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Effect of Lycopene Administration on Necrotic Gene Expression in Renal Epithelial Cell Line (NRK-52E) Exposed to Sodium Fluoride

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ABSTRACT

Objective: The aim of this study was to determine the effects of lycopene administration as a protective agent against necrotic damage of NaF, a fluoride compound found to have high cytotoxic effects in the renal epithelial cell.

Material- Method: The renal epithelial cell was cultured in DMEM high glucose medium, containing 10%FBS, 1%L-Glutamine (2mM) and 1% penicillin/streptomycin. With the MTT viability test, the non-toxic dose of lycopene (1 µM) and the IC_{50} value of NaF at the 24th hour was determined to be 3200 µM. The study groups were divided into four as control, NaF, lycopene and NaF+lycopene (the combination of NaF and lycopene). After the total mRNA obtained from these groups were converted to cDNA, expression levels of the identified necrotic genes were determined by real-time PCR method.

Results: While the Ripk1 gene did not change in the group given lycopene at the 24th hour, it was found that it increased 2.6 times in the group that received only fluoride, while it increased 7 times in the group treated with NaF+lycopene. A significant difference was detected between the groups in terms of gene expression pattern. While the Ripk3 gene increased slightly in the 24th hour applied lycopene group, it was observed that only NaF applied group increased 8 times and NaF+lycopene applied group increased in the 9 times.

Conclusion: Based on the results obtained from this study, it was seen that activation of necrotic genes is important in explaining the molecular basis of cell death from NaF, which is applied as fluoride source, in revealing the molecular basis of the necrotic pathway. It was found that the decrease in cell viability due to NaF increased with lycopene, but the use of lycopene with fluoride also increased necrotic gene expression.

Keywords: *NaF, in vitro, Lycopene, Necrotic Genes*

INTRODUCTION

Fluoride (F) is a highly electronegative element that can be found naturally in water and various nutrients. Prolonged exposure and high concentrations cause damage to teeth, bones and various tissues (Agalakova and Gusev, 2012; Perumal et al., 2013; Cetin et al., 2020).

Fluoride has a high penetrative ability and can easily penetrate the cell membrane. It may enter deeper soft tissues such as the liver, brain, and kidney, and therefore, nephrotoxicity could occur due to the accumulation and retention of inorganic fluoride in the renal tubules (Quadri et al., 2016). In a study in the northern region of Sri Lanka, where the disease of fluorosis is intense, Dharmaratne

(2015) found that the concentration of fluoride directly correlates with renal diseases in the settlements where the drinking water has high levels of fluoride.

Necrosis or necroptosis is an irregular process that develops randomly and cannot be controlled by genes, and it's most common cause is hypoxia. Toxic substances such as arsenic, cyanide, insecticides and heavy metals cause necrosis. During necrosis, mitochondrial ROS production increases, nonapoptotic proteases are activated, ATP production decreases and Ca^{++} channels are opened (Nicotera et al., 2004; Golstein and Kroemer, 2007).

Lycopene, (LYC) has an acyclic structure with 11 conjugated double bonds; the double bonds are in an all-trans form and have antioxidant properties. It has been reported that lycopene has many uses due to its anti-inflammatory, anticancer and antioxidant effects. In addition to protecting cells from free radical damage, LYC strengthens the bonds between cells and improves cell metabolism. It is reported that lycopene is protective against prostate, uterus, liver cancer, aging, Alzheimer's and cardiovascular diseases (Bramley, 2000; Mashima et al., 2001; Pruthi et al., 2003; Cetin et al., 2017).

This study aimed to determine the effects of NaF-induced necrotic damage, a fluoride compound, which is found to be highly cytotoxic in the renal epithelial cell, and the application of lycopene as a preservative.

MATERIALS and METHODS

Cell Culture

The study material comprised rat renal epithelial NRK-52E (ATCC® CRL-1571™) cells. NRK-52E cells were cultured *in vitro* with cultured in a medium containing, 10% fetal bovin serum (FBS), 1% penicillin/streptomycin, 1% L-glutamine and DMEM high glucose at 37°C, 95% humidity, 5%CO_2.

Preparation of Analysis Groups

Stock solutions of NaF and LYC used in the study were prepared by referring to the concentrations in our previous study. NaF and lycopen was dissolved in the medium. The dose that increased lycopen cell proliferation was determined as 1 μM (Cetin et al., 2017). Cell viability was measured by MTT assay to measure the cytotoxic effect of NaF IC_{50}. NRK-52E cells were treated with various NaF concentrations and lycopene in a 24-hour incubation (Figure 1).

Cell Viability Assay (MTT)

MTT (3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyl-tetrazolium bromide) cell viability tests were performed to determine IC_{50} values of compounds. The proliferative doses of lycopene and lycopene were determined. For this purpose, NRK-52E cells were seeded at 2×10^6 cells/well in 96-well plates and incubated overnight at 37°C. After exposure the described doses of compounds for 24 h, medium of wells was discarded and MTT (0.5mg/ml in sterile PBS) solution (10% of completed medium) was added to each well and incubated for 3 h at 37 °C. At the end of the incubation time, MTT medium was discarded and added to lysis solution (1% Triton-X, 10% 0.1mol/l HCl, 89% Isopropanol) to each well for solubilization of the formazan crystals. The absorbance of each well was measured at 570 nm by using a microplate reader. Inhibition and increasing of growth in cells were analyzed Graphad Prism 8 software (San Diego CA). Each experiment in MTT assay was repeated at least four times.

Obtaining RNA

The cells of the experimental groups were collected after 24 hours. RNAs of these cells were isolated by using TRIzol® Reagent (Chomczynski and Mackey, 1995).

cDNA synthesis and real-time PCR analysis (RT-qPCR)

cDNA was obtained using the isolated mRNA and a commercial cDNA synthesis kit (WizScript, Cat. No: w2211). SYBR green master mix (WizPure, Cat. No: w1711) was used in the study. Ct (cycle threshold) was determined at the start of the logarithmic amplification phase. The differences between the Ct values of the control group and the replicates were used for determining the appropriate expression. The reaction content is presented in Table 1

Table 1. Reaction content for real-time-PCR

Reaction content	For a example
Master mix (2X)	10 μl
Primer (F/R)	F: 1 μl, / R: 1 μl
dH_2O	7 μl
cDNA	1 μl
Total	20 μl

Amplification protocol was applied as preliminary denaturation at 95°C for 5 minutes, and denaturation at 95°C for 15 seconds, annealing at 60°C for 60 seconds, in total of 40 cycles, Melting Curve Ramp: 50-99 (1 degree increase)

The difference between the groups was normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the control. Gene products were determined by using ΔΔCt and $2^{-\Delta\Delta Ct}$ values (Livak and Schmittgen, 2001). The expression levels of the target genes were compared with the fold change number and evaluated statistically.

RESULTS

It was revealed that the cell viability of the cells, which were applied for 24 hours in different concentrations of NaF, decreased gradually. It was found that the application of lycopene in association with NaF has significantly reduced the cytotoxication (Figure 1).

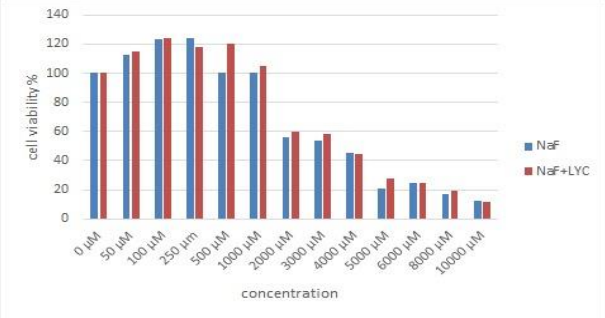

Figure 1. The MTT results

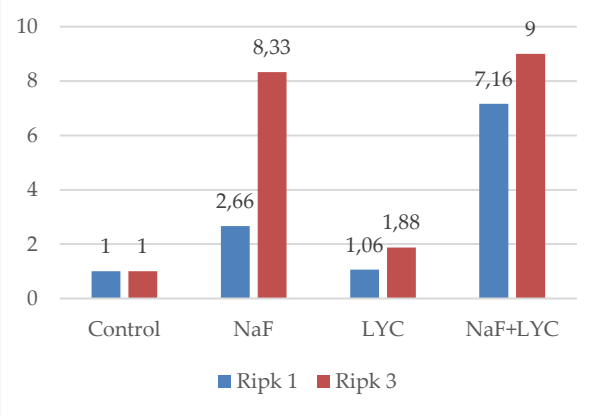

Figure 2. Expression states of necrotic genes relative to the control group

In the NaF group alone, the Ripk1 gene increased 2.5 times more than at the 24th hour, while the Ripk3 gene increased more than 8 times. In the lycopene-treated group, while Ripk1 increased, increase gene was insignificant, Ripk3 gene increased 1.8-fold. In the NaF+lycopene group, the Ripk1 gene increased more than 7 times, while the Ripk 3 gene increased 9 times (Figure 2).

DISCUSSION

High levels of fluoride exposure, causes damage starting from cell, to tissue and organ damage. Many studies, both cellular and experimental, has been made to investigate the damage especially on molecular basis. Parameters seen in apoptotic, autophagic and necrotic pathways have significant role on fluoride dependent cell deaths (Yüksek et al, 2017; Kuang et al, 2018; Tu et al., 2018).

Fluoride reveals various cellular effects depending on time, mixture and cell type. The main toxic effect of fluoride occurs in cells that interact with its enzymes. In most cases, fluoride acts as an enzyme inhibitor, but fluoride ions can occasionally stimulate enzyme activity. Mechanisms depend on the type of enzyme affected (Adamek et al., 2005).

Fluoride at micromole levels is considered to be an effective structural agent because it increases cell reproduction and with the millimolar mixtures stops enzymes such as both living and inanimate phosphates (Mendoza-Schulz et al., 2009). Metabolic, functional and structural damage has been reported due to chronic fluoride poisoning in many tissues. Research data strongly suggests that fluoride inhibits protein synthesis and / or secretion, and effects many pathways such as cell reproduction and apoptosis, mitogen activated protein kinase (MAPK), p53, activator protein-1 (AP-1) and nuclear factor kappa B (NF-B) (Zhang et al., 2007; Zhang et al., 2008; Karube et al., 2009).

Intensive studies are underway to clarify fluoride related toxicity mechanisms. DNA damage due to oxidative stress and activation of apoptotic and necrotic pathways have an important place among these mechanisms (Cao et al., 2015; Yüksek et al., 2017; Tan et al, 2018; Cetin et al., 2019).

Xiong et al. (2007) reports in their study that fluoride levels higher than 2.0 mg/l in drinking water may cause liver and renal damage and tooth fluorosis is independent of liver damage but not independent of renal damage.

Ripk1 and Ripk3 (Receptor interacting protein kinase) are activated as a result of cellular stress or by activation of TNF and Fas receptors. Ripk1 and Ripk3 either directly activate the mitochondria or indirectly affect NADPH oxidase-induced oxygen species (ROS) and induce necrosis (Hengartner et al., 1992). The effects of excess fluoride on the health

of many organisms have been investigated extensively and free radicals have been shown as the mechanism causing fluorosis (Wang et al., 1997). There are literatures that fluoride increases the formation of reactive oxygen species (ROS) and free radicals *in vivo* and *in vitro*, causes excessive oxidative stress and lipid peroxidation, and reduces antioxidant enzyme activities (Lu et al., 2010; Varol et al., 2013). Recently, reactive oxygen species (ROS) induced by excess fluoride have been shown to play an important role in DNA damage (Rzeusk et al., 1998). Fluoride can also cause endoplasmic reticulum (ER) stress and suppress protein synthesis and secretion (Kubota et al., 2005).

According to our findings this situation depending on the time of application of the lycopene, it can be said that it caused the continuation of the already activated path with NaF and that the expected inhibition phase has not yet started.

Fluoride has high penetration ability and can easily pass through the cell membrane. It can enter deeper soft tissues, such as the liver, brain, and kidney, and therefore nephrotoxicity may occur due to the retention and accumulation of inorganic fluoride in the renal tubules (Quadri et al., 2016).

LYC shows a strong antioxidant property *in vitro*, while it is protective against oxidation of DNA, protein and lipids *in vivo* environments (Matos et al., 2011; Karahan et al. 2018). Li et al. (2017) reported that lycopene significantly affected NaF-induced ameloblast and dental fluorosis by reducing oxidative stress and caspase pathway. They also demonstrated that lycopene administration in rats given Sodium Fluoride (NaF) can minimize the toxic effects of fluoride indicating free radical and strong antioxidant activities (Mansour and Tawfik, 2012).

CONCLUSION

As a result, in this study; it was understood that administration of NaF at cytotoxic concentrations accelerated cell deaths by making necrotic genes more active in nephrons. It was understood that the administration of lycopene alone did not affect the necrotic pathway. However, when lycopene was administered together with NaF, considering the dose used in this study and the time of sample collection, the positive effect detected on cell viability was not found positive on the necrotic pathway. In order to confirm this situation, it was concluded that new studies should be planned to apply lycopene at different hours and to follow the necrotic pathway in the samples to be taken at 36. 48. and 72 hours after the application.

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Occurrence and distribution patterns of the diseases of goat in Dhaka, Bangladesh

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ABSTRACT

Objective: The study was conducted to determine the occurrence and distribution patterns of diseases of goat in Dhaka, Bangladesh during the period of January 2018 to December 2018.

Materials and Methods: A total of 452 goats were recorded during the study period. The diseases were diagnosed based on clinical signs, patient owners complain and laboratory findings. The prevalence was measured according to season and age of the goats which were studied. Statistical analysis was done by Chi-square test and P-value was calculated by using SPSS 25 for windows (SPSS, Inc., Chicago, IL).

Results: In this study, 381 (84.29%) goats were found to be diseased from 452 visited goats at Central veterinary Hospital (CVH), Dhaka. The highest prevalence was found with worm infestation (31.42%) followed by PPR (13.72%), miscellaneous (ruminal acidosis, disuria, repeat breeding) (12.16%), pneumonia (10.18%), dermatitis (4.20%), enteritis (3.76%), urolithiasis (3.09%), bloat (2.65%), mastitis (1.55%), tetanus (1.11%) and protozoal diseases (0.44%). Rainy season represented the highest (86.11%) prevalence of goat diseases followed by winter season (82.85%) and summer season (82.44%). PPR was significantly ($p<0.05$) more prevalent in winter season than other two seasons. In this study, Goats had significantly higher ($p<0.01$) prevalence of worm infestation in the summer season (29.00%) and the rainy season (39.35%) and in all three (0-12 months, 13-24 months and >24 months) age groups with 21.64%, 45.06%, and 32.20% respectively. Prevalence of mastitis was lowest in 0-12 months (0.43%) and 13-24 months (0.62%) age group but the prevalence of tetanus was lowest in above 24 months (1.69%) age group.

Conclusions: These findings will help to know about age-wise and season wise variation of different diseases of goat in this area and will help to execute proper preventive measures against those diseases.

Keywords: *Occurrence, Distribution, Diseases, Goat, Bangladesh*

INTRODUCTION

Goat is one of the most important livestock species in Bangladesh because of their short generation intervals, higher rates of prolificacy and the ease with which the goats and their products can be marketed. Goat is called "the cow of poor people" because people like low-income farmers, landless labors and distress women who can't afford to rear cattle, rearing goats can be very useful to them. Goat is easily reared, prolific in climate especially in arid zones (Banerjee, 2004). As subordinate occupation, farmers rear goats to supplement their livelihood. Goat farming can play vital role in improving farmers living status by increasing their income and can contributes substantially in the national economy. It provides the principal dietary animal protein in the form of meat and milk to promote national health. According to the food and Agricultural Organization (FAO), meat and skin of

goats contribute 38% and 28% of whole livestock meat and skin production in Bangladesh (Sarker and Islam, 2011).

In Bangladesh, at present, the approximate number of goats is 26.10 million (DLS, 2018). More than 90% of the total goat population comprises of Black Bengal Goat, which is reputed for their prolificacy, fertility, early sexual maturity, adaptability to hot humid conditions and superior quality meat and skin (Hussain, 1993; Amin et al., 2001) and the remaining ones include the Jamunapari and their crosses. Most of the farmers (80.5%) reared goats in the semi-intensive system but few farmers (7.3%) used confinement systems of rearing while 12.2% farmers used the free-range system (Islam et al., 2009). In Central Veterinary Hospital, Dhaka, most of the goats come are Jamunapari which are reared by the people of old Dhaka in their rooftop on a small scale.

However, various infectious and noninfectious diseases are frequently occurring in goats which considered as a great threat to these animals' survival in Bangladesh. Because of poor management practices and geo-climatic condition, the occurrences of various diseases become conducive in Bangladesh. Productivity and the economy of goat farming are greatly influenced by the occurrence of diseases. The study was conducted to determine the occurrence and distribution patterns of diseases of goat in Dhaka, Bangladesh.

MATERIALS and METHODS

Study Area and Period

This study was performed at Central Veterinary Hospital (CVH), Dhaka, Bangladesh during the one-year study period from January 2018 to December 2018. The necessary information and data were collected from registered record book of the veterinary hospital where all diseased goats were brought for treatment.

Sample Size

During this study period, a total of 452 goats were recorded to visit this hospital. Among those the number of animals in winter (November-February), summer (March-June) and rainy (July-October) season were 102,131 and 219 respectively. The prevalence was measured according to season and age of the goats which were studied.

Clinical Examination and Diagnosis

After visual examination of the patient different parts and system of the body of each of the sick animals were examined by palpation, percussion, auscultation, needle puncture and gait and posture of the animals. The general clinical examination was conducted according to the merit of the individual case, on the basis of disease history and owner's complaint, symptoms and techniques such as microscopic examination and laboratory common techniques that are used (Rosenberger, 1979). Therefore, the temperature, pulse, and respiratory rate from each of these sick animals were recorded. For viral disease diagnosis, close inspection were performed properly in order to observe the presenting signs such as a sharp rise of temperature of 104°F–106°F, occulonasal discharge, diarrhea and respiratory distress. Per rectal temperature was recorded with the thermometer in every case. Respiratory distress was identified with the help of stethoscope and the lung and tracheal sound were observed and recorded. For parasitic diseases, presumptive diagnoses of some parasitic diseases were done based on the history, clinical sign and symptoms and faeces examination (Blood and Radostits, 1989). Gross examination of faeces was made for the detection of living or dead worms or for the detection of the segments of tapeworms. The animal body was examined for the presence of any visible ectoparasites. Ectoparasites were identified according to the keys and descriptions given by Wall & Shearer (1997).

Determination of prevalence

Prevalence is a statistical concept referring to the number of cases of a disease that are present in a particular population at a given time which is determined by following equation,

$$\text{Prevalence} = \frac{\text{Number of existing cases on a specific time}}{\text{Number of total animal on a specific time}} \times 100$$

Statistical Analysis

All the collected data were transferred into Microsoft Excel sheet for descriptive statistics. Chi-square tests were used to determine the differences of prevalence in years, age of the animals and seasons of the year. P values of < 0.05 were considered significant by using SPSS 25.0 for windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Overall prevalence of different diseases of goat

In this study, 381 (84.29%) goats were found to be diseased from 452 visited goats at Central veterinary Hospital Dhaka. The highest prevalence of disease was shown by worm infestation (31.42%) and the lowest was by protozoal diseases (0.44%). Prevalence of other diseases like PPR, pneumonia, dermatitis, enteritis, urolithiasis and miscellaneous (ruminal acidosis, disuria, repeat breeding) were 13.72%, 10.18%, 4.20%, 3.76 % 3.09% and 12.16% respectively. Lower prevalence was observed in case of Tetanus (1.11%), Mastitis (1.58%) and Bloat (2.65%) (Table 1).

Table 1. Overall prevalence of different diseases of goat in Dhaka

Diseases		No. of positive case [b]	Prevalence (95% CI)
Viral	PPR	62	13.72 (10.54-17.09)
Bacterial	Pneumonia	46	10.18 (7.39-13.06)
	Tetanus	5	01.11 (0.14-2.07)
	Mastitis	7	01.55 (0.41-3.08)
Parasitic	Worm infestation	142	31.42 (27.14-36.09)
	Protozoal diseases	2	00.44 (-0.17-1.05)
Non-specific	Enteritis	17	03.76 (2.01-5.51)
	Dermatitis	19	04.20 (2.35-6.05)
	Bloat	12	02.65 (1.17-4.13)
	Urolithiasis	14	03.09 (1.50-4.70)
	Miscellaneous [a]	55	12.16 (9.15-15.20)
Overall		**381**	**84.29 (81.03-88.04)**

[a]: Ruminal acidosis, disuria, repeat breeding; [b]:No. of examined animal =452;

Table 2. Season wise prevalence of goat diseases at Dhaka

	Diseases	Summer (NAE=131)		Rainy (NAE=216)		Winter (NAE=105)		X^2	P
		NPC	Prevalence (%)	NPC	Prevalence (%)	NPC	Prevalence (%)		
Viral	PPR	11	8.40	30	13.88	21	20.00	6.47	0.039*
Bacterial	Pneumonia	13	9.92	16	7.40	17	16.19	5.98	0.050
	Tetanus	-	-	2	0.93	3	2.85	4.47	0.107
	Mastitis	-	-	7	3.24	-	-	7.77	0.021*
Parasitic	Worm infestation	38	29.00	85	39.35	19	18.10	15.31	0.000**
	Protozoal diseases	-	-	2	0.93	-	-	2.19	0.334
Non-specific	Enteritis	3	2.29	7	3.24	7	6.66	3.39	0.183
	Dermatitis	16	12.21	3	1.39	-	-	29.73	0.000**
	Bloat	3	2.29	5	2.31	4	3.80	0.71	0.70
	Urolithiasis	5	3.82	5	2.31	4	3.80	0.844	0.656
	Miscellaneous[a]	19	14.50	24	11.11	12	11.43	0.948	0.622
	Overall	108	82.44	186	86.11	87	82.85		

[a]: Ruminal acidosis, disuria, repeat breeding; NAE=No. of animal examined; NPC = No of positive case; *, Statistically significant (P<0.05), **, Statistically highly significant (P<0.01)

Table 3. Age- wise prevalence of goat diseases at Dhaka

	Diseases	0-12M (NAE=231)		13-24M (NAE=162)		>24M (NAE=59)		X^2	P
		NPC	Prevalence (%)	NPC	Prevalence (%)	NPC	Prevalence (%)		
Viral	PPR	43	18.61	14	8.64	5	8.47	9.58	0.008**
Bacterial	Pneumonia	29	12.55	13	8.02	4	6.78	2.99	0.224
	Tetanus	4	1.73	-	-	1	1.69	2.82	0.244
	Mastitis	1	0.43	1	0.62	5	8.47	21.37	0.000**
Parasitic	Worm infestation	50	21.64	73	45.06	19	32.20	24.25	0.000**
	Protozoal diseases	2	0.87	-	-	-	-	1.96	0.37
Non-specific	Enteritis	10	4.32	7	4.32	-	-	2.65	0.266
	Dermatitis	2	0.87	15	9.25	2	3.39	16.77	0.000**
	Bloat	5	2.16	3	1.85	4	6.78	4.50	0.105
	Urolithiasis	9	3.89	3	1.85	2	3.39	1.34	0.510
	Miscellaneous[a]	28	12.00	16	9.88	11	18.64	3.11	0.211
	Overall	183	79.22	145	89.50	53	89.83		

[a]: Ruminal acidosis, disuria, repeat breeding; NAE=No. of animal examined; NPC = No of positive case; *, Statistically significant (P<0.05), **, Statistically highly significant (P<0.01)

Season wise prevalence of goat diseases

Season wise prevalence of goat diseases were shown in Table 2 in which highest prevalence was in the rainy season (86.11%) and lowest was in the summer season (82.44%). According to table 2, prevalence of worm infestation was found to be significantly higher (X^2 = 15.31, p = 0.000) in rainy (39.35%) and summer (29.00%) seasons while in winter season prevalence of PPR (20.00%) was found to be significantly higher (X^2 = 6.47, p = 0.039). The lowest prevalence was found in case of tetanus and protozoal diseases (0.93%) in the rainy season whereas Enteritis (2.29%) was found lowest in summer and Tetanus (2.35%) was also found lowest in the winter season.

Age- wise prevalence of goat diseases

Age-wise prevalence of goat diseases at Dhaka was shown in Table 3. According to age, prevalence of diseases was found to be highest (89.83%) in above (>) 24 months aged group and lowest was in 6-12 months aged group. In all aged groups prevalence of worm infestation was found significantly higher (X^2=24.25, p=000). Prevalence of mastitis was found significantly lower (X^2=21.37, p=000) in 0-12 months (0.43%) and 13-24 months (0.62%) aged group whereas the prevalence of tetanus was found to be lowest in above (>) 24 months aged old.

DISCUSSION

The goat suffers with various diseases, which are caused by viruses, bacteria, parasites and other non-infectious agents (Taylor, 1984). Among the viral diseases of goat, the present study showed 13.72% prevalence of PPR in goat which is concurrent with other investigations (Poddar et al., 2018) who recorded 13.74% prevalence of PPR at Upazila Veterinary Hospital, Pirojpur, Bangladesh. But in North east India, the prevalence of PPR was detected as 45.2%. This variation may be due to different geographical location and management system (Balamurugan et al., 2014). Our study also showed that the prevalence of PPR was highest in winter (20.00%) and lowest in summer (8.40%) season. In 0-12 month's age group, the prevalence was found highest (18.61%) followed by 13-24 months (8.64%) and above 24 months (8.47%) age group. This study revealed that young goats found to be more susceptible to PPR than the adult. Other studies also showed similar results (Nath et al., 2014; Sarkar and Islam, 2011). The increased susceptibility to young animals might be due to malnutrition, poor immunity and poor management systems.

Among the bacterial diseases, respiratory infections, or pneumonia, are a common and serious disease in goats. In our study, among the

bacterial diseases, pneumonia was recorded to prevalent in 10.18% goats whereas it was observed 17.11% in Ethiopia (Mekibib et al., 2019). The highest prevalence of pneumonia was recorded during winter season (16.19%) followed by 9.92% in summer and 7.40% in rainy season. Increased prevalence in winter might be due to the presence of huge dust in the air. Among study animals, 0-12months (12.55%) age group were more affected by pneumonia than 13-24 months (8.02%) and above 24 months (6.74%) age group. Almost similar result was reported by Sardar et al. (2006) but they found the lowest prevalence of pneumonia in summer season (1.02%). Findings of other studies showed slightly lower prevalence (9.6% and 8.28%) of pneumonia in goats in Magura and Sylhet respectively (Karim et al., 2014; Lucky et al., 2016). This study recorded 1.11% prevalence of Tetanus during the study period. The prevalence was varied according to the season where it was 2.85% in winter and 0.93% in the rainy season but no cases were detected in summer season. Age related prevalence was recorded 1.73% in the 0-12 months age group and 1.69% in the above 24 months age group. Interestingly no cases were found in the 13-24 months age group. Previous studies showed slightly higher prevalence of Tetanus with 4.13% and 3.05% and they also recorded the highest prevalence of tetanus in the summer season (Lucky et al., 2016; Dey et al., 2018). The variation might be due to the geographical location and management system of goats.

Mastitis refers to an inflammation of the mammary glands due to a bacterial infection. Udder damage, often caused by mastitis, is one of the leading causes of culling in goat operations (Scharko, 2008). Findings of the present study showed 1.55% prevalence of Mastitis in goats. These findings support the report of other studies who reported 1.6% mastitis in goat (Karim et al., 2014). But in Eastern Algeria the prevalence of mastitis in goat was 3.55% which was higher than the present study (Gabli et al., 2019). It was found only in the rainy season (3.24%) during our study period. The prevalence of mastitis was found to be increased with age as the study recorded 8.47% cases in the (above 24 months) age group and 0.62% in (13-24 months) and 0.43% in (0-12 months) age group. In rainy season goats were more susceptible to various diseases (Table 2). It might be due to wet environment and poor management during the rainy season. This finding agreed with the findings of other studies (Nath et al., 2014; Sarkar and Islam, 2011).

Parasites pose a significant threat to the health of small ruminants. Parasites can damage the gastrointestinal tract, and result in reduced reproductive performance, reduced growth rates; less productive animals in terms of meat, fiber and milk; and even death (Kate et al., 2006). The present study showed that the prevalence of worm infestation was recorded to be highest with 31.42% which was seasonally distributed like summer (29.00%), rainy (39.35%) and winter (18.10%). Relatively higher age group (13-24) months (45.06%) were found to be more susceptible to worm infestation than 0-12 months (21.64%) and above 24 months (32.20%) age group. The present study showed 0.44% prevalence in case of protozoal diseases and interestingly it was only recorded in rainy season (0.93%) and 0-12 months (0.87%) age group. But previous study recorded 2.01% protozoal diseases in goat at Dinajpur Sadar (Mahfuza Akhtar, 2017). Results are incongruous might be due to geographical location and management practices.

Unawareness about the importance of deworming might be the underlying cause to high worm infestation. Bloat is mainly a dietary in origin and occurs most frequently in ruminants in Bangladesh (Sutradhar et al., 2000). The present study recorded 2.65% prevalence of Bloat which supports the findings of earlier studies (Rahman et al., 2012; Karim et al., 2014). The highest prevalence was observed in winter season (3.80%) followed by rainy (2.31%) and summer (2.29%) season. The prevalence of bloat was 6.78% in the adult (above 24 months) age group followed by 2.16% in (0-12 months) and 1.85% in (13-24 months) age group. Enteritis was found to be prevalent in 3.76% goats. Prevalence was highest in the winter season (6.66%) followed by rainy (3.24%) and summer season (2.29%). Dermatitis was recorded in 4.20% goats. Dermatitis was significantly (X^2=29.73, p=0.000) 12.21% and 1.39% prevalent in summer and rainy season respectively. But no case of dermatitis was found in the winter season. 13-24 months age group had significantly (X^2=16.77, p=0.000) higher prevalence of dermatitis (9.25%) than 0-12 months (0.87%) and above 24 months (3.39%) age group. The overall prevalence of urolithiasis in goats was 3.09%. Seasonal variation was observed in the prevalence of urolithiasis of goat where it was estimated 3.82% in summer season followed by rainy (3.80%) and winter season (2.31%). Earlier

stage of age group (0-12 months) was found to be more (3.89%) susceptible than 13-24 months (1.85%) and above 24 months (3.39%) age group. Alternatively, other study reported higher prevalence of urolithiasis in goat (44.4%) in Magura, Bangladesh (Karim et al., 2014). In this study, along with these non-specific diseases some disorders like ruminal acidosis, disuria, repeat breeding are termed miscellaneous was also observed which affects the goats.

CONCLUSION

From this study, it was observed that goats were most susceptible to worm infestation. Among the diseases found in this area, rainy season showed the highest prevalence of the diseases. Diseases prevalence also varies with the age of the animals. These findings will help to know about age-wise and season wise variation of different diseases of goat in this area and will help to execute proper preventive measures against those diseases.

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Investigation of Brucellosis Information and Applications of Animal Breeders: The Case of Erdemli

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ABSTRACT

Objective: Lack of information about brucellosis can affect patient's health-seeking behaviors and thus cause constant infectation in semi-urban communities. This study aimed to determine the knowledge level of brucellosis of dairy cattle breeders and evaluate the information about brucellosis in 83 people living in 21 different villages and neighborhoods of Erdemli district.

Materials and Methods: In the study, in this cross-sectional study, face to face interviews and data were collected using a 30 question questionnaire to investigate the level of knowledge about individuals about brucellosis. Data were evulated by using SPSS 21.0 statistic program.

Results: While 72.3% the individuals do not know that brucellosis causes disease in humans, 56.6% do not know that it causes disease in animals. Participants said that 56.6% had heard of the about brucellosis before and those who heard said that they had heard from 13.3% of their relatives or neighbors. A majority of the participants (56.6 %) had heard about Brucella, 72.3% of individuals do not know that brucellosis causes disease in humans and 56.6% of the participants do not know that Brucella is an animal disease. It was determined that 65 (73.8%) of the participants did not make cheese from raw milk, 66 (79.5%) did not consume cheese fresh, and 74 (89.2%) did not make butter from raw milk cream. The individuals who participated of in the study 32.5% had bovine animals and 67.5% had small ruminants; the rate of aborted animals is 30.1% in the last year. The total proportion 20.5% was of stillbirths, the rate 51.8% was infertile animals.

Conclusion: In the present study infertility, stillbirth and abortion numbers of individuals who had not heard of brucellosis before were higher than those who had heard and who took the necessary precautions. It is a fact that the herd can threaten all other animals in rapidly spreading infectious diseases such as brucellosis. Therefore, providing the necessary incentives for the establishment of modern business facilities in areas where animal husbandry is intensive, if this is not possible, raising the level of knowledge by providing various trainings to individuals dealing with dairy cattle breeding will contribute to the national economy.

Keywords: *Animal breeders, Brucellosis, Education*

INTRODUCTION

Brucella continues to pose problems in many regions of the world, especially in developing countries, and has been one of the most common zoonotic diseases in the past 15 years (Franco et al., 2007; Nicoletti, 2002). Throughout the world, especially in the Mediterranean region (Portugal, Spain, southern France, Italy, Greece, Turkey, North Africa), the Middle East, Eastern Europe, are highlighted as high-risk zones. It is estimated that

there are 500,000 new cases of brucella annually all over the world (De Bolle et al., 2015). Brucellosis is a common zoonotic disease in humans and subacute or chronic diseases caused by Brucella bacteria that can be transmitted to humans by meat, milk, urine, body fluids and infected animal's pregnancy material (Gotuzzo et al., 1992; Baysal and Ustaçelebi, 1999; Gurturk et al 1999; Bilgehan, 2000; Ilhan et al. 2008). Brucella species are Gram negative, facultative, intracellular coccobacilli, non-spore-forming, non-capsule and non-motile bacteria. In the Brucellaceae family, there are six important species that cause infections and are not specific to the host but can easily breed and infect humans under appropriate conditions (Yuce and Cavus, 2006).

Brucellosis is a disease characterized by nonspecific symptoms and may affect all organs and systems that may present with hematologic, gastrointestinal, skin, genitourinary, cardiovascular, respiratory, osteoarticular and neurological disorders. Due to their intracellular nature, Brucella bacteria are able to survive and proliferate and become chronic in mononuclear phagocytic cells (Dhauk and Nöckler, 2011). Molecular methods have been used in the routine diagnosis of brucellosis in recent years. Various serological and biochemical tests are currently used in practice. However, the exact result takes a long time, so in recent years molecular methods are more preferred for diagnosis, biotyping and species separation of Brucella (Bricker et al., 2003).

Brucellosis is seen in high rates in countries with border neighbors. At the same time, as the majority of the population of this country provides livelihood with livestock, significant economic losses are experienced in the national economies (İzgür, 2007). Brucellosis has become the most important focal point in the world. It seems that this disease is difficult to control and is constantly increasing, becoming a serious public health problem. Brucellosis settles in various tissues and organs of animals, causes offspring and infertility (Young et al., 2000; Bosilkovski et al., 2009).

In this context, our study was carried out in order to determine the knowledge level of brucellosis of the animal breeders living in the district of Erdemli and to determine their educational needs. The aim of this study was to determine the level of knowledge of brucellosis among people living in various villages and neighborhoods in the district of Erdemli. As the people of the district are of Yoruk origin, the tradition of going up to the highlands and grazing in the pasture still continues. It is known that brucellosis is more common in settlements where animal breeding and milk processing techniques performed by traditional methods. In our study, abortus, stillbirth and infertility levels were high in the animals of animal breeders who had not heard of brucellosis before. This situation causes animal breeders to suffer economic losses and endangers the health of consumers. For this reason, it is important to determine the information levels of brucellosis and to provide trainings on this subject in the regions where animal husbandry is widespread.

MATERIALS and METHODS

This is a cross-sectional epidemiological study to determine the knowledge level of animal breeders about brucellosis, a zoonotic disease. In this study, 83 different animal producers living in 21 different settlements in Erdemli district were interviewed. The study was conducted with all animal breeders involved in dairy cattle without discrimination. All of the participants were informed about the method and purpose of the study and they were carried out on a voluntary basis. Before starting the study, ten individuals were pre-tested and the questionnaires were prepared by completing thirty questions.

The research, ethical approval and necessary permission was obtained from Mersin University Non-Clinical Research Ethics Committee and related institutions for conducting the research. After giving information about the purpose and method of the research, written consents were obtained from the participants. The questionnaire form was presented to the people who agreed to participate in the research by face to face interview method.

With the help of SPSS 21.0 statistics program, percentage and frequency values in categorical data obtained from the questionnaire and average values in continuous variables were calculated and the results are presented in tables and figures.

RESULTS

As a result of the study, 83 animal breeders living in 21 different settlements were interviewed. The average age of animal breeders is 50.98. In addition, 10 (12%) are women and 73 (88%) are men. The 80 (96.4%) participants knew how to read and write, while 3 (3.6%) did not know to read and write. The total number of sheep and goats is 6133 (67.5%) and the number of cattle is 512 (32.5%). Of the

individuals dealing with dairy cattle, 47 (56.6%) have not heard of this disease before, the rate of those who have heard of the disease from relatives and neighbors is 13.3%. While all the breeders who heard of brucellosis disease knew that brucellosis caused disease in the animal; the number of people who knew that they had illness was found to be 23 (27.7%). It was determined that the number of people who informed an authorized person "when it was aborted?" was 39 (47%) and the number of those who did not confuse the herd with other flocks was 66 (77.1%). When the level of knowing brucellosis was investigated by looking at the number of animals, no statistically significant difference was found in Table 1 ($p>0.05$).

Table 1. Evaluation of Brucellosis knowledge levels according to the number of animals

Number of Animals Raised	Breeders knowledge of Brucella disease				Total
	Yes		No		
	n	%	n	%	
1-20 animals	14	38.9	22	61.1	36
21-50 animals	6	46.2	7	53.8	13
51 and above animals	16	47.1	18	52.9	34
Total	36	43.4	47	56.6	83

Table 2. Abortion incidence rates in animals by number of animals

Number of Animals Raised	Abortion in animals				Total
	Yes		No		
	n	%	n	%	
1-20 animals	3	8.3	33	91.7	36
21-50 animals	4	30.8	9	69.2	13
51 and above animals	18	52.9	16	47.1	34
Total	25	30.1	58	69.9	83

Table 3. Investigation of infertility levels by number of animals

Number of Animals Raised	Infertility cases in animals				Total
	Yes		No		
	n	%	n	%	
1-20 animals	9	25	27	75	36
21-50 animals	9	69.2	4	30.8	13
51 and above animals	22	64.7	12	35.3	34
Total	40	48.2	43	51.8	83

Of the 25 (30.1%) animal breeders, 196 (30.1%) animals had abortion in the past year and it was found that 71 (17%) animals of 17 (20.5%) animal owners still gave birth. When the abortion rate of animal breeders according to the number of animals was investigated, a statistically significant difference was found in Table 2 ($p<0,005$).

The participants of only 20 (24.1%) had previously received information about brucellosis. In addition the animal producers of 64 (77.1%) performed barn disinfection after birth; used gloves 40 (48.2%) for the birth of each animal, 79 (95.2%) wash their hands before milking animals and 59 (71.1) before milking the animals the animal has been found to wash its udder. Based on these data, infertility 237 (49.4%) of 40 (48.2%) was detected in animal owners. When the infertility numbers of animal breeders are analyzed, a statistically significant difference was found between them a statistically

significant difference was found between Table 3 ($p<0.05$).

The participants of 65 (73.8%) did not make cheese from raw milk; 66 (79.5%) did not consume cheese freshly. It was determined that 74 (89.2%) did not make butter from raw milk cream. In parallel, 77 (92.8%) of the participants have not had brucellosis disease and treatment before. The number of those who immediately added the new animal to the herd was found to be 44 (53%), while the number of those who immediately used the milk of the low or stillbirth was 9 (10.8%) and the number of those who immediately brought the low or stillbirth to the herd was 54 (65.1%). The number of people who think that the vaccine protects brucellosis is 68 (89.1%).

DISCUSSION

Brucella disease which is an important public health problem worldwide is one of the important zoonotic diseases neglected by the World Health Organization (OIE, 2009). Brucellosis is an important health problem in regions where animal husbandry, widespread in our country and its morbidity is quite high. Brucellosis is characterized by offspring, infertility, stillbirth and various clinical symptoms in animals such as sheep, cattle and pigs. In addition to causing serious infections in humans, it is an infectious disease that causes economic losses (Badur, 1970; Bilgehan, 2000).

In our study, the participants of 43.4% stated before that they had heard of brucellosis. In a study done in animal breeders in Tajikistan 31% of the participants stated that they heard and know brucellosis (Grahn, 2013). In a study conducted in Uganda this rate was 99.3% (Kansiime et al., 2004), in a study conducted in Italy this rate 74.6% (Angelillo, 2001) and in a study conducted in Kars province this rate 66% (Akkuş et al., 2011). The participants of 86.7% stated that the source of information about the disease is professional. Information about brucella disease appears to be obtained from professional individuals, but compared to other studies, the rate of hearing brucella disease is low. It is very important for the eradication of the disease that professional community organizations, health personnel and academic institutions provide information and counseling services to the society about brucellosis.

Considering the level of knowledge about the ways of transmission of the disease the number of animal breeders who have heard of brucellosis disease 43.4%, while all of these animal breeders know that brucella is a disease only in animals; it causes disease in humans do not know of 72.3%. In the study of it was found that 80% of the participants could count joint pain and fever as symptoms and 4.5% of them knew at least one symptom (Lindahl, 2015; Babaoğlu and Demir, 2017). The animal breeders of 77.1% had disinfected the barn after birth; 49.4% had infertile animals; 30.1% of the offspring in the last year. It was seen that 20.5% had stillbirth. In other studies, 34% offspring, 31% stillbirth, 66.7% vaccinate animals but 15.7% do not know how to protect animals from brucellosis, 30.7% do not properly dispose of their abort determined (Babaoğlu and Demir, 2017). In our study, the participants of 81.9% did not believe in the protection of the vaccine.

The participants of 7.2% were diagnosed with brucellosis and treated. This rate 59.8% in Uganda (Kansime et al., 2004), 5.2% in a study in Kars (Akkuş et al., 2011) and 40.9% in a study in Van in the past was diagnosed and treated with brucellosis (Kuşaslan et al., 2017). It is a fact that developed countries are known as a chronic disease of developing and underdeveloped countries. In the study area, it was determined that most people do not know how to protect their animals from brucellosis. In our study, 21.7% of the participants made cheese from raw milk; 20.5% of the fresh consumed cheese; It was determined that 10.8% made from butter raw milk cream. It was found that the risk for consumption of raw dairy products of 84.5% was much higher a study conducted in Kars (Akkuş et al., 2011). In a similar study conducted in Van province, it was determined that raw milk and dairy products of 13.6% were consumed fresh by the participants (Kuşaslan et al., 2017). One of the ways of transmission of Brucellosis is the body secretions of the infected animal. It is known that individuals with impaired skin integrity in contact with these secretions are also at risk (Kılıç et at., 1994). When the individuals participating in our study were examined; risky behaviors such as milking by hand, not cleaning the breast of the animal before milking and not using gloves at birth are common. However, 51.8% of the participants used prenatal or postnatal gloves, 95.2% were washing their hands before and after milking and 71.1% were washing their breasts before milking the animals. In a study, it was found that 35.6% of those assisting birth did not use gloves (Kuşaslan et al., 2017). In the study conducted in Kars, it was stated that 44% of the participants did not use gloves

(Kansime et al., 2004). The rate of animal breeders who applied to the veterinary surgeon after stillbirth or 92.8% abortion; abortion and stillbirth in 10.8% of the animal's immediate use of milk, while 98.8% of the participants take care of the herd. While most of the developed countries have eradicated the disease, in developing and underdeveloped countries, the disease causes significant health problems and economic losses. In our study, the high level of veterinary cooperation of animal breeders is pleasing while it is known that the cattle that produce bacteria with their milk for weeks and even months after abortions, stillbirths and the calves that throw off spring. Due to Brucellosis begin to disappear 30-40 days after the abortion date. As a result of our research it was seen that the animal breeders of 22.9% mixed the herd with other herds while 53% did not keep the herd apart from the other herds. They were buying a new animal and 65.1% immediately added the low and stillbirth animal to the herd (Özcan and Şahin, 2012). According to the data obtained from the study, 39 (47%) people inform a competent person when they are abort. Inadequate reporting of suspected cases of abortion and stillbirth, such as abort, indicates that the fight against brucellosis still does not reach the desired levels. It was determined that animal breeders did not show the necessary care when adding animals to the herd. It is understood that brucella disease causes big losses in livestock economy. Diseases in humans are caused by direct contact with the infected animal and consumption of raw dairy products obtained from this animal (Akdeniz et al., 2000; Seleem and Boyle 2010). The fact that animal breeders who are at high risk for brucellosis does not have sufficient information about the transmission, symptoms and ways of infection increases the importance of the problem. Not only animal breeders, but also animal breeders are at risk for brucellosis. Disinfection of the barn, keeping the abort materials away from the herd and destroying them as necessary is important in preventing the spread of the disease. It is possible to prevent the epidemic by washing the hands of the people who make milking with the disinfectants. The transition between animals changing the titles after each milking with milking machines fighting with the village people in the fight against the disease and showing the necessary attention by separating the suspicious animal from the herd.

CONCLUSION

As a result, brucellosis should be considered primarily in long-term fever and joint pain in individuals working in animal farms. Brucellosis is a rapidly spreading infectious disease. It is a fact that an infected animal that will be brought to one of the village households or enterprises will soon threaten all the village animals. The study looking at the data the individuals only of 24.1% included in the sample stated that they had received information about brucellosis before. However, disease and economic losses can be prevented by simple training on brucellosis. During our study, most of the participants stated that they wanted to learn about brucellosis. As the findings of the fight against disease are specific, their complications are high, they cause loss of labor force and they can affect large masses, especially in endemic regions, relevant professional organizations should cooperate and develop a common national struggle program against epidemics.

In developing countries, efforts are needed to establish an infrastructure, such as training in the risks of brucellosis, to people. Training and informative brochures should be organized for the consumer, especially about the consumption of risky products. Between the public health and veterinary sectors in providing health education and information about the cause, symptoms, transmission and prevention of brucellosis for better management of the disease. The need for cooperation is emphasized. The most important transmission route is the consumption of milk and dairy products without pasteurization. To be protected from brucella is to be careful in the control of milk and dairy products. Following this will be the control of animals with the reservoir of the brucella and the decrease in the incidence of brucella in humans.

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Anatomical and histological structure of cervix uteri, corpus uteri and cornu uteri of the Anatolian wild goat

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ABSTRACT

Objective: This study aimed to determine the anatomical and histological structure of the uterus of the Anatolian wild goat.

Materials and Methods: Measurements were taken from the uterine segments using digital callipers. The animal material consisted of three wild goats of similar ages (2-3 old). The uterus was studied in three sections: Corpus uteri, cornu uteri and cervix uteri. Each section was examined anatomically and histologically.

Results: The mean length of cervix uteri was 33.99±1.22 mm, width of the cervix uteri was 13.39±0.92 mm, thickness of the cervix uteri was 8.42±1.68 mm and weight of the cervix uteri was 5.45±0.80 g. The mean corpus uteri length was 29.61±5.14 mm, corpus uteri width was 21.22±1.98 mm, corpus uteri thickness was 9.05±1.45 mm and corpus uteri weight was 4.30±0.77 g. The mean cornu uteri length was 41.34±8.02 mm, the cornu uteri width was 12.49±2.59 mm, the cornu uteri thickness was 6.73±0.86 mm and the cornu uteri weight was 5.46±0.31 g. Histologically, the uterine tissue was consist of three different regions, namely, cervix uteri, corpus uteri and cornu uteri also this parts were composed of endometrium, myometrium and perimetrium layers were located from the inside to the outside.

Conclusion: The findings of this study related to the Anatolian wild goat, which contributes to the wildlife diversity in Turkey, however, the population of which has been decreasing due to illegal and uncontrolled hunting or traffic accidents in recent years.

Keywords: *Anatomy, Anatolian wild goat, Histology, Uterus*

INTRODUCTION

Goats are mammals that make up the *Capra* genus of the *Bovinae* family. The mountain goat (*Capra*) is a genus that includes nine species. The domestic goat (*Capra aegagrus hircus*) is a domesticated subspecies of wild goats (Albayrak *et al*., 2007). The wild goat, spreading in some countries of the Caucasus and the Middle East in the world, can be found in the Aegean, Mediterranean, Southeastern Anatolia, Eastern Anatolia and Black Sea regions up to 4000-4500 m above sea level (Pellicena, 2014).

The cervix, corpus and cornu uteri are the three parts of the uterus (Budras and Wünsche, 2009; Popesko, 2010). The cervix uteri is a curved, muscular organ with a thin canal in the middle that connects the corpus uteri to the vagina. The anatomy, length and width of the cervix uteri varies among mammals. The mucosa of the cervix canal

(tunica mucosa) has branched folds (plica) extending towards the canal cavity (Alaçam, 2005). These plicas are plica circulares in ruminants. The canalis cervicis uteri is the gateway between the ostium uteri externum and the ostium uteri internum (König and Liebich, 2015). The vaginal protrusion of the ostium uteri externum can be found in many different shapes including, slit, bud (papilla), cover, duckbill, rose (Kershaw *et al*., 2005), spiral, rosette (Naqvi *et al*., 2005), star, bunch, tuber (Dayan *et al*., 2010). The type of the shape vary according to age. While sheep generally have a rose shaped ostium uteri externum, lambs have been reported to have papilla shaped (Kershaw *et al*., 2005). The corpus uteri is the part of the uterus that lies between the origin of the cornu uteri and the cervix uteri. Cornu uteri are cylindrical, curved, arc-shaped tubular formations located in the cavum abdominis, extending forward from the cranial of the corpus uteri (Semacan *et al*., 2012; Bahadır and Yıldız, 2014). In the ruminants, the uterus is composed of three layers, namely, the endometrium, myometrium and perimetrium, starting from the lumen. It has been reported that the endometrium is composed of five different regions: surface epithelium, capillary surface, stratum compactum, stratum spongiosum and stratum basale (Agrawal and Bhattacharya, 1980; Bhattacharya and Saigol, 1984; Budras and Wünsche, 2009). It has been stated that prismatic epithelial uterine glands are located under the epithelial layer in which the cilium and cilium-free prismatic epithelium are located on the surface of the endometrium (Trautmann and Fiebiger, 1957; Salih and Abbas, 2014). The myometrium layer consisting of circular, inner vascular and longitudinal muscles the perimetrium constituted the outer most layer of the uterine wall (Agrawal and Laloraya, 1978).

The aim of the present study was to investigate the anatomical and histological structure of the uterus (cervix, corpus, cornu uteri) of the Anatolian wild goat (*Capra aegagrus aegagrus*) living in the Caucasus and North Anatolia Region. For the continuation of life, it is essential that zygote be formed and the embryo clings to the uterus wall. Cervix uteri is a difficult barrier to be used in artificial insemination applications. For these reasons, it was inevitable to study an extinct type of uterus.

MATERIALS and METHODS

The necessary permissions were obtained from the General Directorate of Nature Conservation and National Parks (21264211-288.04 / E.3790788) of the Ministry of Agriculture and Forestry to carry out this study. The animal material consisted of three wild goats of similar ages (2-3 old) that had been brought to the Kafkas University Wildlife Rescue and Rehabilitation Center for various reasons (traffic accidents, firearm injuries) and could not be saved despite all interventions.

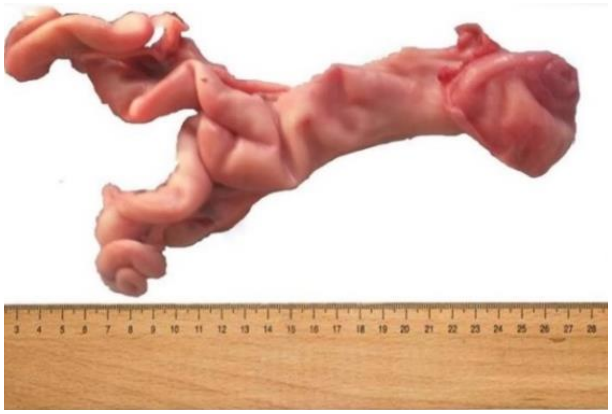

Figure 1. The study material

The uterine material seen in Figure 1 was dissected and photographed using the Kodak Easyshare M320 Digital Camera (Eastman Kodak Company, Rochester, NY, USA) during necropsy while preserving the anatomical structure. *Nomina Anatomica Veterinaria (2017)* was used for naming the anatomical structures. The uterus was studied in three sections: corpus uteri, cornu uteri and cervix uteri. The cervix uteri length was measured from the ostium uteri internum to the ostium uteri externum. Corpus uteri length was measured from the junction of the two cornu uteri to the ostium uteri internum. The length of cornu uteri was measured from the cornu uteri junction to the tubouterinal junction. The measurements were taken from the uterine segments using digital callipers (stainless steel 1- to 150-mm). The weights of the uterine segments were measured on a precision scale (min: 0.0001 g–max: 220 g, code: XB220A, Precisa®, Swiss). The mean and standard error values of all measurements obtained were examined in the SPSS (version 20.0) packaged software program. For the histological examination of the uterine tissue, the tissues were fixed with 10% formol-aldh and routine histological tissue follow-up was performed. After being processed through graded alcohols and polished, the fixed blocks were embedded in paraffin. 5 μm thick sections were

taken from the paraffin blocks and stained using Crossman's triple staining method. The stained and fixed samples were examined under a light microscope (Carl Zeiss Microscopy, Göttingen, Germany) and their photographs were taken.

RESULTS

Anatomical results

The portio vaginalis, the protrusion of the ostium uteri externum, one of the holes that shaped cervix uteri, was found to be rose-shaped in wild goats. As shown in Figure 2, the mean cervix uteri length was 33.99±1.22 mm, cervix uteri width was 13.39±0.92 mm, cervix uteri thickness was 8.42±1.68 mm and cervix uteri weight was 5.45±0.80 g (Table 1).

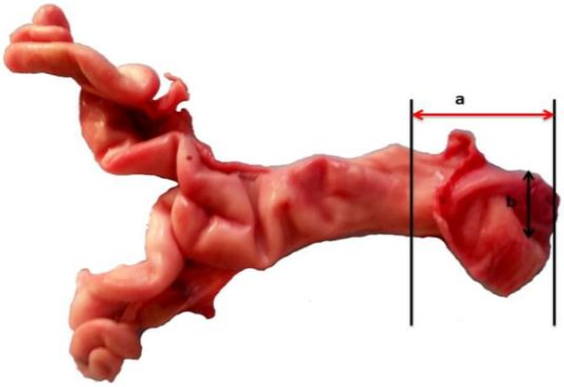

Figure 2. Measurements taken over cervix uteri and the rose shaped portio vaginalis.
(a: Length of cervix uteri, b: Thickness of cervix uteri)

Table 1. Some values taken from the cervix uteri of the Anatolian wild goat.

Measurement	Mean±SE
Length of cervix uteri (mm)	33.99±1.22
Width of cervix uteri (mm)	13.39±0.92
Thickness of cervix uteri (mm)	8.42±1.68
Weight of cervix uteri (g)	5.45±0.80

SE: Standart error

We measured the mean length and thickness of corpus uteri as shown in Figure 3, 29.61±5.14 mm, 9.05±1.45 mm and corpus uteri weight was 4.30±0.77 g. The mean corpus uteri width was 21.22±1.98 mm and corpus uteri weight was 4.30±0.77 g (Table 2).

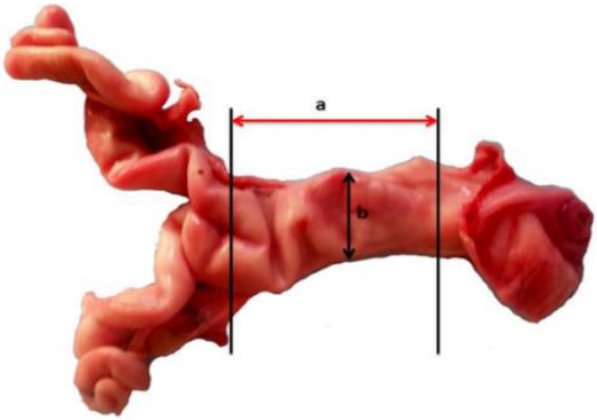

Figure 3. Measurements taken from the corpus uteri.
(a: Length of the corpus uteri, b: Thickness of the corpus uteri)

Table 2. Some values taken from the corpus uteri of Anatolian wild goat.

Measurements	Mean±SE
Length of corpus uteri (mm)	29.61±5.14
Width of corpus uteri (mm)	21.22±1.98
Thickness of corpus uteri (mm)	9.05±1.45
Weight of corpus uteri (g)	4.30±0.77

SE: Standart error

As shown in Figure 4, the average cornu uteri length was 41.34±8.02 mm, cornu uteri width was 12.49±2.59 mm, cornu uteri thickness was 6.73±0.86 mm and cornu uteri weight was 5.46±0.31 g (Table 3).

The macroanatomic examination revealed no ligamentum intercornule.

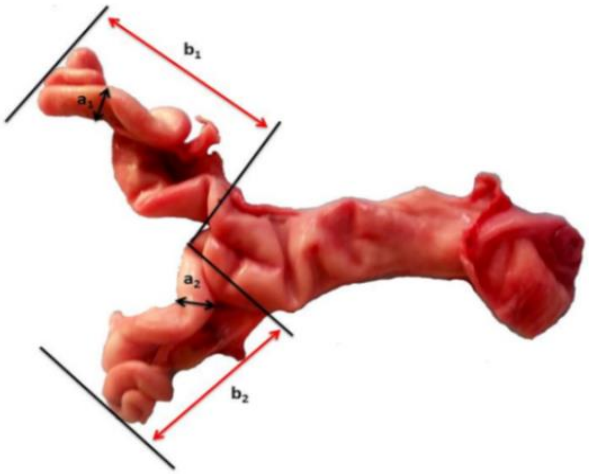

Figure 4. Measurements taken from the cornu uteri.
(a_1, a_2: Thickness of the cornu uteri, b_1, b_2: Length of the cornu uteri)

Table 3. Some values taken from the cornu uteri of the Anatolian wild goats.

Measurements	Right mean±SE	Left mean±SE	General mean±SE
Length of cornu uteri (mm)	42.60±9.78	40.42±6.50	41.34±8.02
Width of cornu uteri (mm)	13.12±2.63	11.87±2.60	12.49±2.59
Thickness of cornu uteri (mm)	6.80±0.95	6.78±0.81	6.73±0.86
Weight of cornu uteri (g)	5.03±0.34	5.95±0.67	5.46±0.31

SE: Standart error

Histological results

Histologically, the wall of cervix, corpus and cornu uteri were comprised of three different layers from within outwards; endometrium, myometrium and perimetrium.

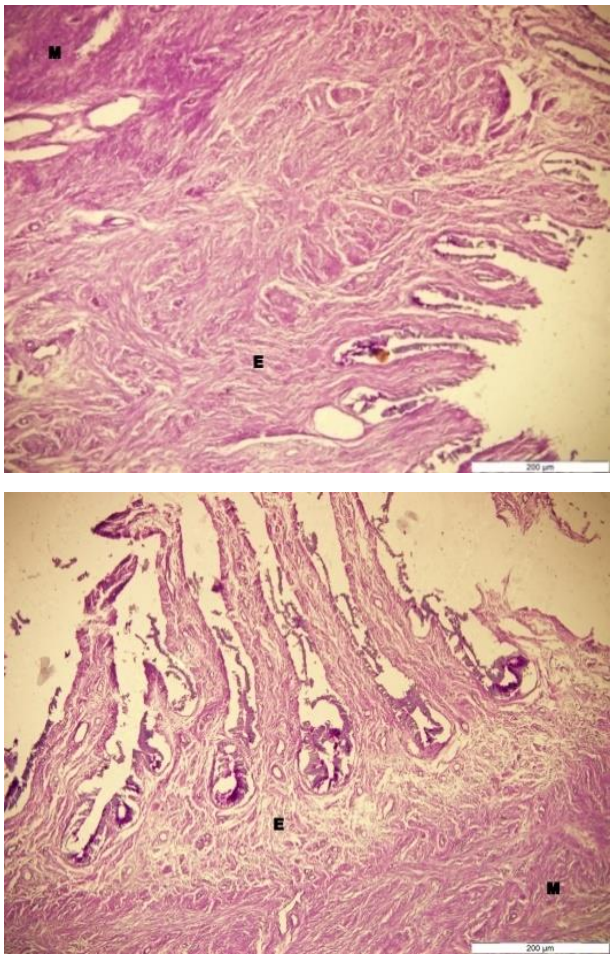

Figure 5. Triple staining of the Anatolian wild goat cervix uteri. Endometrium (E), myometrium (M).

The cervix uteri was the caudal most part of the uterus. We observed the cervix was thick walled, highly muscular and connective structure. It had a narrow lumen the canalis cervicis uteri. It was revealed that mucosa of cervix was thrown into several cervical crypts. The lining epithelium of cervical crypts was pseudostratified columnar epithelium. The cervical glands were formed of the lining epithelium into the endometrium. The muscular layer of the cervix consisted of inner circular and outer longitudinal smooth muscle cells. The perimetrium comprised of connective tissue (Figure 5).

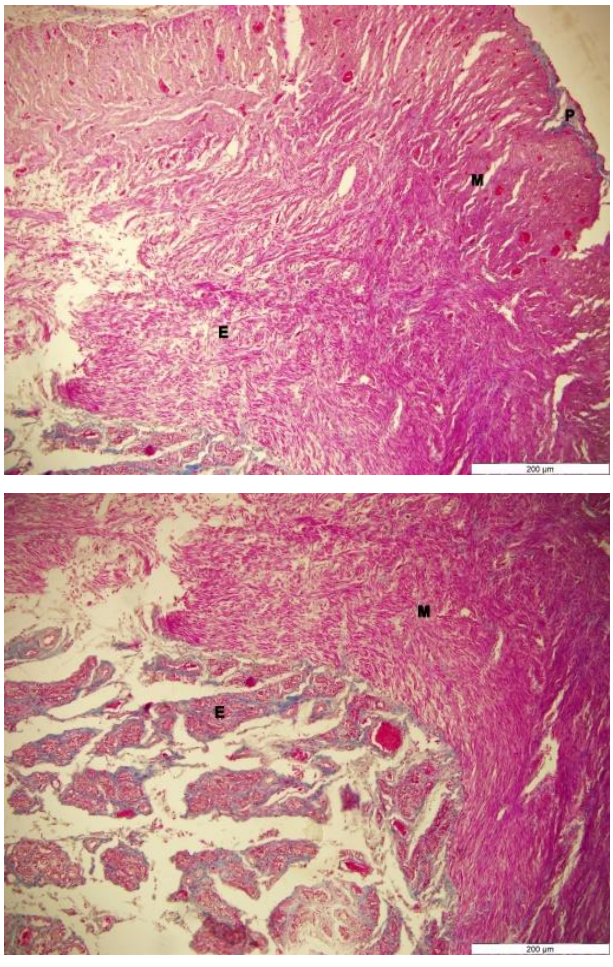

Figure 6. Triple staining of the Anatolian wild goat cornu uteri. Endometrium (E), myometrium (M), perimetrium (P).

It was determined that the corpus and cornu uteri consisted of pseudostratified columnar epithelium cells with cilium and non-cilium in places on the lumen-facing surfaces. Under these cells, the endometrium, which is rich in blood vessels and

endometrial glands, was found. The myometrium was composed of inner circular and outer longitudinal smooth muscle cells layers. These layers were followed by the perimetric layer consisting of connective tissue (Figure 6).

DISCUSSION

Harris (1962) and Zeuner (1963) reported that today's domestic goat is genetically involved in the domestication process of three wild goat species called bezoar (*Capra aegagrus*), markhor (*Capra falconeri*) and ibex (*Capra ibex*). It has been reported that *Capra aegagrus* main lines from the origin of the Turkey indigenous goat breeds (Cinar *et al*., 2015). The age, species, physiological status, number of births given, etc. affect the length of the cervix uteri (Abiaezute *et al*., 2017; Kershaw *et al*., 2005). The shape of the ostium uteri externum, one of the holes that shapes the cervix uteri, was found to be rose shaped in sheep, papilla shaped in lambs (Kershaw *et al*., 2005) and a bump in Ankara goats (Dayan *et al*., 2010). When we compare the materials, we examined with the studies in the literature, it was the same as that of adult sheep (Kershaw *et al*., 2005). The length of the cervix uteri has been reported to vary between 80-100 mm in ruminants (Budras and Wünsche, 2009). In a study by Gültiken *et al*. (2009) the length of the cervix uteri was reported to be 36.9±6.5 mm in Karakaya sheep and 28.9±3.5 mm in Karakaya lamb. In another study conducted by Naqvi *et al*. (2005), the cervix uteri length of sheep was determined as 53.00±1.5 mm, while this was determined as 38.00±1.2 mm for lambs. This value can vary between 30-40 mm in goats (Semacan *et al*., 2012), 33.48±1.13 mm in Black Bengal goats (Gupta *et al*., 2011), 21.9±4.9 mm in Gaddi goats (Shalini, 1997) and 25.9±6.1 mm in red Sokoto goats (Adigwe and Fayemi, 2005). In the present study, the length of the cervix uteri was determined as 33.99±1.22 mm. This value is compatible with the data obtained in the literatüre. This value was nearest to that of the Black Bengal goat. The width of the cervix uteri was 13.39±0.92 mm in Anatolian wild goats, while it was 10.7 ± 1.7 mm in red Sokoto goats (Gültiken *et al*., 2009), 14.1±2.0 mm in Gaddi goats (Shalini, 1997), and 17.55±0.42 mm in Black Bengal goats (Gupta *et al*., 2011). Cervix uteri width was also found to be among the determined reference values.

The mean corpus uteri length was reported as 20-40 mm in ruminants (Budras and Wünsche, 2009; Mahre *et al*., 2016), 21.75±2.5 mm in sheep (Hyacinth *et al*., 2016), and 20-30 mm in domestic goats (Semacan et al., 2012). The findings of the present study are generally higher compared to those in the literature, however they are still within the maximum size limit. The corpus uteri width for red Sokoto goats was reported as 21.00±4.2 mm (Adigwe and Fayemi, 2005), while this length was determined as 21.22±1.98 mm for the Anatolian wild goats. The width of the cervix uteri obtained in the present study was found to be within the determined reference values.

Cornu uteri length has been reported to be 120-150 mm in domestic goats (Semacan *et al*., 2012). In the present study, the mean length of the cornu uteri of Anatolian wild goats was determined to be 41.34±8.02 mm. The difference is a wavy structure of this difference was considered wild goat uterus and can vary depending on the age of the animals used. In the Rusa deer, the length of the left cornu uteri was 110±5 mm and the length of the right cornu uteri was 106±7 mm (Mahre *et al*., 2016). In the wild goat, the mean length of the cornu uteri was 42.60±9.78 mm on the right side and 40.42±6.50 mm on the left side. Some studies have indicated that the left cornu uteri is longer than the right cornu uterus (Adigwe and Fayemi, 2005; Hyacinth *et al*., 2016; Saleem *et al*., 2017). In the present study, it was observed that the left cornu uteri was longer in two materials, while the right cornu uteri was longer in one material. In general, it was determined that the right cornu uteri was longer. In the Rusa deer, the left cornu uteri width is 15±5 mm, while the right cornu uteri width is 12 mm (Mahre *et al*., 2016). Therefore, it can be said that the left cornu uteri is wider. However, in the wild goats the right cornu uteri was wider. When the relation between the length and width of cornu uteri in the Anatolian wild goats is observed, it can be said that there is a proportion between the two measurements. The absence of the ligamentum intercornuale in both the left and the right cornu uteri in ruminants was incompatible with the findings of this study, but similar to those found for Rusa deer (Mahre *et al*., 2016).

CONCLUSION

In conclusion, it should not be forgotten that these findings of the Anatolian wild goat uterus, which are part of the diverse wildlife of Turkey but have decreased in terms of population due to uncontrolled hunting or traffic accidents in recent years, may be the first or last study. Studies on the

female genital system of Anatolian wild goat are limited (Kırbaş Doğan *et al*., 2019). The results of the present study may contribute to future studies, when these animals, which are on the verge of becoming extinct (Anonymous, 2019) are taken under protection and propagated by artificial insemination.

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Antimicrobial and Antioxidant Activity of Different Herbal Tea Combinations

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ABSTRACT

Objective: Today, the use of components obtained from plant extracts is rapidly increasing, especially in the pharmaceutical industry. Eight different plants, which are used as winter tea and are frequently consumed among herbal teas, were selected in the study. The aim of study was to investigate the antimicrobial and antioxidant activities of teas obtained from medicinal and aromatic plants such as Linden, Ginger, Cinnamon, Sage, Daisy, Turmeric, Clove and Rosehip. Five different pathogens (*Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa)* were selected from common disease-causing pathogens.

Materials and Methods: A total of 21 combinations were made for each plant. Disc diffusion and Minimum inhibition concentration methods were used to determine antimicrobial activity. DPPH (2,2 Difenil-1-Pikrohidrozil) method was used to determine antioxidant activity. The amount of total phenolic and tannins contents contained of herbal teas were also determined using the Folin-Ciocalteu reagent (FCR) method.

Results: The highest value among the antimicrobial activities of herbal teas (triple combination) was measured against *E. faecalis* (25.11 mm). The herbal combination with the highest value measured was found in the ginger+cinnamon+clove group. The highest antioxidant value was measured in this mixture (36.8 mg/mL).

Conclusion: Because some plants have more bioavailability, these benefits can be suppressed in a mixture. When determining these mixtures, the consumption will be more beneficial for public health, given the recommendations of researchers and experts.

Keywords: *Herbal tea, Antimicrobial, Antioxidant, Pathogen, Public health*

INTRODUCTION

Medical plants are used effectively in the treatment of diseases from past to present. In recent years, the acceptance of traditional medicine as an alternative healthcare has led researchers to investigate the bioactive properties and bioavailability of these plants. Plant originated medicines continue to be important resources to combat serious health problems, especially in developing countries (Mothana *et al*., 2010).

According to the report of the World Health Organization (WHO), about 60-80% of the world population benefits from traditional medicinal plants in the treatment of common diseases (Schuster and Wolber, 2010; WHO, 2013).

Today, the rapid increase of infectious diseases and the development of resistance against existing drugs by pathogens microorganisms lead to an increase in new and improved potential searches against bacterial and viral infections (Gibbons, 2004). Despite the latest developments in drug combinations developed using different technologies; it is supported by research that plant-

based compounds can still be important drug sources for humans (Salim *et al*., 2008).

Plant-derived antimicrobials have a long history of research among new therapeutic agents. Plants can constantly interact with free radicals, with external environmental factors that can rapidly change metabolism and potentially cause harm. Thus, they support anabolic reactions in the body by combating harmful free radicals that may occur in metabolism. Plants develop alternative defense strategies by creating various chemical metabolites to overcome stress conditions that may occur with their unique metabolic interactions. Therefore, the use of plants in both traditional and modern health systems is highly preferred by researchers (Avila *et al*., 2008).

Various traditionally used medicinal plants have been involved in many studies in terms of their various biological activities and bioavailability using in vivo and in vitro study models. It is in the literature that extracts obtained from plants that have therapeutic properties and the phytochemicals isolated from them are preferred in developing modern medical practices (Abdalla *et al*., 2013). The phytochemicals that plants contain in their structure provide significant benefits on the immune system. In addition, when more than one phytochemical is combined, the bioactive property increases. Thus, with these compounds in a single plant structure, it can show antimicrobial, antifungal, antidiabetic, anti-inflammatory, anticancer and antiviral properties (Puangpronpitag and Sittiwet, 2009; Pundir *et al*., 2010; Sasidharan and Menon, 2010; Gupta *et al*., 2015; İlkimen and Gülbandılar, 2018; Demir *et al*., 2019; Vatlak *et al*., 2019; Rovna *et al*., 2020). This study was carried out to determine the antimicrobial and antioxidant properties of some herbal tea combinations.

MATERIALS and METHODS

Preparation of Plant Extracts

The plants used in the study were obtained from local sales outles of Sivas province. Linden, sage, daisy and rosehip were dried in an oven (40°C). While cloves are ground into powder, cinnamon, ginger and turmeric are taken as direct powder. In addition, it was brought to the same particle size by mixing (milling) the plants in triple combinations (56 combinations).

Table 1. Herbal teas and combinations

	A:Linden	B:Ginger	C:Cinnamon	D:Sage	E:Daisy	F:Turmeric	G:Clove	H:Rosehip
1	**ABC**	**BCD**	**CDE**	**DEF**	**EFG**	**FGH**	ABG	ABH
2	**ABD**	**BCE**	**CDF**	**DEG**	**EFH**	ABF	ACG	ACH
3	**ABE**	**BCF**	**CDG**	**DEH**	**EGH**	ACF	ADG	ADH
4	**ABF**	**BCG**	**CDH**	**DFG**	ABE	ADF	AEG	AEH
5	**ABG**	**BCH**	**CEF**	**DFH**	ACE	AEF	AFG	AFH
6	**ABH**	**BDE**	**CEG**	**DGH**	AEF	AFG	AGH	AGH
7	**ACD**	**BDF**	**CEH**	ABD	AEG	AFH	BCG	BCH
8	**ACE**	**BDG**	**CFG**	ADE	AEH	BCF	BDG	BDH
9	**ACF**	**BDH**	**CFH**	ADF	BDE	BDF	BEG	BEH
10	**ACG**	**BEF**	**CGH**	ADG	BEF	BEF	BFG	BFH
11	**ACH**	**BEG**	ABC	ADH	BEG	BFG	BGH	BGH
12	**ADE**	**BEH**	ACD	ACD	BEH	BFH	CDG	CDH
13	**ADF**	**BFG**	ACE	BCD	CDE	CDF	CEG	CEH
14	**ADG**	**BFH**	ACF	BDE	CEF	CEF	CFG	CFH
15	**ADH**	**BGH**	ACG	BDF	CEG	CFG	CGH	CGH
16	**AEF**	ABC	ACH	BDG	CEH	CFH	DEG	DEH
17	**AEG**	ABD	BCD	BDH	DEF	DEF	DFG	DFH
18	**AEH**	ABE	BCE	CDE	DEG	DFG	DGH	DGH
19	**AFG**	ABF	BCF	CDF	DEH	DFH	EFG	EFH
20	**AFH**	ABG	BCG	CDG	BCE	EFG	EGH	EGH
21	**AGH**	ABH	BCH	CDH	ADE	EFH	FGH	FGH

The herbal tea combinations analyzed are shown in bold.

Herbal teas were stored at +4°C until analysis. Ground plants were mixed with water in a ratio of 10:1 (ml/g). Then it was extracted in a shaking water bath at 80°C. At the end of the extraction period, the samples were centrifuged (10 min at 5000 rpm). The combinations of herbal teas and mixtures are shown in Table 1.

Antimicrobial activity

Microorganisms (*Staphylococcus aureus* (ATCC 29213), *Streptococcus pyogenes* (ATCC 19615), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) used in antimicrobial activity were obtained from Sivas Cumhuriyet University Research Hospital Microbiology Laboratory. All chemicals used as analytical standard were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Merck KGaA, Darmstadt, Germany).

Preparation of bacterial cultures

Nutrient Agar was used as medium for bacterial cultures. Sterilized media were poured into petri dishes and incubated at 37ºC for 20 hours in order to grow bacterial cultures. Nutrient Broth was used to determine the minimum inhibition concentration (Ebrahimabadi *et al*., 2010).

Disc diffusion method

Bacterial cultures produced in solid media of microorganisms were used. Serum was suspended in physiological and dilutions of 10^8 cfu/ml were prepared by comparison with a 0.5 McFarland turbidity tube. 100 µl of cultivation was made to the Mueller Hinton Agar (MHA, Merck; 70191, Germany) medium from bacterial dilution. Then incubated at 37 ºC in the over for 18-24 hours. Inhibition zones formed at the end of the incubation were measured (Ebrahimabadi *et al*., 2010).

Determination of minimum inhibition concentration (MIC)

Bacterial cultures were incubated at Nutrient Broth (Merck; 70122, Germany) at 37 °C for 24 hours. The inoculum suspension was prepared. MIC analysis of the prepared herbal teas was determined by the macrobroth dilution method. 25 µl (10^8 cfu/ml) of each bacterial culture was taken into the test tubes with 3 ml Mueller Hinton Broth (MHB, Merck; 70192, Germany) and herbal tea (starting from 25 mg/ml to a concentration of 0.78 mg/ml). It was then incubated at 37°C for 24 hours. The lowest dilution concentration in the tubes where bacterial growth was not seen at the end of the incubation was determined as the MIC value (Oskay *et al*., 2007).

Determination of total phenolic compound content

The total phenolic compound content was determined by the Folin-Ciocalteu method. The results were expressed as g gallic acid equivalent/kg of tea leaves (g GAE/kg). Measurements were conducted in triplicates (Radhakrishnan *et al*., 2014).

Determination of tannin content

The tannin content was determined by a method described by Nakamura *et al*. (2003). The tannin content was calculated from a calibration curve using a catechin as standard and expressed as g catechin equivalents/kg of tea leaves. Measurements were conducted in triplicates.

Antioxidant activity

Free radical screening of each herbal tea extract with DPPH (2,2 Difenil-1-Pikrohidrozil) radical sweep was performed and the results were given as total antioxidants. DPPH solution in methanol was prepared just before starting the analysis. Then 3 mL of this solution was mixed with 100 µl of herbal tea extracts. The samples were incubated at 37°C for 20 minutes in water bath and their absorbance was measured (515 nm). An empty cuvette containing 100 µl of methanol in DPPH solution was prepared and its absorbance was recorded. All experiments were done in three replicates. Antioxidant activity was calculated using the formula below (Brand *et al*., 1995).

$$\% \text{ inhibition: } [(AB-AE)/AB] \times 100$$

AB: Absorbance of the blank sample and AE: Absorbance of the herbal tea extract.

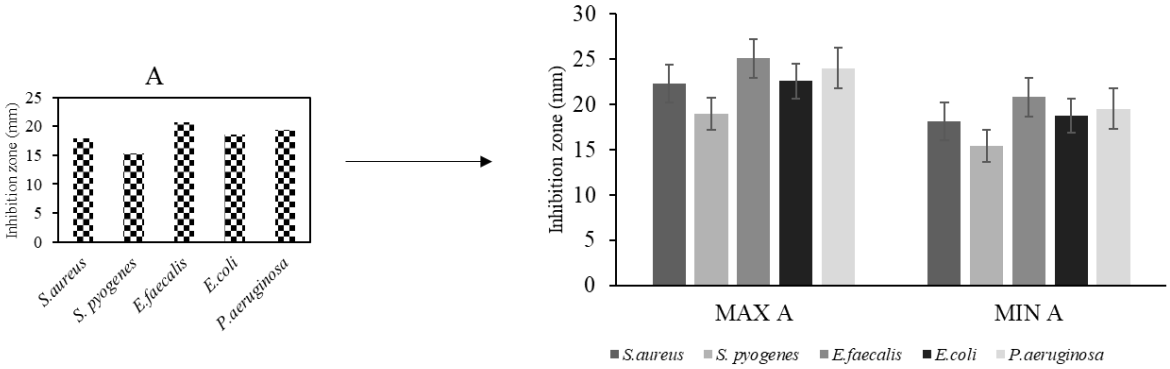
Statistical analysis

The results are given as three repeat means (±) standard deviation. SPSS was used to analyze statistical computer program results (IBM SPSS Statistics 22, Inc., Chicago, IL, USA). The variance analyzes of the results were made (ANOVA) and the differences were evaluated statistically in 95% confidence interval by Duncan multiple comparison test.

RESULTS

In this study, eight different plants which are used as winter tea and are frequently consumed among herbal teas were selected. Five different pathogens were selected from common disease-causing pathogens. A total of 21 combinations were made for each plant and the antimicrobial activity of these 21 combinations on each pathogen was determined using disc diffusion and minimum inhibition

concentration methods. The antimicrobial effects of herbal teas used in our study on five different pathogens are given in Figure 1-8. When the graphs are examined, inhibition zones measured by disc diffusion method different antimicrobial activities in the combination (triple; 21 combinations) of each plant are also shown comparatively. Maximum (MAX: Highest in 21 combinations) and minimum (MIN: Lowest in 21 combinations) values are given in Figure 1-8.



MAX: Highest in 21 combinations, **MIN**: Lowest in 21 combinations

Figure 1. Antimicrobial activities of linden tea measured by disc diffusion method against different microorganisms **MAX**: Highest in 21 combinations, **MİN**: Lowest in 21 combinations

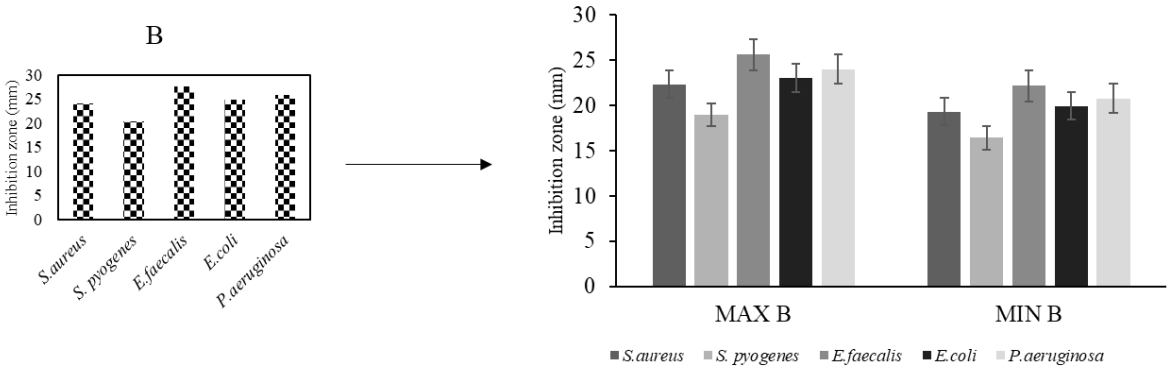


Figure 2. Antimicrobial activities of ginger tea measured by disc diffusion method against different microorganisms.

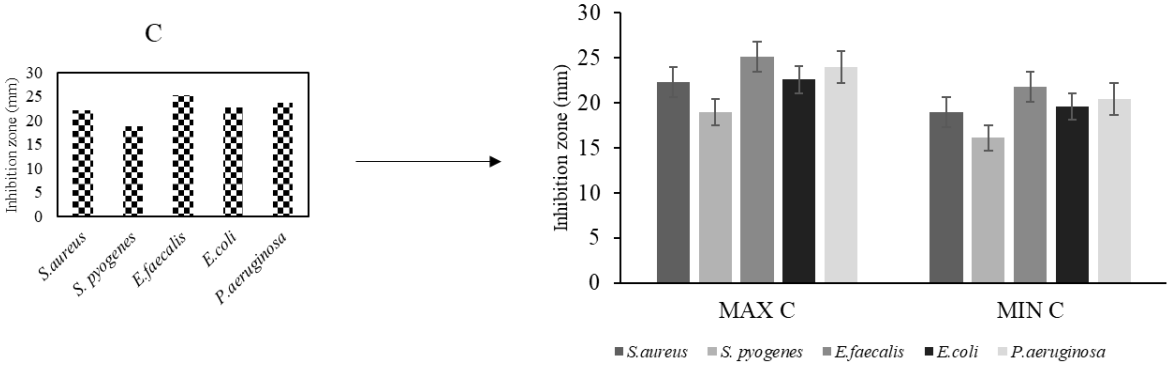


Figure 3. Antimicrobial activities of cinnamon tea measured by disc diffusion method against different microorganisms.

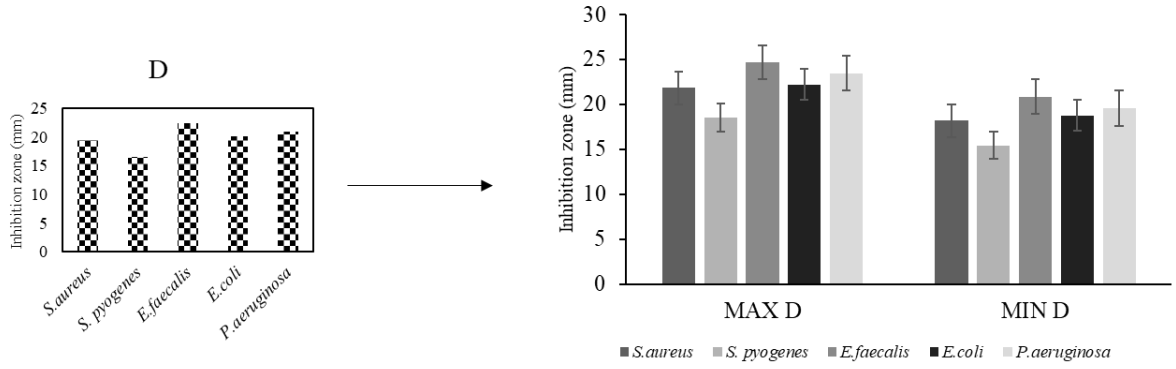


Figure 4. Antimicrobial activities of sage tea measured by disc diffusion method against different microorganisms.

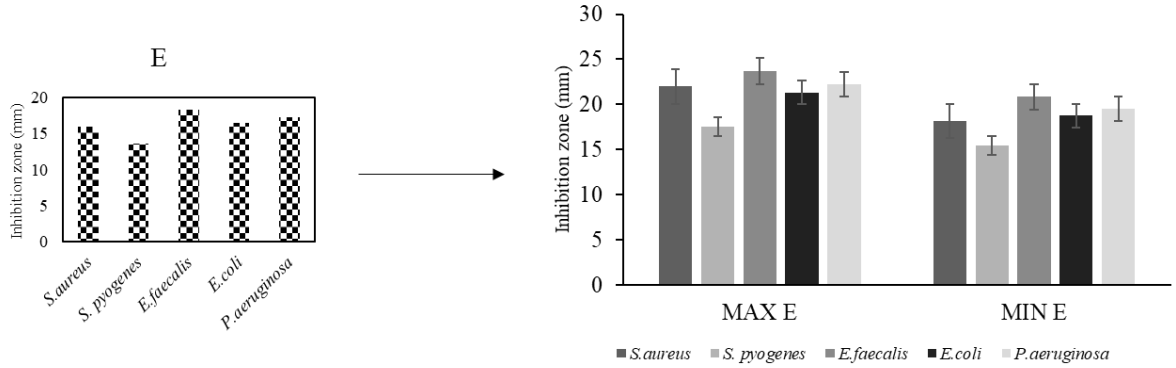


Figure 5. Antimicrobial activities of daisy tea measured by disc diffusion method against different microorganisms.

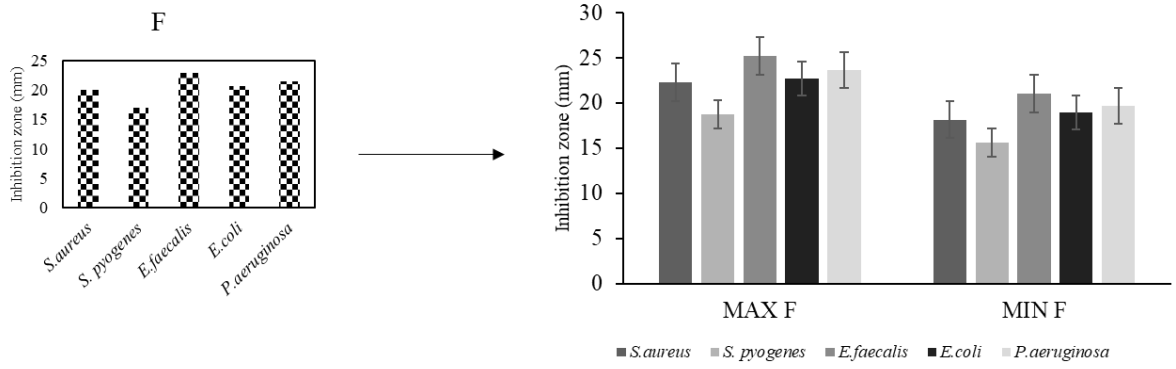


Figure 6. Antimicrobial activities of turmeric tea measured by disc diffusion method against different microorganisms.

When the antimicrobial activity results were evaluated, the extract (single combination), which acts on both gram positive and Gram negative bacteria, was measured as ginger (26 mm) extract. Daisy extract showed the lowest inhibition against gram positive bacteria *S. pyogenes* (14 mm). All extracts showed the highest inhibition on *E. faecalis*. Extracts in which *E.coli*, the fecal contamination indicator, were the most resistant were ginger (23 mm) and cinnamon (23 mm). When the synergistic effects were evaluated, eight combinations (ACG, BCG, CAG, DBG, EBC, FBC, GBC and HBC) showed a positive synergistic effect (Table 2).

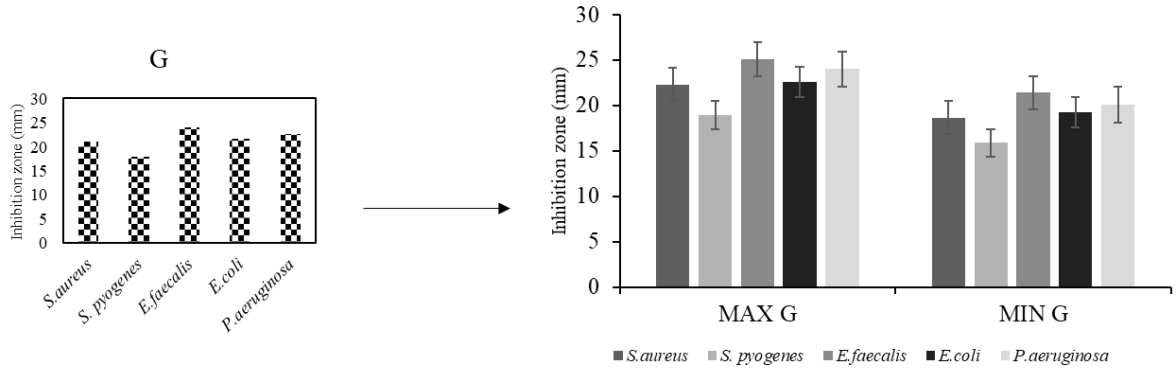


Figure 7. Antimicrobial activities of clove tea measured by disc diffusion method against different microorganisms.

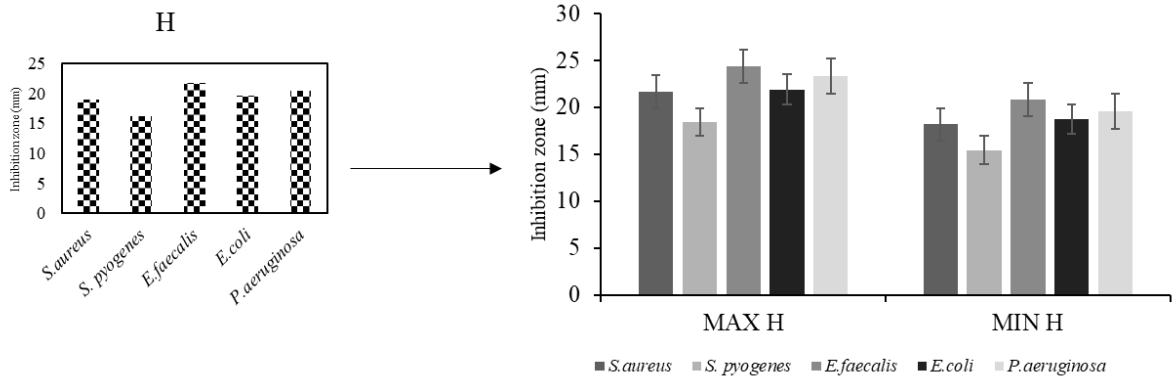


Figure 8. Antimicrobial activities of rosehip tea measured by disc diffusion method against different microorganisms.

Table 2. Positive and negative synergistic effects of herbal tea combinations

Herbal tea	Single highest (İnhibition zone mm)	Synergy (+)↑	Combination	Single lowest (İnhibition zone mm)	Synergy (-)↓	Combination
A	19.00 *	24.01	ACG	15.50 ***	15.44	AEH
B	25.25 **	25.62	BCG	20.40 ***	16.43	BAE
C	25.00 **	25.11	CAG	18.70 ***	16.15	CEH
D	22.38 **	24.60	DBG	16.58 ***	15.44	DEH
E	18.36 **	23.70	EBC	13.60 ***	15.44	EAH
F	22.95 **	25.25	FBC	17.00 ***	15.58	FEH
G	24.11 **	25.11	GBC	17.85 ***	15.87	GEH
H	21.80 **	24.35	HBC	16.15 ***	15.44	HAE

*: *P. aeruginosa*; **: *E. faecalis* ; ***: *S. Pyogenes*

MIC results obtained, disc diffusion results are in line with the results, and the lowest concentration in which each plant extract is effective on the same bacterial group was determined (Table 3). The minimum inhibition concentration of ginger extract was seen in containing *S. pyogenes* with <0.78 mg/mL.

In the literature, the pharmacological effects of plants have been mentioned in many studies and it has been stated that the expected benefits of the mixtures are at different levels. In this study, apart from each plant itself, triple combinations were made with 7 different plants and 21 different combinations. While some extracts retained their

inhibition when it was mixed, some of them overcame the effect. Some of the plant combinations could not show their main inhibiting ability in mixtures of combinations. When the study results were evaluated, the most resistant group against the microorganisms from the triple combinations of herbal teas was the ginger + cinnamon + clove (BCG) group. Antimicrobial activity results of this group are 22.33 mm, 18.98 mm, 25.11 mm, 22.6 mm, 24.01 mm against *S. aureus, S. pyogenes, E. faecalis, E. coli, P. aeruginosa* pathogens, respectively. Chloramphenicol, the standard antibiotic used in the disc diffusion method, showed an inhibition zone of 30-35 mm against these pathogens. Within this study limits, BCG group data is close to the standard antibiotic value. Therefore, this group in the study is thought to be a strong antimicrobial agent.

Table 3. MIC values measured against different microorganisms

	S. aureus	*S. pyogenes*	*E. faecalis*	*E. coli*	*P. aeruginosa*
A	>6.25[b]	12.50[c]	<3.125[a]	6.25[b]	>6.25[b]
B	>1.56[b]	<3.125[c]	>0.78[a]	<0.78[a]	<0.78[a]
C	3.125[b]	6.25[c]	>0.78[a]	<3.125[b]	1.56[a]
D	<12.50[b]	12.50[b]	<3.125[a]	3.125[a]	3.125[a]
E	12.50[b]	>12.50[b]	6.25[a]	12.50[b]	6.25[a]
F	<3.125[a]	6.25[b]	>3.125[a]	3.125[a]	3.125[a]
G	3.125[b]	6.25[c]	>1.56[a]	<3.125[b]	>3.125[b]
H	6.25[b]	12.50[c]	3.125[a]	>6.25[b]	3.125[a]

[a, b, c] Averages marked with different letters in the same line are statistically different from each other according to Duncan test ($p<0.05$), Chloramphenicol was used as a control (MIC value;> 0,78 mg/ml)

The fact that the antimicrobial activity shown by an extract alone could not show the same level (zone) in the plant mixture made can be evaluated in the sense that the antimicrobial mechanism of this plant does not work at all performance. Plants show the antimicrobial mechanism of action together with the mechanism of action of volatile compounds such as alkaloids and terpenoids.

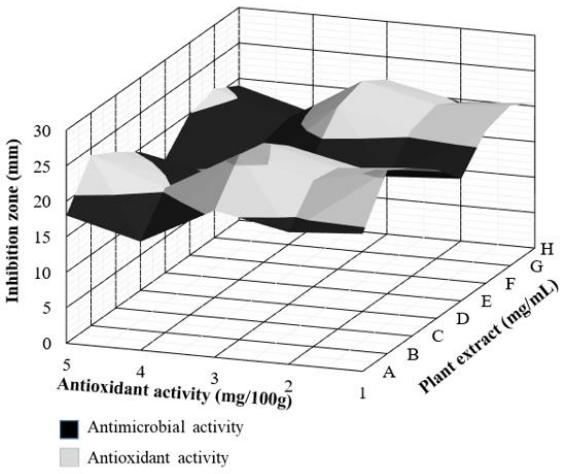


Figure 9. The relationship between antioxidant properties and antimicrobial activities of herbal tea extracts (There was a significant correlation; R^2> 0.87)

Although the chemical reaction caused by the molecular weight of alkaloids in the mixtures made suppressed the antimicrobial effect of the plant, other antioxidant mechanism of action still exists. The chemical composition of herbal teas is shown in Table 4.

Table 4. Chemical composition of herbal teas

	Total Polyphenols content (mg/100 g)	**Tannin contents (mg/100 g)**	**Total antioxidant content (mg/mL)**
A	9.2±1.01	0.28±2.11	27.7±1.13
B	15.6±1.88	0.31±1.18	34.9±2.58
C	12.7±0.81	0.25±1.05	36.8±3.05
D	9.3±0.78	0.28±3.08	23.7±0.08
E	7.2±2.07	0.29±2.18	22.4±0.90
F	13.4±1.19	0.44±1.09	38.9±1.18
G	11.8±3.09	0.32±0.44	31.3±3.05
H	11.9±2.08	0.34±0.87	34.1±1.58

The phenolic and flavonoid compounds released by the extraction step were tasked with destroying the cell wall of the pathogen with different defense systems and exhibited different levels of antimicrobial action. In addition, in our study, the

total phenolic contents of the herbal teas and the contents of the total tannins were measured and recorded. When these results were evaluated, the highest polyphenols content showed ginger (B: 15.6 mg/100g), followed by turmeric (F: 13.4 mg/100g) (Table 4). The relationship between antioxidant activities and antimicrobial activities of extracts is presented in Figure 9. A significant correlation was determined between the antimicrobial activity of all herbal teas examined against different microorganisms and total polyphenol contents. The highest antioxidant value was measured in BCG mixture (36.8 mg/mL).

DISCUSSION

Plant polyphenols are aromatic hydroxylated compounds that are the most potent and therapeutically useful bioactive substances (Apak *et al*., 2007). In this study, the difference in the all between formulizations of antimicrobial activity concurs with the results of studies that reported that the secondary metabolites may vary between plants of the same species and of different species (Achakzai *et al*., 2009). In a study examining the antimicrobial properties of herbal extracts, the highest antibacterial activity of linden extract was measured against to gram negative bacteria *P. aeruginosa* used with disc diffusion method (Vatlak *et al*., 2019). In our study, the inhibition zone measured as a result of the antimicrobial activity of linden measured using disc diffusion method is 19 mm (*P. aeruginosa*). When the results are evaluated, it is thought that this situation may be effective on the microorganism load and extraction solution and extraction time.

Tshivhandekano *et al.* (2014) were investigated the chemical composition and antimicrobial activities of different herbal teas and the synergistic effects of combined herbal teas. The antimicrobial activity exhibited by some extracts alone differed in the combined extracts. In this study, positive and negative synergistic effects of combinations of herbal teas were investigated. The single highest and lowest antimicrobial activities of herbal teas were evaluated. The highest and lowest antimicrobial activities were then recorded in combination. When comparing the most inhibited bacteria groups at the lowest concentration, gram positive bacteria were more inhibited than gram negative bacteria. In a different study, ginger reported that the minimum inhibition concentration on *S. pyogenes* was measured between 1.25 mg/mL and 2.50 mg/mL. Results of conducted the study by Onianwah and Stanley (2018) were found to be higher compared to our study findings. The MIC results in Table 3 are statistically different from each other according to the Duncan test, with the mean letters marked in different letters on the same line ($P < 0.05$). Antioxidants play an important role in neutralizing oxidative damage caused by free radicals in tissue fluids (Saleh *et al.* 2010). Our study results show that F>C>B plants have significantly high antioxidant activity content. The primary source of total antioxidant activity of these plants is thought to be total polyphenols. Therefore, the significantly higher antioxidant activity found in these plants may be associated with the high content of polyphenols analyzed. Composition of plant bioactive compounds has also been reported to play a role in the antimicrobial activity. Phytochemicals have an important place in plant bioactive components (Güzel and Akpınar, 2017). There are many phytochemical agents that plants naturally possess in their structure. These components, in addition to antioxidant and antimicrobial activity, have antidiabetes, anti-inflammatory and cytotoxic effects (Demir *et al.* 2019). In recent years, research has deepened to make use of plant phytochemicals in antiviral agents. In addition, bacterial inhibition may also vary according to plant extract; the solvent is used for extraction and the organism tested. Polyphenols content are related to the antibacterial activity of tea extracts. Our results indicated that bitki çaylarının had significantly high polyphenols content.

CONCLUSION

Plants that are frequently consumed by the people (winter tea) have been the subject of research in this study. Therapeutic properties of herbal teas that have for public health continue to be the subject of new studies day by day. Unconscious diversification of herbal tea blends may not always be right. Because some plants have more bioavailability, these benefits can be suppressed in a mixture. When determining these mixtures, the consumption will be more beneficial for public health, given the recommendations of researchers and experts.

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Relationship between Cystatin C with some hematological and biochemical parameters in neonatal calf diarrhea

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ABSTRACT

Objectives: The purpose of this research is to determine the relationship between cystatin C (Cys-C) and some hematologic and biochemical parameters in neonatal calves diarrhea.

Materials and Methods: In this research the animal material of the study was obtained from different breeds, genders and ages (0-30 days) 10 samples have been taken from healthy neonatal calves and 22 samples from diarrhea calves which didn't received any medicine. Otherwise, the general examination has been done for all the calves. The levels of hematologic, biochemical and blood gas have been determind for both healthy calves and neonatal calves diarrhea.

Results: Depending on the control group, we have observed that the neonatal calves diarrhea hematologic parameters WBC, Neu, Hct, Hb levels ($p<0.05$) and biochemical parameter BUN ($p<0.01$) and Cr ($p<0.05$) level statistically have been increased. On the other hand, Alb ($p<0.05$) and glucose ($p<0.01$) levels have been decreased. In term of blood gas analysis and depending on the control group the level of K^+ ($p<0.05$) has been increased, the levels of pH, pO_2 and base ($p<0.05$) have been decreased. We evaluate the Cys-C level in the neonatal calves diarrhea and we have found that Cys-C level is statistically increased this was detected comparing to the control group ($p <0.01$).

Conclusion: In this research the obtained level of Cys-C can be used as normal for calves; statistically there is no relationship between Cys-C and some of the hematologic and biochemical parameters, the Cys-C level in the calves diarrhea is an important parameter it can be used to determine the diagnosis, treatment and prognosis of the disease; but still much more research about the topic should be done.

Keywords: *Neonatal calf, Diarrhea, Cystatin C*

INTRODUCTION

Diarrhea is one of the most important causes of neonatal calf deaths. Calf diarrhea is reported as one of the most important problems of cattle breeding, high morbidity and mortality associated with diarrhea occurance and causes significant economic losses (Altuğ et al., 2013; Uetake, 2013).

The early diagnosis and the effective treatment of calf diarrhea can reduce the mortality in general the expected results from early diagnosis reduce losses of existing cases and prevent the occurrence of new cases (McGuirk, 2008; Smith, 2012).

Deaths can be observed due the occurance of diarrhea that causes fluid loss in a short time which causes kidney failure as a result of hypovolemia, metabolic acidosis as a consequence of electrolyte losses HCO_3^- and/or its exchange (Na^+, K^+, H^+) and heart blockade as a result of hyperkalemia. Kidney reduces the urine production to recover the

increased fluid losses during diarrhea (Berchtold, 2009; Altuğ et al., 2013).

The main parameters used to diagnose acute and chronic kidney diseases is circulating Creatinin (Cr) and Blood Ure Nitrogen (BUN) concentrations and urine specific gravity (Almy et al., 2002; Cobrin et al., 2013; Ghys et al., 2014). In the previous years many studies examine the correlation between glomerular filtration rate (GFR) and Cys-C (Etem and Mızrak, 2015; Ustaalioğlu et al., 2015).

Serum Cr is affected by many variables such as age, gender, muscle mass. In addition, many studies observed that serum Cr values didn't change remarkably in the early period when kidney functions started to deteriorate (Etem and Mızrak, 2015). Cys-C serum concentration is independent of gender, age or muscle mass, meaning it typically reflects GFR assessment (Onopiuk et al., 2015).

Cys-C has many properties ideal for endogenous GFR marker applications; without GFR variation they are known to have constant production and plasma concentration, low individual variability, no plasma protein binding, no tubular secretion, no tubular reabsorption without catabolism and no extrarenal excretion (Briguori et al., 2010; Ghys et al., 2014; Onopiuk et al., 2015).

Cys-C is freely filtered by glomeruli and after glomerular filtration Cys-C is reabsorbed and catabolized by proximal tubular cells; that is why it does not return to its circulation and the remaining minimum part which is low in concentration is eliminated in the urine (Miyagawa et al., 2009). As a indication for GFR, serum Cys-C is more advantageous than Cr and Cys-C shows an increasing rate at it clinical usage (Toprak, 2013).

In the literature review, there are studies on Cys-C in dogs (Almy et al., 2002; Braun et al., 2002; Pagitz et al., 2007; Miyagawa et al., 2009; Monti et al., 2012; Ghys et al., 2014), but no study on Cys-C in ruminants has been found especially in calves.

In this study by revealing the relationship between kidney biochemical parameters and Cys-C levels in diarrhea calves, it was aimed to contribute to the literature how it changes and its levels according to the healthy control group.

MATERIALS and METHODS

Working approval was obtained from Van YUHADYEK with the ethical committee permission dated at 28.03.2019 and numbered 2019/03. In this study, 22 calves with diarrhea brought to clinics with the complaint of diarrhea from Van province and its districts and 10 calves with no health problems as a control group (0-30 days old, 32 in total) were included in the study.

General examinations of the calves brought to the clinic were carried out. Body temperature, heart and breathing frequencies and age were recorded. Anamnesis of calves with diarrhea was obtained, clinical examinations were performed and data were recorded. Later, fecal samples were taken to sterile fecal container with rectal stimulation. The Speed V DIAR 5 (BVT® Diagnostica Veterinaria, France) test, which enables etiological diagnosis from faeces, was performed on the faeces samples. This kit detected the antigens of the pathogen on the strip membrane by a rapid immuno-chromotographic method. According to the agents that were determined by the previous method, the rates of agents was Giardia 15.789%, E. coli 21.05%, Coronavirus 15.789%, Rotavirus 15.789%, Cryptosporidium 5.26%, Rotavirus and Coronavirus 21.05%, E. coli and Coronavirus 5.26%. Several treatment plans were followed depending on the aetiology and dehydration that were mild, moderate or severe and in most cases varying degrees of metabolic acidosis were determined. The aim of the treatment was to preserve the life of the calves, which aimed at intravenous rehydration through serum and sodium bicarbonate, and then appropriate therapeutic applications were used, whereas bacterial pathogens with antibiotics and the causes of parasites with specific anti-parasites and for the viral agents, their treatment was aimed as continuous intravenous rehydration and giving nutrients and antibiotics to prevent secondary infection.

For hematological, biochemical and blood gas examinations, blood samples were taken duly from the vena jugularis of calves to tubes- with and without anticoagulants. From the anticoagulated blood samples, hematologically red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC), Neutrophil (Neu), Lymphocyte (Lym), Eosinophil (Eo), Monocyte (Mo), Hematocrit (Hct) and Hemoglobin (Hb) levels were determined by veterinary blood count device (MS4-s® Veterinary Blood Count Device, Melet Schloesing Laboratoires co., France). Blood samples in tubes without anticoagulants were centrifuged and the serum obtained used to determine the biochemically; glucose, total protein (Tp), albumin (Alb), BUN, Cr and creatine kinase (CK) levels

(Mindray® BS 400 Veterinary Biochemistry, Mindray co., China) were measured.

The blood taken into the heparinized syringe evaluated by the blood gas device according to the method and the data were recorded according to the patient protocol. Blood gases pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), HCO_3^-, and serum Na^+, K^+ and Cl^- values were evaluated with the blood gases device (Radiometer® ABL80, Rdiometer co., Denmark).

The absorbance levels in the blood serum was determined by proceeding procedure of the specific ELISA test kit (YLbiont®) (Catalog No: YLA0386BO) Cys-C ELISA device (DAS®, Italy). Descriptive Statistics for the features mentioned; it is expressed as median, mean, standard deviation, minimum and maximum value. In terms of these features, Mann-Whitney test was used to compare groups.

For determining the relationship between variables, Spearman Correlation Coefficients were calculated separately in the groups. Statistical significance level was taken as 5% in calculations and SPSS (ver:13) statistical package program was used for calculations.

RESULTS

Clinically all data of calves included in the control group were at normal physiological limits. In most of the diarrhea calves presenting to the clinic, lymph nodes are swollen especially prescapular and body temperatures average 37.84±0.36 °C, heart rate average 125.28±5.12 per minute and breathing frequency average 36.28±4.17 per minute. Conjunctiva hyperemic, sunken eyes and dehaydration were detected; their stools were soft and waterly in others.

Hematological parameters in blood samples taken with anticoagulants shown at Table 1. Comparing to the control group a statistically significant increase ($p<0.05$) in WBC, Neu, Hb and Hct levels was detected in diarrhea calves.

From the serum obtained biochemically; glucose, Tp, Alb, BUN, Cr and CK levels, as well as the absorbance values obtained in Cys-C in blood serums were determined (Table 2). In diarrhea calves, there was a statistically significant increase in Cys-C, BUN ($p<0.01$) and Cr ($p<0.05$) levels, and a statistically significant decrease in glucose levels ($p<0.01$) and Alb levels ($p<0.05$) compared to the control group.

Table 1. Hematologic data of healthy and patient group

Parameters	Control Group (Mean± St. dev.) (n=10)	Patient Group (Mean± St. dev.) (n=22)
WBC (m/mm3)	8.64±1.04	14.93±8.28*
Lym (%)	56.12±8.35	40.51±20.32
Mo (%)	3.97±0.88	5.30±2.43
Neu (%)	37.87±7.81	59.01±33.87*
Eo (%)	1.63±1.10	1.86±2.20
RBC (m/mm³)	9.63±1.37	9.17±1.98
MCV (fl)	32.43±1.63	35.53±5.08
MCHC (g/dl)	38.90±3.85	39.35±4.44
Hct (%)	25.48±1.20	33.78±1.52*
Hb (g/dl)	9.38±0.82	12.73±0.59*

The difference depending on control group *: $p<0.05$, **: $p<0.01$, and ***: $p<0.001$ statisically

Table 2. Biochemical data healthy and patient group

Parameters	Control Group (Mean± St. dev.) (n=10)	Patient Group (Mean± St. dev.) (n=22)
Glycose (mg/dl)	111.86±21.86	81.63±17.18**
Tp (g/dl)	5.82±0.21	5.97±0.32
Alb (g/dl)	3.31±0.30	2.92±0.33*
BUN (mg/dl)	9.85±0.40	44.62±8.06**
Cr (mg/dl)	1.11±0.11	3.38±0.70*
CK (U/l)	242.71±39.24	607.93±123.28
Cys-C (ng/ml)	6.96±0.86	10.54±3.75**

The difference depending on control group *: $p<0.05$, **: $p<0.01$, and ***: $p<0.001$ statisically

Table 3. Blood gas data of healty and patient group

Parameters	Control Group (Mean± St. dev.) (n=10)	Patient Group (Mean± St. dev.) (n=22)
pH	7.39±0.06	7.24±0.16*
pCO2 (mm Hg)	37.45±9.84	38.38±6.29
pO2 (mm Hg)	37.13±7.51	30.92±11.53*
HCO3- (mmol/l)	22.36±6.03	17.38±7.78
Base (mmol/l)	-1.89±5.73	-9.38±9.98*
Na+ (mmol/l)	136.88±3.36	132.92±8.38
K+ (mmol/l)	4.90±0.63	6.20±1.63*
Cl- (mmol/l)	99.50±3.78	95.87±8.84

The difference depending on control group *: $p<0.05$, **: $p<0.01$, and ***: $p<0.001$ statisically

Table 4. The correlation analysis between Cys-C with pH, pCO_2, pO_2, HCO_3^-, base, Na^+, K^+, Cl^-, BUN, Cr, Hct and Hb

Parameters		Cys-C	pH	pCO2	pO2	HCO3-	Base	Na+	K+	CL-	BUN	Cr	Hct	Hb
Cys-C (ng/ml)		1.000												
	P													
pH		0.264	1.000											
	P	0.235												
pCO2 (mm Hg)		0.009	0.184	1.000										
	P	0.968	0.390											
pO2 (mm Hg)		0.145	0.016	-0.254	1.000									
	P	0.521	0.939	0.231										
HCO3- (mmol/l)		0.215	0.941**	0.458*	-0.021	1.000								
	P	0.337	0.000	0.024	0.923									
Base (mmol/l)		0.216	0.957**	0.411*	-0.055	0.990**	1.000							
	P	0.334	0.000	0.046	0.797	0.000								
Na+ (mmol/l)		0.093	-0.042	0.080	-0.084	-0.039	-0.097	1.000						
	P	0.679	0.845	0.709	0.697	0.858	0.651							
K+ (mmol/l)		-0.001	-0.354	0.081	-0.046	-0.247	-0.238	-0.598**	1.000					
	P	0.998	0.090	0.707	0.832	0.245	0.263	0.002						
CL- (mmol/l)		0.132	-0.257	-0.118	-0.117	-0.280	-0.329	0.858**	-0.485*	1.000				
	P	0.557	0.225	0.582	0.585	0.186	0.116	0.000	0.016					
BUN (mg/dl)		-0.337	-0.496	-0.245	0.166	-0.447	-0.456	-0.557*	0.688**	-0.494	1.000			
	P	0.202	0.051	0.360	0.540	0.082	0.076	0.025	0.003	0.052				
Cr (mg/dl)		-0.035	-0.218	-0.276	0.473	-0.226	-0.218	-0.628**	0.655**	-0.632**	0.784**	1.000		
	P	0.579	0.417	0.300	0.064	0.399	0.418	0.009	0.006	0.009	0.000			
Hct (%)		-0.316	-0.502*	-0.490*	0.163	-0.557**	-0.579**	0.103	0.280	0.243	0.556*	0.412	1.000	
	P	0.152	0.012	0.015	0.447	0.005	0.003	0.633	0.185	0.252	0.025	0.113		
Hb (g/dl)		-0.342	-0.545**	-0.385	0.121	-0.565**	-0.597**	0.028	0.320	0.247	0.494	0.322	0.900**	1.000
	P	0.119	0.006	0.063	0.572	0.004	0.002	0.895	0.127	0.245	0.052	0.223	0.000	

Correlation *: p <0.05, **: p <0.01, and ***: p <0.001; the value in the row is statistically significant according to the value in the column

The blood taken into the heparinized syringe in accordance with the procedure was immediately scanned in the blood gas device and the data were recorded according to the patient protocol. Blood gases (pH, pO_2, pCO_2, HCO_3^-), and serum Na^+, K^+ and Cl^- were determined by the blood gas analyzer (Table 3). In diarrhea calves a statistically significant increase in K^+ levels compared to the control group ($p<0.05$). A statistically significant decrease ($p<0.05$) was detected in pH, pO_2 and base levels. Data of the correlation analysis between Cys-C with pH, pCO_2, pO_2, HCO_3^-, base, Na^+, K^+, Cl^-, BUN and Cr were determined (Table 4).

DISCUSSION

Diarrhea can be fatal in neonatal calves as a result of dehydration and acidosis, which can result from various pathogens or factors that play a role in the development of diarrhea (Sobiech et al., 2013; Cho and Yoon, 2014; Pereira et al., 2017).

The 22 diarrhea calves that used in this study were devided into three groups depending on their daily age, and sometimes there was one or more causes of disease was observed. By evaluating these results, it was observed that Giardia, *E. coli*, Coronavirus, Rotavirus and Cryptosporidium were among the factors causing diarrhea expressed by researchers (Xiao et al., 1993; Bazeley, 2003; Gomez et al., 2013). In the clinical examination of 10 healthy calves included in the control group, all physiological parameters are in the reference range that reported by the researchers (Wilson et al., 2000; Piccione et al., 2010; Silva et al., 2016) and this is an indication that the calves that make up the control group are healthy.

In 22 diarrhea calves included in the study, clinical results support the results stated by the researchers (Millemann, 2009; Sobiech et al., 2013; Dawes et al., 2014; Bednarski and Kupczynski, 2015; Pereira et al., 2017). All clinical signs may not occur at the same time in a diarrhea calves. While some calves only have fluid and electrolyte losses, others may show severe clinical symptoms (high fever, weakening etc.) (Pereira et al., 2017). Therefore, laboratory tests in diarrhea calves can provide important information about the diagnosis, treatment and prognosis of the disease (Mohri et al., 2007). Along with hematology and biochemical analyzes, blood gas analysis is a good method to determine the severity of metabolic acidosis (Berchtold, 1999; Kasari, 1999).

The control group in this study, hematological parameters of healthy calves were found by researchers (Knowles et al., 2000; Mohri et al., 2007; Ježek et al., 2011; Bellino et al., 2012; Klinkon and Ježek, 2012; Panousis et al., 2018) found in the reference ranges expressed (Table 1). This situation supports that the calves included in the study are healthy.

By comparing the hematological parameters of the calves in the patient group and the control group, there was a significant increase ($p<0.05$) in WBC 14.93±8.28 m/m3, Neu 59.01±33.87, Hct 33.78±1.52, and Hb 12.73±0.59 g/dl values (Table 1). This situation coincides with the statements (Cambier et al., 2001; Dawes et al., 2014; Heller and Chigerwe, 2018). This situation thought to be caused by fluid and electrolyts losses and infection.

The glucose level of healthy calves included in this study was between 111.86±21.86 mg/dl (Tablo 3). This value was within the reference ranges expressed by the researchers (Knowles et al., 2000; Bellino et al., 2012). This is an indication that the control group calves included in the study are healthy.

In this study, the glucose level of diarrhea calves included in the patient group was 81.63±17.18 mg/dL (Table 2). When this situation was compared to the control group, a statistically significant decrease was observed ($p<0.01$). These data are similar to the data of the researchers (Sobiech et al., 2013; Bednarski and Kupczynski, 2015; Trefz et al., 2017) and it thought that the reason for the decrease in serum glucose levels, loss of the sucking reflex, problems with food, malnutrition, nutration and diarrhea-related losses.

In healthy calves forming the control group, the Tp level was between 5.82±0.21 g/dl, and the Alb level was between 3.31±0.30 g/dl (Table 2). These values were within the reference ranges expressed by the researchers (Knowles et al., 2000; Mohri et al., 2007; Klinkon and Ježek, 2012; Panousis et al., 2018). This is an indication that the control group calves included in the study are healthy.

In diarrhea calves included in the study, the level of Tp was 5.97±0.32 g/dl, Alb was 2.92±0.33 g/dl, and there was only a statistically decrease in Alb level compared to the control group ($p<0.05$) (Table 2). This situation is thought to occur as a result of infectious factors in the etiology of diarrhea affecting the liver, increased protein catabolism in diarrhea, protein loss through the digestive system, loss of sucking reflex, malnutrition and passive

transfer failure in neonatal calves. These findings are coincided with the statement that the decrease of serum Alb levels in calves may be a result of liver damage or protein catabolism in long term diarrhea as the researchers proved Seifi et al. (2006), Klinkon and Ježek, (2012) and Heller and Chigerwe (2018).

The control group BUN level included in this study was between 9.85±0.40 mg/dl (Table 2). These values were within the reference ranges expressed by the researchers (Mohri et al., 2007; Klinkon and Ježek, 2012; Başer and Civelek, 2013). This is an indication that the control group calves included in the study are healthy.

In diarrhea calves included in the study, a statistically significant increase ($p<0.01$) was detected in BUN levels 44.62±8.06 mg/dl compared to the control group. It was observed that this level coincided with the statements of the researchers (Klinkon and Ježek, 2012; Başer and Civelek, 2013). It is thought the reason for its appearance is due to metabolic acidosis, hypoglycaemia and increased catabolism. However, it indicates that increased dehydration and hyperkalemia affect kidney functions.

The Cr level of the healthy calves included in the study was 1.11±0.11 mg/dl (Table 2). This value appears to be within the reference ranges expressed by the researchers (Mohri et al., 2007; Gomez et al., 2013). While the Cr level in diarrhea calves included in the study was measured 3.38±0.70 mg/dL, a statistically significant increase ($p<0.05$) was found compared to the control group. In this situation, for diarrhea calves the levels appears to be coincide with the researchers expressing (Almy et al., 2002; Seifi et al., 2006; Mohri et al., 2007; Cobrin et al., 2013) as well as, kidney functions are affected due to increased Hct, fluid and electrolyte loss that associated with diarrhea.

Başer and Civelek (2013) in their study; CK level of healthy calves are 404.80±234.16 U/l, Özkan et al. (2011), in their study, reported that it is between 316.18±37.64 U/l. CK levels were found between 242.71±39.24 mg/dl in the healthy calves that constituted the control group, and these values were found to be close to the data of the researchers.

Başer ve Civelek (2013) in their study, the CK level was between 944.94±269.65 U/l for diarrhea calves, Özkan et al. (2011) reported between 884.80±196.00 U/Ll. In diarrhea calves included in the study, CK level was determined as 607.93±123.28 mg/dl (Table 2). Compared to the control group, the level of CK in diarrhea calves is not statistically significant, although it increases ($p>0.05$) (Table 2). These data are among the values expressed by the researchers (Özkan et al., 2011; Klinkon and Ježek, 2012; Başer and Civelek, 2013), this situation is due to diarrhea which causes increased metabolic acidosis, Hct, fluid and electrolyte loss, glucose and Alb losses. It is thought that these are the reasons of varying degrees of muscular dystrophy.

Blood gas analysis has been routinely used in the diagnosis and treatment of diarrhea calves (Seifi et al., 2006; Heller and Chigerwe, 2018). The pH of the healthy calves included in the study was detected as 7.39±0.06, pCO_2 37.45±9.84 mm Hg, pO_2 37.13±7.51 mm Hg (Tablo 4). These levels appear to be within the reference ranges expressed by the researchers (Bouda and Jagos, 1984; Bellino et al., 2012; Gomez et al., 2013; Sobiech et al., 2013). In the light of these data, it is an indication that the calves included in the control group are healthy.

In the blood gas analysis of diarrhea calves included in the study, a statistically significant decrease ($p<0.05$) was detected at pH 7.24±0.16 and pO_2 30.92±11.53 mm Hg compared to the control group (Table 3). However, there was no statistically change in pCO_2 level. It was observed that the values obtained coincided with the statements of the researchers (Gomez et al., 2013; Sen and Constable, 2013). The decrease in pH and pO_2 may be an indicator of malnutrition, dehydration due to diarrhea, fluid and electrolyte loss, the pCO_2 level does not change because the kidney and respiratory system are trying to respond to metabolic acidosis.

For the healthy calves included in this study HCO_3^- level was determined as 22.36±6.03 mmol/l and the base level was -1.89±5.73 mmol/l (Table 3). These levels were within the reference ranges expressed by the researchers (Bouda and Jagos, 1984; Bellino et al., 2012; Gomez et al., 2013; Sobiech et al., 2013). This indicates that the control group calves included in the study did not have any metabolic and systemic diseases.

In diarrhea calves included in this study, the HCO_3^- level was measured as 17.38±7.78 mmol/l and the base level as -9.38±9.98 mmol/l (Table 3). It was found that these levels coincide with the statements of the researchers (Gomez et al., 2013; Sen and Constable, 2013). Although there was no significant change in HCO_3^- level in diarrhea calves compared to the control group, a statistically significant decrease was observed in the base ($p>0.05$). It is thought that this condition may be due to diarrhea which causes Na^+ and HCO_3^- loss, fluid loss,

bacterial fermentation of the carbohydrates in the intestines which leads to a base excess associated with the apperance D-lactate, furthermore venous pO_2 reduction, L-lactic acid accumulation in ineffective tissue perfusion, and a decrease in H^+ excretion in ineffective renal perfusion.

Na^+ value of healthy calves included in the study was 136.88±3.36 mmol/l, K^+ 4.90±0.63 mmol/l and Cl^- 99.50±3.78 mmol/l (Table 3). It is seen that these levels are within the reference ranges given by the researchers (Maach et al., 1991; Mohri et al., 2007; Bellino et al., 2012; Klinkon and Ježek, 2012; Gomez et al., 2013).

Serum Na^+ level is significantly lower in calves with acute diarrhea (131.2±7.2 mmol/l) compared to healthy calves of the same age (140.0±9.9 mmol/l) (Klinkon and Ježek, 2012). During acute diarrhea in calves, the amount of stool may increase 40 times and electrolyte loses take place with the stool. In these calves, the Cl^- level (95.6±6.9 mmol/l) was significantly lower compared to healthy calves (103.3±6.9 mmol/l) (Klinkon and Ježek, 2012). Although this situation decreases at Na^+ 132.92±8.38 mmol/l and Cl^- 95.87±8.84 mmol/l, this decrease is not statistically significant ($p>0.05$). Gomez et al. (2013). Na^+ (78%) and Cl^- (68%) in diarrhea calves reported that they were within normal ranges.

In diarrhea calves included in the study, there is a statistically significant increase in K^+ 6.20±1.63 mmol/l level ($p<0.05$). This coincides with the statements of the researchers (Maach et al., 1992; Constable and Grünberg, 2013; Sen and Constable, 2013; Sobiech et al., 2013). Diarrhea is thought to cause dehydration, acidemia, and hyperkalemia and impaired cardiovascular and kidney functions losses.

Serum Cys-C is reported to be of clinical importance in the early diagnosis and treatment of acute kidney injury and chronic renal failure in the evaluation of renal dysfunction (Cobrin et al., 2013; Toprak, 2013; Nakhjavan-Shahraki et al., 2017). Determination of Cys-C in serum and urine can be routinely used. Their level in serum almost entirely depends on kidney function (Onopiuk et al., 2015).

In the literature searches, no research on Cys-C level in calves and even ruminants have been detected until this time. There are studies on Cys-C in dogs (Almy et al., 2002; Braun et al., 2002; Pagitz et al., 2007; Miyagawa et al., 2009; Monti et al., 2012; Ghys et al., 2014).

Normal serum Cys-C values in humans are between 0.6 and 1 mg/l (Villa et al., 2005). An overlap of plasma Cys-C concentration was observed (0.12 to 1.10 mg/lin adult dogs, 0 to 1.73 mg/l in young dogs and 0 to 1.60 mg/lin older dogs) (Ghys et al., 2014). In another study, the average Cys-C concentration in healthy dogs and dogs with kidney failure was 1.08±0.16 mg/l and 4.37±1.79 mg/l, respectively (Almy et al., 2002;). In a study on healthy dogs, they express the upper limit of Cys-C as 1.3 mg/L using an immunoturbidimetric procedure for human Cys-C (Braun et al., 2002). Onopiuk et al. (2015) they found serum Cys-C level as 0.96 µg/ml in their study on adult people.

The Cys-C level in the healthy calves that constitute the control group included in this study is 6.96±0.86 ng/ml, and in diarrhea calves; it was determined as 10.54±3.75 ng/ml. According to the control group, this increase was found to be statistically significant in diarrhea calves. This situation, thought that it may have been occurred because of the association with the buffering of metabolic acidosis resulting from anaerobic respiration due to decreased fluid volume and electrolyte loss such as pO_2 and HCO_3^- loss, excessive effort and consequently decreased glomerular filtration and destruction of glomeruli, all of these associated with diarrhea.

In the correlation test, it is seen that although there is positive and negative relation between hematological, biochemical and blood gas parameters measured in the study was detected, this relationship was not statistically significant.

CONCLUSION

As a result; due to the physiopathological changes in diarrhea, WBC, Hct, K^+, BUN and Cr levels increased, pH, base, glucose and Alb levels decreased; Cys-C level which is an indicator of kidney degeneration increased statistically significantly compared to the healthy control group, it is believed that this increase may have diagnostic significance, the Cys-C levels determined in the control group may be the normal value for calves, and there was a conviction that more researches should be conducted with more sampling.

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The Effect of Atropine on Post-operative Cardio-Respiratory Effect and Body Temperature in Cats That Undergoing Elective Ovariohysterectomy

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ABSTRACT

Objective: The purpose of the study reported here, to investigate the effect of atropine on cardio-respiratory parameters and body temperature in cats undergoing ovariohysterectomy with the combination of medetomidine-ketamine anesthesia.

Materials and Methods: Twenty-six adult female intact domestic cats were admitted to Ankara University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology for routine elective ovariohysterectomy. The animals were divided into two groups by the randomized grouping method before the operation. Trial animals (n=14) received atropine together with medetomidine and ketamine anesthesia. In control animals (n=12) received the same anesthesia without atropine injection. At the end of the operation, animals were moved to the critical care unit and the measurements of vital parameters were performed. Heart, respiration rates, deep rectal temperature and status of anesthesia recovery were recorded after the operation every 10 minutes for 1 hour.

Results: There was a group, time, and group x time interaction noted for heart rate. Treatment cats showed greater heart rate during measurement. The mean respiratory rate and deep rectal temperature were in reference ranges for cats and similar for both groups. In both groups significant sedation induced, however, the scores were not statistically significant among groups.

Conclusion: In conclusion, atropine is an effective drug preventing decrease of heart rate and patients have shown less undesirable side effects when it is used before the administration of medetomidine in cats that operated for ovariohysterectomy.

Keywords: *Atropine, Anesthesia, Cat, Ovariohysterectomy*

INTRODUCTION

General anesthesia a controlled drug-induced reversible intoxication of the nervous system. In this method, the patient neither perceives nor recalls noxious or painful stimuli (Hall et al., 2001). Anesthesia is a requirement for the success of surgical procedures to ensure petitive analgesia and muscle relaxation. General anesthesia is important for performing large operations in small animal applications (Haque et al., 2019).

Medetomidine, a powerful and specific α2-adrenoceptor agonist, is used worldwide as a sedative and analgesic drug for veterinary use in small animals (Cullen, 1996). Medetomidine has dose-dependent biphasic cardiovascular effects. After the administration, hypertension, and bradycardia lasting within 15 to 20 minutes. After

that, sympathetic tone decreases due to vasodilation, hypotension, and bradycardia. The monitoring of vital parameters such as heart rate per minute and blood pressure should be performed. (Fossum, 2007).

Ketamine is a dissociative anesthetic that has been used in veterinary medicine for a very long time. It has cardiovascular effects similar to sympathetic nervous system stimulation with increased heart rate and blood pressure, cardiac output, and cardiac oxygen demand, especially if high doses of ketamine are used, dramatic drops in heart rate and blood pressure may occur (Fossum, 2007). The medetomidine-ketamine combination is used for anesthesia of cats that undergoing ovariohysterectomy. This method of anesthesia has some advantages such as adequate respiration, provision of analgesia, and useful for intramuscular administration (Kalchofner Guerrero *et al.,* 2014). Both medetomidine and ketamine cause bradycardia. Blood pressure should be measured before bradycardia is treated. If necessary, anticholinergic drugs can be used to treat the second phase of bradycardia when hypotension is also present. Anticholinergic drugs such as atropine and glycopyrolate are used in combination with α2 adrenoceptor agonists by many anesthesia practitioners based on early studies assessing only heart rate. However, the use of such combinations is controversial. Although anticholinergics have been shown to reduce heart rate, cardiac output, and decreased oxygen delivery, these drugs increase the size and duration of the hypertensive phase caused by α2 agonists (Fossum, 2007; Monterio *et al.,* 2009).

Atropine is an anticholinergic medication often added to premedication. Atropine reduces salivation and increases heart rate by acting in parasympathetic cholinergic areas. Useful to intravenous administration for the treatment of bradycardia in an anesthetized patient. The administration of low doses results in a paradoxical decrease in heart rate in consequence of the presynaptic cholinergic receptors inhibition (Stoelting *et al.,* 2006; Fossum, 2007).

The purpose of the study reported here, to investigate the effect of atropine on cardio-respiratory parameters and body temperature in cats undergoing ovariohysterectomy with the combination of medetomidine-ketamine anesthesia.

MATERIALS and METHODS

Twenty-six female intact domestic cats among the different breeds (mix breed, Angora cat, British Shorthair, Siamese) were admitted to Ankara University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology for routine elective ovariohysterectomy. The animals weighing were between 2.5-4.5 kg and the ages were between 7 months to 12 months. The animals were considered healthy based on normal physiological examination. The cats were vaccinated routinely and free of intestinal parasites.

Study design

The animals were divided into two groups by the randomized grouping method before the operation. Trial animals (n=14) received atropine together with medetomidine and ketamine anesthesia. In control animals (n=12) received the same anesthesia without atropine injection. Measurement of vital parameters was performed and recorded after operation for 60 minutes.

Anesthesia Protocol and operation

At least 12 hours before the operation food and water access were ceased. The cats were placed in unheated cages at least 30 min before the operation. Premedication was performed with subcutaneous atropine (50 μg/kg; Atropin, Vetas, Turkey) and intramuscularly (i.m.) medetomidine (80 μg/kg medetomidine; Domitor, Zoetis, Finland) in trial group and alone medetomidine (i.m.) in control group. Ten minutes after premedication, ketamine (5 mg/kg ketamine; Alfamine, Ege Vet, Turkey) was injected intramuscularly. After then, cats were moved stainless steel preoperative preparation table. An intravenous catheter was replaced to the cephalic vein and Ringer's lactate solution was administered at the beginning of the operation (10ml/kg/h). The left flank was clipped, cleaned and disinfected in cats for cleaning at the preparation of the operation area. A left flank blunt incision was performed and both ovaries and uterine horns and corpus uteri were removed. Absorbable suture material (Polyglycolic acid, Katsan, Turkey) was used for closing the abdomen wall and skin. Injectable amoxicillin-clavulanic acid (25 mg/kg; Synulox, Zoetis®, Finland) was administered subcutaneously at the end of the operation. Atipamezole (200 μg/kg; Antisedan, Zoetis, Finland) was injected intramuscularly 45 minutes after the ketamine injection. The cats were discharged from the clinic on the day of operation and animals were presented to the clinic 3 days after

the operation. Then the owners continued the treatment with antibiotics for 7 days. The abdominal skin sutures were removed on the 7th day after the operation.

Measurements of vital parameters

At the end of operation animals were moved to the critical care unit and the measurements of vital parameters were performed. Heart rate was measured and recorded after the operation. The measurement was performed by auscultation with a stethoscope. Respiration rates were determined by following the intercostal muscle movement. Deep rectal temperature was measured by a rectal thermometer. The heart rate, respiration rate, and deep rectal temperature were measured every 10 minutes for 1 hour.

Anesthesia recovery

Assessment of recovering from anesthesia were adapted from Ko et al., (2001) and started just after operation and lasted 1 hour later. For assessing the following measures were used, motor activity, respiration, circulation, level of consciousness, the coloration of the mucosa. Grades from 1 to 4 were assigned to the degree of functional recovery. The fourth degree was deep anesthesia and the first degree was recovered anesthesia. In the first degree, cats successfully attempt to walk unassisted.

Statistical Analyses

Before performing the statistical analysis, data were examined with the Shapiro-Wilk test for normality and Levene test for homogeneity of variances as parametric test assumptions. Descriptive statistics for each variable were calculated and presented as "Mean ± Standard Error of Mean". The student t-test was used to evaluate the differences between groups for the duration of the operation and weight of cats. The effect of group, time of measurement, and their interaction on hearth rate, respiration, body temperature, and the score of sedation was analyzed linear mixed models by using the following model with repeated measures:

$$Yijk = + Gi + Tj + (G\ T)ij + eijk$$

Where, Yijk, dependent variable; , overall mean; Gi, the effect of the group (i = Control and Trial groups); Dj, effect of time of measurement (j = 1,2,3,4,5,6 measurement points); (G D) ij, the interaction between group i and time of measurement j; and eijk, residual error. Animals within the group were assessed as a random effect, while group, period, or day of sampling, and their interaction were assessed as a fixed effect. When a significant difference was revealed, any significant terms were compared by Simple effect analysis with Bonferroni adjustment. P<0,05 was considered as significant in all analyses.

RESULTS

Cats among the different breeds (mix breed, Angora cat, British Shorthair, Siamese) were allocated to the present study. The mean age was similar among groups. The mean time of operations was similar. Also, the mean body weights were not statistically different among groups (Table 1).

Table 1. Mean operation time and body weight in trial and control cats.

Parameters	Groups	Mean	Standard Error	*P*
Time of Operation	Trial	25.15	2.01	0.878
	Control	24.73	1.81	
Weight	Trial	3.00	0.13	0.579
	Control	2.89	0.15	

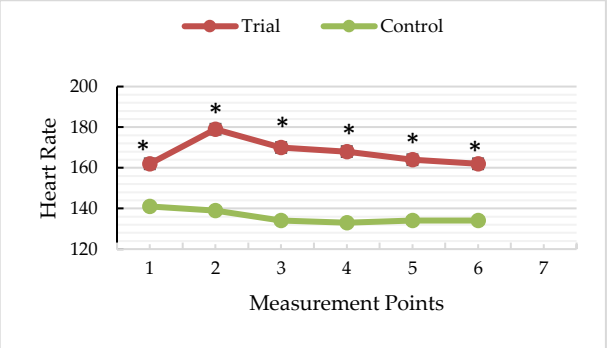

Figure 1. Heart rate (bpm) after the operative approach in Atropine treated and control cats.

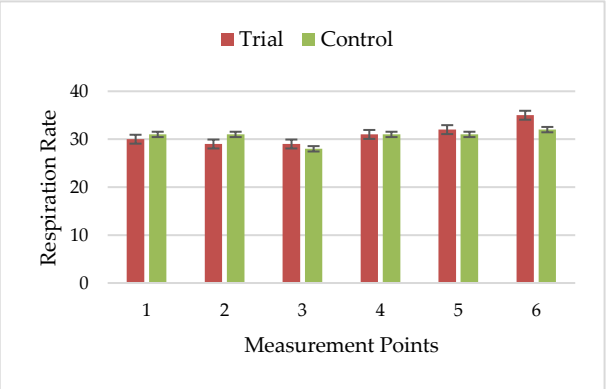

Figure 2. Respiratory rate per minuteafter the operative approach in Atropine treated and control cats.

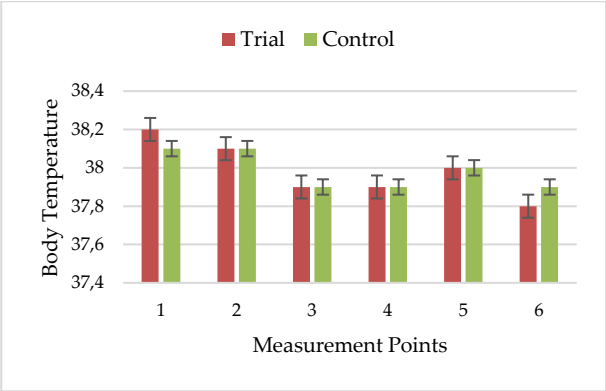

Figure 3. Body temperature changes after the operative approach in Atropine treated and control cats.

There was a group, time, and group x time interaction noted for heart rate. The mean heart rate was greater in trial group for all measurement points (Figure 1). However, none of the cats experienced bradycardia or tachycardia during the study period. There was no interaction noted between group x time for respiration rate. The mean respiratory rates were presented in Figure 2. There was no group, time, or group x time interaction recorded for deep rectal temperatures. The body temperatures were in normal ranges for all measurement points (Figure 3). In both trial and control groups, all cats assigned a sedation score of 0 at baseline. In both groups significant sedation induced, however, the scores were not statistically significant among groups.

DISCUSSION

In the present study we evaluated the post-operative role of atropine on selected vital parameters in cats that had elective ovariohysterectomy. α-2 adrenergic receptor agonist administrations resulted in cardiovascular changes. Especially heart rate parameter decreases under the baseline value (Lamont *et al.,* 2001; Graholm *et al.,* 2006). Under the influence of medetomidine and ketamine anesthesia central venous pressure and systemic vascular resistance increases although, cardiac index decreases (Lamont *et al.,* 2001). Interestingly, the increased dose of medetomidine did not affect the severity of the decrease of heart rate in dogs (Monteiro *et al.,* 2008). However, the dose depended heart rate evaluation was not measured in the present study (Ko *et al.,* 2001). The present study showed that the use of medetomidine alone resulted in decreased heart rate during the measurement period. The role of anticholinergics in combination with α-2 adreno-receptor agonists is not exactly known the data about combine used has inconsistent results in dogs (Sinclair *et al.,* 2003). Especially combination using α-2 adrenoreceptor agonists and anticholinergics prevented reduction in heart rate in dogs. Also, Monteiro *et al.,* (2009) showed that atropine prevented medetomidine caused bradycardia in cats. However, in that study, the authors evaluated the role of different doses of medetomidine. Different than that, in the present study we have used the same doses per kg for all animals and evaluated the role of atropine directly. Similar to both previous studies (Selmi *et al.,* 2002; Monterio *et al.,* 2009) we have encountered decreased heart rate post-operative measurement points in control cats. And atropine prevented heart rate to decrease. Unlike the other studies, we have just evaluated the post-operative period until anesthesia recovery for 1 hour. Monterio *et al.,* (2009) reported that atropine has a shorter duration of effect than medetomidine. Our study has no information because we have determined the post-operative 1 hour to evaluate the early effect of drug administration, but Monterio *et al.,* (2009) evaluated animals for 3 hours. Short (1991), reported that atropine administration increases the risk of cardiac dysrhythmia which associated with increased heart rate and not affected respiratory function. Possibly, the way of administration is a factor. Intravenously injected atropine and cardiac arrhythmias such as multiple premature ventricular contractions observed (Short, 1991; Lamont *et al.,* 2001). On the other hand, Ko *et al.,* (2001) atropine administered only intramuscularly in combination with medetomidine and reported only a few dogs had premature contractions. Different than others, we have administered atropine subcutaneously maybe that is a factor we did not record any cardiac arrhythmias.

In dogs and cats α-2 adrenoreceptor agonists decrease the body temperature. This process-related due to decreased heat production, muscle relaxation, and the influence of the drug in the thermoregulatory system. There is another mechanism that affects body temperature loss (Granholm *et al.,* 2006). The duration and area of operation might be effective in body temperature. In the present study, we have used an automated critical care unit to keep animals in a normal temperature environment and oxygenated. Heat loss can be originated from surgical sites well especially major body cavities such as the abdominal cavity and thorax are exposed. The style

of operation maybe has a role in heat loss (Kelly *et al.*, 2012). In the present study, after the operation deep rectal temperatures were similar and not decreased under the reference value. We have operated cats from the left flank. This small invasive operation technique might decrease heat loss.

CONCLUSION

In conclusion, atropine is an effective drug preventing decrease of heart rate and patients have shown less undesirable side effects when it is used before the administration of medetomidine in cats that operated for ovariohysterectomy.

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Clinical Toxoplasmosis in Two Cats and its Treatment with Clindamycin

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ABSTRACT

Toxoplasmosis is a zoonotic disease, affecting birds, human beings and most warm-blooded animals throughout the world. On the following case report *Toxoplasma gondii* infection was detected in two cats. Primary clinical findings were defined as involuntary and continuous contraction of the hind limb muscles, incoordination and pain. *Toxoplasma gondii* generally progresses asymptomatically however when clinical signs do appear, *T. gondii* shows itself with neurological symptoms. In these cases, the diagnosis of the disease was made by enzyme-linked immunosorbent assays (ELISA) method. In both of the cases Clindamycin was given at a dose of 25mg/kg/24h for the first week and the dosage was rearranged to 12,5mg/kg/q12h. Clinical improvement was observed after one week and treatment was discontinued at the third week.

Keywords: *Toxoplasma gondii, ELISA, Diagnosis, Treatment, Cats*

INTRODUCTION

Toxoplasmosis is caused by an intracellular protozoal parasite *Toxoplasma gondii* and it is one of the most common parasitic infections of man and other warm-blooded animals (Dubey, 1998). It has been found globally, infecting nearly one-third of the human population (Dubey, 1998; Tenter et al., 2000).

While *T. gondii* can multiply asexually in all of its hosts, the only known definitive ones are feline species, including the domestic cat, Iriomote cat, leopard, tiger and lions (Lukesová & Literák, 1998). Humans, birds, and all other animals are mostly infected by contamination of the environment with feces which passes through immune-competent cats (Smith et al., 1992). Also in neonatal or young dogs and cats (e.g., transplacentally infected kittens), clinical toxoplasmosis is often persistent, disseminated, and has a high mortality rate (Davidson, 2000).

In humans *T. gondii* infection can result with clinical toxoplasmosis in the fetus; stillbirths, intensive neurologic and ocular diseases are common if the mother is infected during pregnancy. Most humans acquire the disease by ingesting oocyst-contaminated soil and water, or from undercooked meat containing tissue cysts, by transplantation, blood transfusion, laboratory accidents, or congenitally (Elmore et al., 2010).

Clinical signs of feline toxoplasmosis include; anorexia, cough, dyspnea, fever followed by hypothermia, lethargy, vomiting, diarrhea, peritoneal effusion, icterus, myocardial dysfunction encephalitis and sudden death (Dubey et al., 1990, 1996; Patton et al., 1991). Ophthalmic manifestations consists of retinochoroiditis and both anterior and posterior uveitis (Piper et al., 1970; Dubey et al., 1990, 1996; Davidson, 2000; Michael R. Lappin, 2010).

Clinicopathological findings and test results are not definitive for toxoplasmosis diagnosis. However, Toxoplasmosis should be on the differential list for

cats with nonregenerative anemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, neutropenia, eosinophilia, proteinuria, bilirubinuria, as well as increases in serum protein and bilirubin concentrations, and creatinine kinase, alanine aminotransferase, alkaline phosphatase, and lipase activities (Dubey et al., 2009; Michael R. Lappin, 2010).

Definitive diagnosis of feline toxoplasmosis is quite hard to make. Radiographic, hematologic, and biochemical changes sometimes occur but these results are not pathognomonic. High levels of Immunoglobulin G (IgG) antibody titers are not always an indication of the disease. ELISA can diagnose subclinical infections by using immunoglobulin M (IgM) antibody titers Michael R. Lappin et al., 1989a) and circulating antigen (Ag) (Michael R. Lappin et al., 1989b) detection, but their use with clinically ill animals has not been reported (Michael R. Lappin et al., 1989).

CASES

First Case

The first case was a 1-year-old, female, mix breed cat showing convulsions, pain, head flexed towards the chest and instability on hind limbs without problems with defecation or urination. She was brought to a clinic in Hatay, Turkey with suspicion of trauma and the treatment was applied accordingly with methylprednisolone sodium succinate, vitamin B complex, ranitidine and fluid therapy. The condition of the cat did not improve and it was referred to Small Animal Hospital, Faculty of Veterinary Medicine in Ankara. First the cat's neurological examination was performed and the results was normal and according to that the cat's treatment started at the Department of Internal Medicine.

During general examination ptosis on the left eye, pale mucous membranes with hyperemic areas noted on the soft palate was observed. Lower back and pelvic pain was noted with palpation. Heart rate was 140 bpm, respiratory rate was 80/min and capillary refilling time was < 2 seconds. Femoral arterial pulse was normal and there was no color change on footpads of the hind legs. Crackles and dyspnea were detected after auscultation. In radiographic images of the thorax bronchial structures had an increased opacity.

A Complete Blood Count (CBC) showed Leukocytopenia, lymphocytopenia, anemia and thrombocytopenia. Alanine aminotransferase (ALT) value was measured as 331 IU / L. FCOV / FIP IgG antibody titer was lower than 1:10 and Toxoplasma / Chlamydia IgG antibody titer was 1:64 resented by ELISA (Table 1, Table 2). Toxoplasma and Chlamydia antibody titer were checked. Toxoplasma antibody titer 1:64, Chlamydia antibody titer was less than 1:16.

Contractions of the hind limbs continued after the initial fluid and O_2 therapy initiated to improve the general condition and breathing problems. Following the test results Clindamycin 25 mg/kg/q24h IM was used for treatment. Within the first few days' response to treatment was positive but the treatment was continued for another 2 weeks 12.5mg / kg / 12h IM due to complaint of lameness in right hind leg. Lameness slowly decreased to normal at the end of third week of treatment. At the end of the third week Toxoplasma and Chlamydia antibody titer were checked again for control and the results were; Toxoplasma antibody titer 1:16, Chlamydia antibody titer was less than 1:16. Three months after the treatment, recurrence of disease has not occurred.

Table 1. Biochemical parameters of cases

Parameters	First Case		Second Case		Normal Range
	Lab. Value	Result	Lab. Value	Result	
Urea (mg/dl)	41.1	L	27.70	L	42.80- 64.20
Creatine (mg/dl)	0.71	L	12.24	N	0.80- 1.80
Total Protein (g/dl)	7.15	N	8.73	H	5.40- 7.80
Albumin (g/dl)	3.02	N	2.87	N	2.40- 3.80
ALT (IU/L)	331.0	H	53.00	H	0.00- 50.00
ALP (IU/L)	50.0	H	14.00	N	0.00- 70.00
CK (IU/L)	95.2	N	101.00	N	0.00 – 130.00

ALT alanine aminotransferase, ALP alkaline phosphatase, CK Creatine Kinase

Second Case:

The second case was an 11 years old, male, mix breed cat showing convulsions and pain on the hind legs. Owners consulted to a veterinary clinic after the cat has lost its appetite. Clinic referred the patient to Small Animal Hospital, Faculty of Veterinary Medicine in Ankara suspecting from an underlying neurological condition after the general examination.

Table 2. Hematologic parameters of cases

Parameters	First Case		Second Case		Normal Range
	Lab. Value	Result	Lab. Value	Result	
WBC	1.60	L	18.10	N	5.50- 19.50
LYM	0.90	L	0.60	L	1.10- 7.00
MONO	0.30	N	0.40	N	0.20- 1.50
NEUT	0.40	L	15.80	H	2.80- 13.00
EOS	0.00	L	1.30	N	0.10- 99.90
LYM%	53.50	N	3.30	L	15.00- 60.00
MONO%	19.00	H	2.20	N	0.50- 11.00
NEUT%	27.10	N	87.20	H	25.00- 85.00
EOS%	0.40	N	7.30	N	0.10- 99.90
RBC	1.82	L	7.96	N	5.00- 45.00
HGB	2.60	L	12.70	N	8.00- 15.00
HCT	6.80	L	34.90	N	25.00- 45.00
MCV	37.40	L	43.80	N	39.00- 50.00
MCH	14.60	N	16.00	N	12.50- 17.50
MCHC	39.10	H	36.60	N	31.00-38.50
RDWa	17.00	L	20.30	L	20.00- 35.00
RDW%	14.60	N	13.80	L	14.00- 18.50
PLT	16.00	L	10.10	N	200.00- 500.00
WBC	1.60	L	18.10	N	5.50- 19.50

WBC White blood cells, LYM Lymphocytes, MONO Monocytes, NEUT Neutrophil, EOS Eosinophils, LYM% Lymphocytes percent, MONO% Monocytes percent, NEUT% Neutrophil percent, EOS% Eosinophils percent, RBC red blood cells, HGB Haemoglobin, HCT Haematocrit, MCV Mean corpuscular volume, MCHC Mean corpuscular haemoglobin concentration, RDWa Red blood cell distribution, RDWa% Red blood cell distribution percent, PLT Platelet

General condition of the cat was good but it was struggling to urinate and defecate and it was not relating to a trauma or operation history. Right hind leg was hyperextended and show more pain compared to the other one. Lung auscultation revealed no abnormalities. The heart rate was measured as 120 bpm and the number of breaths were 64 per min. The color of the mucous membrane was normal and capillary refilling time was determined as normal. Femoral arterial pulse was normal and no color change was observed in the footpads of the hind legs.

Lymphocytopenia and thrombocytopenia was noted on CBC. Alanine aminotransferase was measured as 53 IU/L. (Table 1, Table 2). In Toxoplasma/ Chlamydia test performed by ELISA method, IgG antibody titer was 1:64. Toxoplasma and Chlamydia antibody titer were checked. Toxoplasma antibody titer 1:64, Chlamydia antibody titer was less than 1:16.

Treatment started with Clindamycin 25mg/ kg/ 24h IM. Since oral intake did not reach to a sufficient level on the 5th day of treatment jaundice was detected on the mucous membranes. Clindamycin dosage was rearranged to 12.5 mg/kg/q12h IM. Ornipural Solution® was used 2 ml/sc /48h and forced feeding was performed. The treatment process was completed at the end of the 3rd week. General condition improved to normal and problems with hind limbs completely resolved. After treatment clinical recovery maintained. The patient didn't show up for controls therefore patient doesn't have antibody titer after treatment.

DISCUSSION

Extraintestinal toxoplasmosis develops from intracellular replication of tachyzoites in hepatic, pulmonary, Central Nervous System (CNS) and pancreatic tissues (Michael R. Lappin, 2010). In both of our cases permanent contracture of the muscles of the hind limb and generalized fatigue were the first clinical sings of the disease. Symptoms relating to CNS problems might be seen in feline toxoplasmosis cases, *T. gondii* should be in the differential list when such symptoms are encountered.

Toxoplasmosis shows a variety of clinical signs, most commonly anorexia, lethargy, interstitial pneumonia, fever, hepatitis, gastrointestinal signs, hyperesthesia from myositis, and a variety of neurologic signs (Davidson, 2000). Decrease or loss of appetite and respiratory problems were also recognized in our cases and they are prevalent findings in other toxoplasmosis cases as well.

Variety of clinicopathologic abnormalities are recognized in feline toxoplasmosis including nonregenerative anemia, neutrophilic leukocytosis, lymphocytosis, monocytosis, neutropenia, eosinophilia, proteinuria, bilirubinuria, as well as in serum protein and bilirubin concentrations, creatinine kinase, alanine aminotransferase, alkaline phosphatase, and lipase activities might increase (Michael R. Lappin, 2010). Out of these nonspecific hematological and serum biochemical abnormalities we have recognized include leukopenia, lymphopenia and increase of alanine aminotrasferase activity in our two cases. Our

second patient also developed jaundice due to loss of appetite which leads to hepatic lipidosis and damage to the paranchyma as a direct effect of the parasite.

Various publications for the treatment of feline toxoplasma suggest the administration of clindamycin at a dose of 10-12 mg / kg p12h for 4 weeks (Michael R. Lappin, 2010). During our treatment, clindamycin was administered intramuscularly at a dose of 25mg / kg p24h during the first week. For the next 2 weeks, 12.5mg / kg p12h was administered and the treatment was completed.

A study done in Ankara region with samples taken from 129 cats 43.4% had positive results in titer of 1/16, 5.4% had in 1/64 and 17.8% had in 1/256 (Yücesan et al., 2019). Another study on seroprevalence of *T. gondii* in humans obtained 30.80% IgG, 3.78% IgM and 7.02% IgG and IgM positive results (Yereli et al., 2006). *Toxoplasma gondii* seroprevalence was found to be high in these studies. This report is written to draw attention to *T. gondii* since it has high prevalence and asymptomatic symptoms which could be easily overlooked by the veterinarians.

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Ectoparasites detected on a red fox (Vulpes vulpes Linnaeus, 1758) in Turkey and the first case of Hippobosca longipennis (Diptera: Hippoboscidae)

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ABSTRACT

The aim of this case presentation is to report ectoparasites detected in one red fox. The adaptation of red foxes to urban environments and their increasing number result in an increased risk of transmission of some ectoparasites and pathogens originating from ectoparasites to humans and domestic animals. In this study, one red fox (*Vulpes vulpes*) which was after a traffic accident was examined for ectoparasites in the Clinic of Hatay Mustafa Kemal University, Faculty of Veterinary. A total 14 flies, 13 ticks and 4 fleas were collected from the red fox. As a result of the microscopic examinations of ectoparasites, *Hippobosca longipennis* (9 ♀, 5 ♂), *Rhipicephalus turanicus* (8 ♀, 5 ♂), *Ctenocephalides felis* (1 ♀) and *Pulex irritans* (2 ♀, 1 ♂) were identified. With this study, *Hippobosca longipennis* was recorded from foxes for the first time in Turkey.

Keywords: *Ectoparasite, Fly, Tick, Flea, Vulpes vulpes*

INTRODUCTION

The red fox (*Vulpes vulpes*) is a canid species which has adapted to various habitats and climate conditions. It has a long nose, large ears in comparison with its head size and a long tail with a white tip (Larivière and Pasitschniak-Arts, 1996). Red foxes, animals and human beings are the final hosts of numerous endoparasites including zoonosis pathogens such as *Echinococcus multilocularis* and *Toxocara canis* (Gıcık et al., 2009). Red foxes that have adapted to urban environments and are increasing in number are reported to carry the risk of transmitting some ectoparasites and ectoparasite-induced pathogens to humans and domestic animals (Kočišová et al., 2006).

The aim of this case presentation is to report ectoparasites detected in one red fox.

CASE

One young male red fox (*Vulpes vulpes*) was wounded after a traffic accident on the Antakya-İskenderun road was examined for ectoparasites in the Clinic of Hatay Mustafa Kemal University Faculty of Veterinary on 26.05.2018. As a result of the examination, a total 14 hippoboscid flies, 13 Ixodid ticks and 4 fleas were collected from the red fox and taken to the laboratory in glass bottles containing 70% ethyl alcohol. Ticks and hippoboscid flies from the ectoparasites brought to the laboratory were examined under stereo microscope. Fleas were examined under binocular light microscope after being made transparent in 10% KOH solution during one day and by passing through alcohol series (Girişgin et al., 2018). As a result of the examination carried out by taking into consideration the morphological criteria in the related literature (Iwasa and Choi, 2013; Estrada-Peña et al., 2018), *Hippobosca longipennis* (9 ♀, 5 ♂) from flies (Figure 1), *Rhipicephalus turanicus* (8 ♀, 5 ♂) from Ixodid ticks, *Ctenocephalides felis* (1 ♀) and *Pulex irritans* (2 ♀, 1 ♂) from fleas were identified.

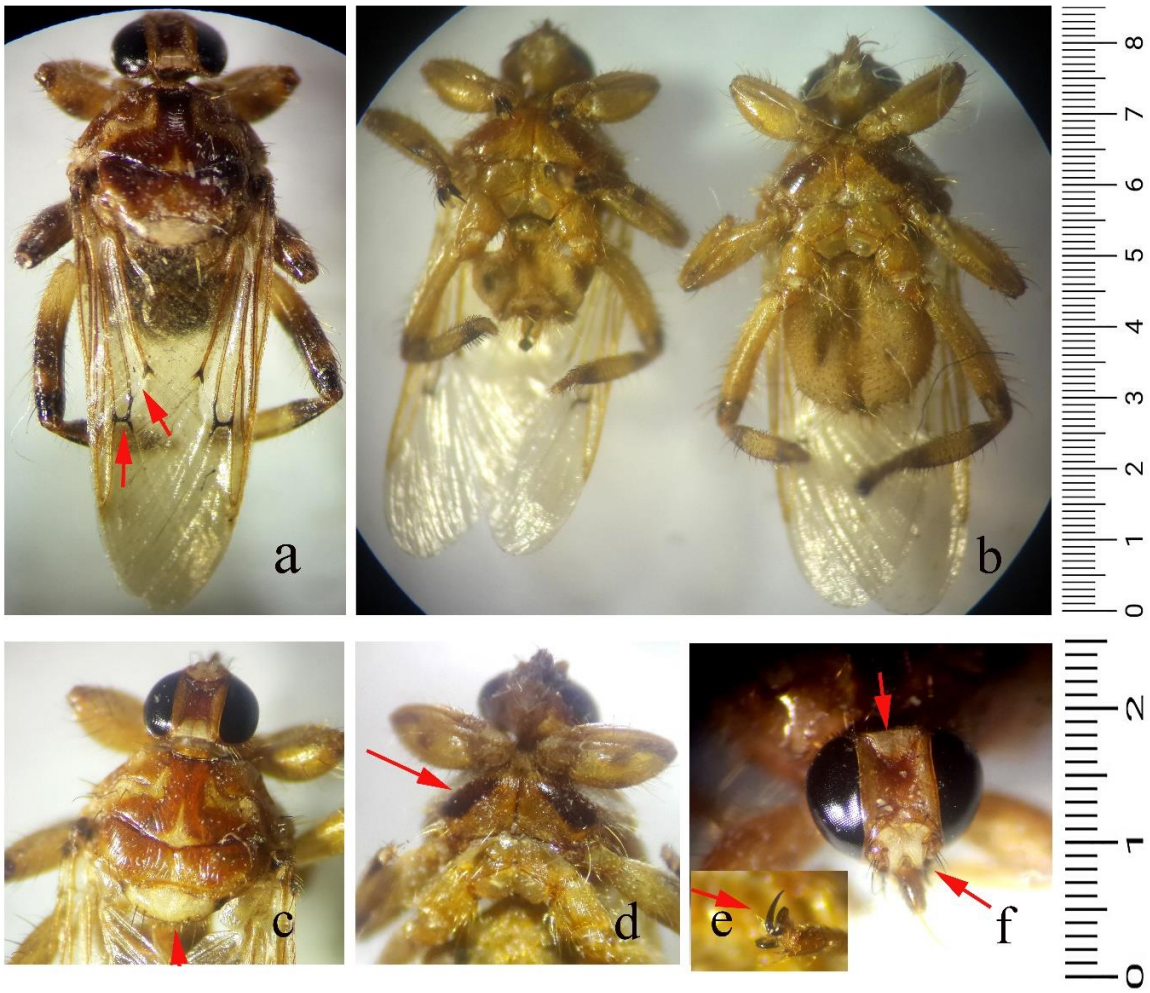

Figure 1. **a)** *Hippobosca longipennis* dorsal view, wing has two cross veins **b)** ventral view, left ♂, right ♀ **c)** Scutellum ivory-white **d)** Prosternum width greater than length **e)** nail structure **f)** The apical lobe of fronto-clypeus is regular and sharp triangular, the vertical plate on the medio-vertex has a semi-elliptical appearance, original

DISCUSSION

In studies that were conducted on red foxes around the world, cestodes, nematodes and trematodes (Letková et al., 2006; Gıcık et al., 2009; Jankovska et al., 2016) and protozoans such as *Hepatozoon canis* (Orkun and Nalbantoğlu, 2018) and *Isospora* spp. (Martínez-Carrasco et al., 2007) and various different louse, flea, tick, mite and fly species were reported (Sréter et al., 2003; Millán et al., 2007; Perrucci et al., 2016). In studies conducted in Turkey, *Ixodes kaiseri* (Orkun and Karaer, 2018), *Ixodes hexagonus*, *Haemaphysalis numidiana* (Aydın et al., 2011), *Haemaphysalis parva* (Orkun and Nalbantoğlu, 2018; Yaya et al., 2019), *Haemaphysalis sulcata*, *Haemaphysalis erinacei*, *Dermacentor reticulatus* (Yaya et al., 2019) *Rh. turanicus* (Orkun and Emir, 2019) tick species and *Ct. felis* (Aydın et al., 2011), *Ct. canis*, *P. irritans*, *Chaetopsylla globiceps* (Aydın et al., 2011; Yaya et al., 2019), *Chaetopsylla trichosa*, *Xenopsylla cheopis* and *Spilopsyllus cuniculi* (Yaya et al., 2019) flea species were reported to be found.

In the present study, four ectoparasite species, namely *H. longipennis*, *R. turanicus*, *Ct. felis* and *P. irritans* were identified in the red fox.

Hippobosca longipennis is a fly species which is extremely common in domestic dogs. This fly species, which can easily adapt to mild climates such as South Europe and the Mediterranean region, is a parasite that is originally found in wild carnivores in East Africa and is also commonly seen in foxes (Millán et al., 2007). The *R. turanicus* tick species, which shows a tendency to live in habitats in the Mediterranean region and across both Africa and Asia and uses various domestic and wild animals and sometimes even human beings for their developmental stage, is also seen in foxes (Millán et al., 2007; Chochlakis et al., 2014). The *Pulex* and *Ctenocephalides* flea species, which are important vectors of flea-based diseases, were determined in various carnivores, herbivores and omnivores mammals such as wild cats, canidae, skunks and badgers, Ground squirell, hares (Uslu et al., 2008; Dik and Uslu, 2018; López-Pérez et al.,

2018). In the present study, similar to various studies conducted around the world on red foxes, *Ct. felis* and *P. irritans* flea species were reported (Martínez-Carrasco et al., 2007; Foley et al., 2017).

In addition, the *R. turanicus* Ixodid tick, which is one of the ectoparasites determined in the red fox, was found in sheep, goat, cattle, red hawk (*Buteo rufinus*), fox and human beings (İnci et al., 2003; Ica et al., 2007; Açıcı et al., 2012; Oğuz et al., 2015; Gökpınar et al., 2017; Orkun and Emir 2019), while the *Ct. felis* (Aydın et al., 2011) and *P. irritans* (Aydın et al., 2011; Yaya et al., 2019) flea species were found in foxes (Aydın et al., 2011).

With this study, *H. longipennis* was determined for the first time in foxes in Turkey.

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