of subcutaneous fat layer thicknesses in different body condition groups were also calculated. Although the number of animals in the group is low, these data can guide the body composition of cats to be evaluated more quickly and objectively based on the thickness of the subcutaneous fat layer, which can be easily measured from the abdomen during ultrasound examination in clinics.

In the later stages of obesity, in addition to subcutaneous fat, fat accumulation can occur in muscle and liver tissue (Fabbrini et al., 2010). From this point of view, obesity is important in

terms of the risk of hepatic lipidosis. However, the number of studies examining the relationship between body condition score and hepatic lipidosis in veterinary medicine is limited. In a study with cows, it was shown that increases in body condition score also cause an increase in the degree of liver fat detected by biopsy (Šamanc et al., 2010). In the human studies, a highly significant positive correlation was obtained between the hepatic lipidosis grade determined by biopsy and the grades obtained in the ultrasonographic hepatic lipidosis grading system used in the presented study (Hamaguchi et al., 2007). Based on this, the study examined the relationship between the degree of hepatic lipidosis detected by Hamaguchi's grading system and the hepatorenal index and significant positive correlation was found between both parameters ($p<0.001$).

In addition, the distribution of hepatic lipidosis grades in different body condition groups was also examined in the study. Unexpectedly, mild hepatic lipidosis was observed even in some cats in the ideal body condition group. For this reason, it is thought that performing ultrasonographic examinations in terms of liver fat at regular intervals in cats may be beneficial in preventing possible cases of hepatic lipidosis.

Today, hepatorenal index has also been used in human medicine, in addition to the liver fat grading method using ultrasonography (Ferraioli & Monteiro, 2019; Marshall et al., 2012). Hepatorenal index values corresponding to different degrees of hepatic steatosis have already been defined in human medicine (Avramovski et al., 2020; Chauhan et al., 2016). In veterinary medicine, there are limited studies in which mean hepatorenal index values are determined in cats (Drost et al., 2000; Yabuki et al., 2008). In the present study, a positive and significant correlation was found between the degrees of liver fattening determined by ultrasonography and the hepatorenal index values. In addition, mean hepatorenal index values corresponding to different degrees of hepatic lipidosis were also calculated. It is thought that these data will guide the faster and more objective evaluation of hepatic lipidosis in clinics.

CONCLUSION

The fact that mild hepatic steatosis was observed even in cats with normal body condition in this study suggests that it is necessary to monitor hepatic lipidosis in cats that are generally kept at home and lead a relatively sedentary life. Subcutaneous fat layer measurement and hepatorenal index, among the parameters evaluated in the study, are relatively easier and more objective than other methods. A high correlation of these parameters with the increase in body condition score and hepatic lipidosis grade, even in a small number of cats, shows that they can be considered as a practical option for the prediction and grading of hepatic lipidosis.

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Ethical approval:

The ethical approval of the study was provided by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (approval number: 19.02.2021 / 64583101/2021/021). In this study, a signed Consent form was obtained from the owners for the study.

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A case of papillary and infiltrative urothelial carcinoma of the urinary bladder in a terrier dog

ABSTRACT

Urothelial carcinomas are malignant tumours originating from the epithelial layer of the urinary bladder. In this instance, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a 2-year-old terrier dog was defined clinically, histopathologically and immunohistochemically. The material of the presented case consisted of urinary bladder tissue samples that were surgically extirpated from a two-year-old terrier-breed female dog that applied to the Department of Surgery of the Faculty of Veterinary Medicine, Selcuk University with the complaint of hematuria. The tumour brought to the pathology laboratory was 11x10x12 cm in size and had finger-shaped extensions. Its outer surface was rough and hemorrhagic. Tissues were fixed in 10% buffered formalin and paraffin blocks were obtained by going through the necessary routine follow-up procedures. Afterwards, sections were taken and subjected to Hematoxylin-Eosin, Masson's Trichrome and immunohistochemical staining. As a result of the pathological and immunohistochemical examinations of the tumoral tissue samples taken from the urinary bladder, the diagnosis of papillary and infiltrative urothelial carcinoma was reached, and the case was discussed with the information provided by the literature. In addition, immunohistochemically, intense Proliferating cell nuclear antigen (PCNA) and Vascular endothelial growth factor (VEGF) staining has been associated with malignancy.

Keywords: Urinary bladder, urothelial carcinoma, dog, histopathology

INTRODUCTION

The prevalence of urinary bladder tumours is quite low in dogs. They constitute only 0.5-1% of the tumours seen in dogs. It has been reported that almost all urinary bladder tumours have malignant features, and approximately 97% are of epithelial origin (Güzel, 2006; Maxie, 2015; Meuten, 2017). Urothelial carcinomas are malignant tumours originating from the epithelial layer of the urinary bladder. In the urinary system, the urinary bladder is the area where malignancy most commonly occurs (Meuten, 2017). This is probably due to the prolonged contact of the urinary bladder with carcinogens excreted through the kidneys and found in the urine (Maxie, 2015; Meuten, 2017; Yönez, 2016). The most common urinary bladder tumour in dogs is urothelial carcinoma. It is mostly seen in dogs of 11 years and older. Urothelial carcinomas are especially seen in older female dogs weighing more than 10 kg and exposed to benzene-containing insecticides (Fulkerson and Knapp, 2015; Knapp et al., 2014; Meuten, 2017; Moulton, 1990; Norris et al., 1992).

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Case Report

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Blood vessels are required for the invasion and metastasis of a solid tumour. There are many growth factors in tumour angiogenesis, especially vascular endothelial growth factors (VEGFs) (Haigh et al., 2000). Studies on tumour prognosis focus on VEGF, and it is defined as a critical regulator of angiogenesis (Sia et al., 2014; Simons et al., 2016).

Proliferative cell nuclear antigen (PCNA) is a nuclear protein that participates in DNA synthesis, is synthesised in the normal cell cycle and plays a role in the regulation of the cycle. PCNA, first synthesised in the G1 phase, reaches its peak level in the S phase. Afterwards, it decreases significantly in the M and G2 phases (Maga and Hubscher, 2003; Pradhan et al., 2019). PCNA is frequently used as a marker of cell proliferation in healthy and tumour tissues (Tehseen et al., 2019). In addition, it is stated that neoplastic cells are closely related to their biological activities and play an effective role in carcinogenesis, and there is a positive correlation between PCNA expression and malignancy (Ahmed and Sozmen, 2020; Ozdemir et al.2022; Ye et al., 2020).

In this instance, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a 2-year-old terrier dog is defined clinically, pathologically, and immunohistochemically. It was deemed appropriate to publish the case because of its uncommonness.

CASE REPORT

The material of the presented case consisted of urinary bladder tissue samples that were surgically extirpated from a two-year-old terrier-breed female dog that applied to the Department of Surgery of the Faculty of Veterinary Medicine, Selcuk University with the complaint of hematuria. From the anamnesis, it was learned that the patient, even though frequently adopting the urination position, could only urinate with a few drops of bloody urine, that there was no change in appetite and body condition, and that these symptoms had been observed for approximately a month. In the macroscopic examination of the urine taken with the catheter, it was determined that it contained dirty yellow turbid colour and sediment. In the examination of urine centrifuged at 1000 g for 5 minutes, hematuria was detected. A drop of the centrifuged urine sample was taken from the sediment on a slide, and a microscopic examination was performed. The microscopic examination revealed that erythrocytes, leukocytes, urine crystals and epithelial cells were found in masses in each microscope field. During the operation, it was noted that tumour structures were also found in the serosa of the urinary bladder and the adipose tissue surrounding it. In addition, it was determined that the tumour formed plaques spreading to all layers of the urinary bladder wall and made papillary extensions connected to the mucosa, reaching into the urinary bladder.

The tumour brought to the pathology laboratory was 11x10x12 cm in diameter and had finger-shaped extensions. Its outer surface was rough and hemorrhagic. Although the finger-shaped masses presented a red appearance, it was determined that some areas had grey-white foci too. The masses with a hard consistency had a whitish pink colour on the cross-sectional area and included hemorrhagic areas (Figure 1). Tissues were fixed in 10% buffered formalin. After routine follow-up procedures, 4-5 µm thick sections were taken from the prepared paraffin blocks. Sections were stained with Hematoxylin and Eosin (H&E), Masson's Trichrome methods and examined under a light microscope. Immunohistochemistry staining was performed according to Tuzcu et al. (2022). Paraffin extraction and rehydration processes were performed on the sections. The antigen retrieval process was performed by boiling in citrate buffer solution (pH: 6) for 2x5 minutes at 500 watts. Immunohistochemistry staining was performed with the UltraVision Detection

System Anti-Polyvalent, HRP (Ready-to-Use, TP-060-HL, Lab Vision, USA) ihc kit following the recommendations of the manufacturer. Monoclonal Anti-PCNA (1:200, Dako, clone PC10, M0879, 1-hour incubation) and monoclonal Anti-VEGF (1:200, Santa Cruz Biotechnology, sc-7269, 1-hour incubation) were used as primary antibodies. 3.3 diaminobenzidine (DAB) was used as chromogen and counterstained with Mayers Hematoxylin. It was then passed through a series of alcohol and xylene, covered with a coverslip and examined under a light microscope (Olympus BX51, Tokyo, Japan).

In the microscopic examination, it was observed that tumoral growths made papillary extensions from the mucosa to the lumen, as well as growths in the propria and significant connective tissue formation (desmoplasia) around them. It was determined that different microscopic areas of the tumour mass differed in terms of malignancy criteria. It was noted that in some sections examined; in the neoplastic cells, had nuclei of varying sizes and randomly ordered in a abundant eosinophilic cytoplasm, polarity was completely lost, marked atypia and pleomorphism, along with increased mitotic figures, were observed (Figure 2. A-C). Papillary extensions with urothelial proliferation were noted in some regions. In various zones, it was observed that tumour cells were surrounded by connective tissue. In addition, signet ring cells and Melamed wolinska bodies were detected sporadically (Figure 2. B-C). Furthermore, inflammatory cell infiltrations were also detected in the sections along with foci of necrosis (Figure 2. B-C). It was determined that the blood vessels were filled with erythrocytes (congestion, Figure 1. A, Figure 2. A-D), and sporadically there were bleedings. The Masson's Trichrome staining showed that a large connective tissue surrounded the tumour masses located on the urinary bladder wall (Figure 2. D). In the staining with VEGF, significant immunopositivity was observed in both neoplastic cells (cytoplasmic) and endothelial cells in the regions (Figure 3. C-D). Intense nuclear immunopositivity was determined in atypical tumour cells in staining with anti-PCNA (Figure 3. A-B).

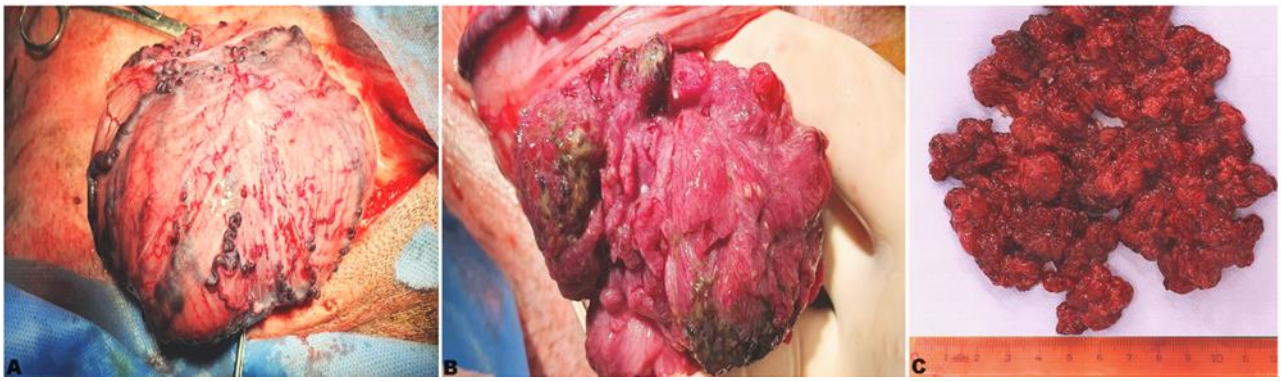

Figure 1. A. Tumor foci in the serosa of the urinary bladder, **B.** Tumor foci formed in the mucosa of the urinary bladder, **C.** Macroscopic view of the surgically removed tumor tissue

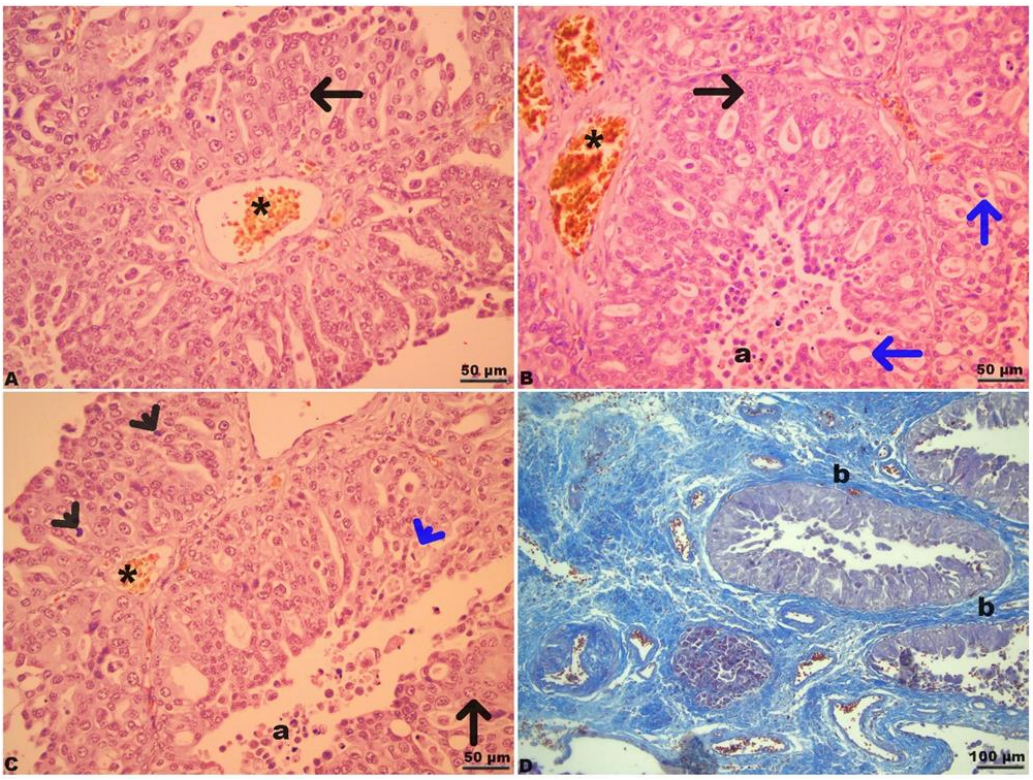

Figure 2. A-B-C. Histopathological examination of the tumoral mass, H&E, hyperemia (star), atypical cell features (black arrow), epithelial cells spilled into its lumen, and inflammatory cell infiltrates (a), mitotic figures (black arrowhead), signet ring cells (blue arrow), Melamed-wolinska bodies (blue arrowhead). **D.** View of the connective tissue surrounding the tumor mass (b), Masson's Trichrome

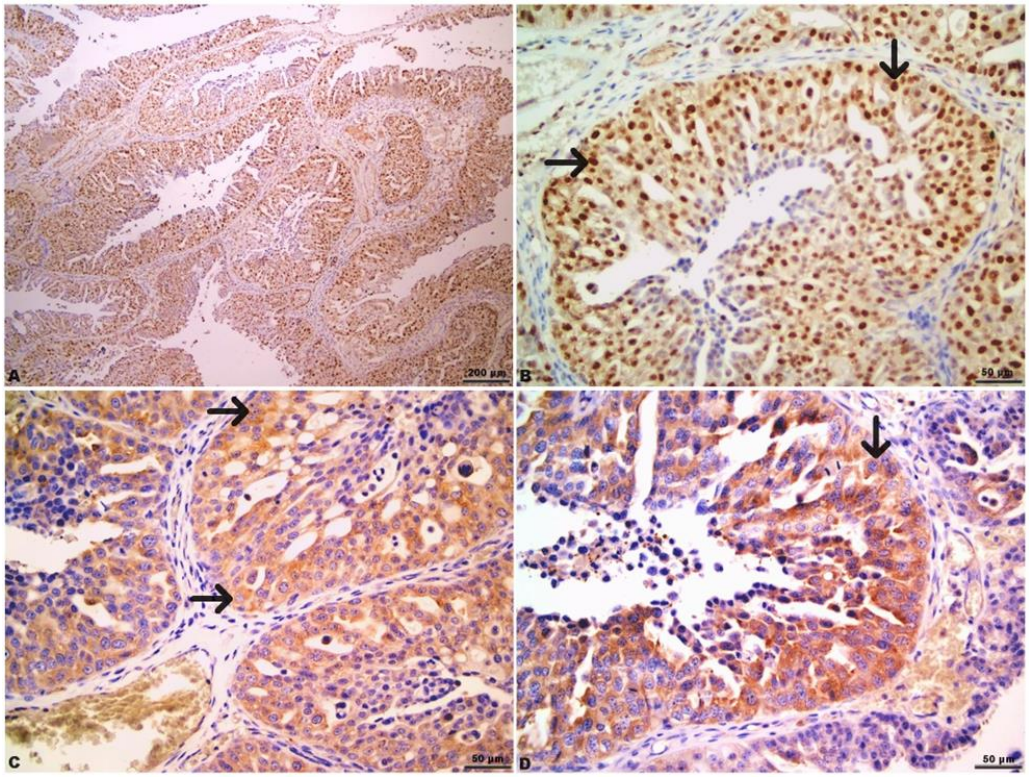

Figure 3. Anti-PCNA immunohistochemical staining (DAB), dense nuclear immunopositive cells (**A-B**) in atypical and other cells in the tumoral area (arrow). Anti-VEGF immunohistochemical staining (DAB), intense cytoplasmic staining (**C-D**) of cells in the tumoral area (arrows).

DISCUSSION

In the presented report, a case of papillary and infiltrative urothelial carcinoma in the urinary bladder of a terrier dog is described clinically and pathologically. Since this was the first case encountered in our laboratory and the dog was young, it was deemed appropriate to publish it.

Urothelial carcinomas are divided into four groups according to their macroscopic and microscopic appearances as non-papillary and non-infiltrating carcinoma, papillary carcinoma, papillary and infiltrative carcinoma, and infiltrative carcinoma (Maxie, 2015; Meuten, 2017). When the macroscopic and microscopic findings of the presented case were evaluated, they were consistent with the findings of papillary and infiltrative urothelial carcinoma.

Studies indicate that the incidence of tumours in dogs increases with age (Kent et al., 2018; Kok et al., 2019; Yönez, 2016). Although it has been reported that urinary bladder tumours are mostly seen between the ages of 4 and 16, it is informed that these tumours develop on average around 9-10 years of age (Knapp et al., 2014; Meuten, 2017; Moulton, 1990; Strafuss and Dean, 1975). In this case, it was noteworthy that the dog was 2 years old. The appearance of this tumor at a young age can be interpreted as breed susceptibility of terrier dogs to this tumor (Meuten 2017), although it is not certain.

Urothelial carcinomas are single or multiple. The latter presents a differing appearance among them, and they have mostly a papillary structure. Polyp-like and broad-based tumours may also be encountered. They often vary in size, and some fill the bladder completely (Maxie, 2015; Meuten, 2017; Norris et al., 1992). In the presented case, plaques infiltrated into the urinary bladder wall were detected next to the papillary structures. In addition papillary structures were observed filling the urinary bladder, similar to the literature.

It is stated that for the diagnosis of urothelial carcinoma, anaplasia, invasion of the urinary bladder wall, and metastasis in tumour cells should be evaluated (Meuten, 2017). In dogs, urothelial carcinomas metastasis to the regional lymph nodes and lung are encountered in the late stage, along with peritoneal implants or retrograde lymphatic spread to soft tissues and hind leg bones also (Fulkerson and Knapp, 2015; Meuten, 2017; Moulton, 1990). In the presented case, anaplasia and invasion into the sac wall were evident in tumour cells, similar to the literature. Tumour structures located in the serosa of the urinary bladder were determined, whereas metastases in neighbouring organs could not be determined.

In the presented case, it was determined that different microscopic areas of the tumour mass were different regarding malignancy criteria. In some sections examined, it was noted that the nuclei were randomly ordered and of different sizes, while a significant atypia and pleomorphism were found in the tumor-forming cells. While papillary extensions with urothelial proliferations were observed in some areas, it was observed that tumor cells were surrounded by connective tissue in some areas. The microscopic findings determined in this case were consistent with the literature (Mantovani et al., 2006; Maxie, 2015; Meuten, 2017). Necrosis, which is known as a characteristic of malignant tumours (Erer and Kıran, 2009), was observed in the form of foci ranging from small areas of necrosis formed by two or three cells to large areas of necrosis that can be noticed macroscopically.

Restucci et al. (2003), reported that they found VEGF expression to be higher in canine seminomas compared to the control group and reported that it was a useful criterion regarding malignancy and growth potential in seminomas. Campos et al. (2012) reported that VEGF levels increase significantly in hemangiosarcomas and are associated with malignant vascular

proliferation. Martano et al. (2016) reported that VEGF expressions were significantly increased in neoplastic tissues compared to normal tissues in a study on oral squamous cell carcinomas of dogs. In the presented case, the very prominent staining of VEGF in neoplastic tissues and vascular endothelium was consistent with the literature showing a correlation between malignancy and VEGF.

Krishna et al. (2022), in a study on mammary tumours in dogs, reported that PCNA scores increased in malignant tumours and were associated with malignancy. Aydogan et al. (2018) stated that PCNA expression has prognostic value in canine mammary tumours. In the study conducted by Ahmed and Sozmen (2020) on feline and canine fibrosarcomas, it was reported that PCNA expressions increased as the tumour malignancy grade increased. In the present case, intense nuclear immunopositivity was detected in atypical tumour cells in PCNA staining, which is consistent with the literature showing a correlation between malignancy and PCNA.

CONCLUSION

In conclusion, in this case, the diagnosis of papillary and infiltrative urothelial carcinoma was reached as a result of the clinical, pathological and immunohistochemical examinations of tumour samples taken from the urinary bladder of a two-year-old terrier dog, and the case was discussed with the literature. In addition, intense PCNA and VEGF staining immunohistochemically has been associated with malignancy.

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Ethical approval:

Ethical committee is not required as it consists of inanimate material (SÜVDAMEK, directive number 10.4)

Conflict of interest:

The authors declared that there is no conflict of interest.

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