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A molecular investigation of Extended Spectrum Beta-Lactamase genes in *Escherichia coli* and *Klebsiella spp.* in raw cow milk

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ABSTRACT

Objective: Raw milk is an important source of nutrients. Therefore, today, there is a great demand for raw milk consumption. The positive side of milk consumption on growth and development cannot be ignored, but unfortunately, pathogens in raw milk are always potential public health risks for transmission pathogens. Bacteria such as Enterobacteriaceae in normal flora can cause serious problems due to their extended-spectrum beta-lactamase (ESBL) production. These bacteria and their resistance genes have been reported in raw milk. In this matter, the aim of the study is to determine the status of blaCTX-M-1, blaCTX-M-2, blaTEM and blaSHV genes responsible for the production of ESBL enzyme in Escherichia coli and Klebsiella spp. strains to identify risk factors in raw milk consumption and to gain an understanding of the epidemiology of these resistant strains.

Materials and methods: A total of different 50 raw milk samples were collected and subjected to phenotypic microbiological analysis and Real-time PCR targeting blaCTX-M-1, blaCTX-M-2, blaTEM and blaSHV genes. In the phenotypic analyses, suspicious isolates were identified by classical microbiological methods and antibiotic resistance profiles were revealed.

Results: These results indicated that raw milk is a potential reservoir for ESBL producing E. Coli, Klebsiella spp. strains are obviously significant. And It was determined that CTX-M-basedESBL genes are predominant in ESBL production.

Conclusion: The present study revealed that raw milk is epidemiologically involved in the transmission of ESBL genes. Raw milk could be distributed to ESBL genes widely and is consumed in Şanlıurfa.

Keywords: *E. coli, ESBL genes, Klebsiella spp., Raw Cow milk, qPCR*

INTRODUCTION

Raw milk is a particularly nutritious food having proteins, fats, carbohydrates, vitamins, minerals, and essential amino acids. Due to its near-neutral pH level and high-water activity, raw milk also provides ideal conditions for the growth of many microorganisms (Altun et al., 2002; Kim et al., 2017). More and more people are choosing to consume unpasteurized raw milk. The thought that some nutrients in raw milk will be lost after pasteurization is advocated as the reason for the increased interest in raw milk consumption. However, many epidemiological studies expressly show that raw milk can be contaminated with pathogens, some of which are incorporated with human diseases, as it also provides optimal

conditions due to the growth of many microorganisms (Oliver et al., 2009; Yun et al., 2020). While most studies about foods such as raw milk focus on zoonotic pathogens, there is a lack of data focused on bacteria associated with antimicrobial resistance in the normal flora (Gaffer et al., 2019; Shi et al., 2020; Plassard et al., 2021). In contrast, Gram-negative (GN) bacteria, such as Extended-spectrum beta-lactamase (ESBL), cephalosporins (AmpC), and carbapenemase (CP)-producing *Enterobacteriaceae*, have been identified in numerous environments worldwide, including bovine (Tóth et al., 2020). ESBL/AmpC/CP producing GN bacteria have also been reported in raw milk (Ansharieta et al., 2021). The increasing prevalence of antimicrobial resistance (AMR) continues to be a significant threat to global health. The extensive use of antibiotics in both human health and control of animal diseases is gradually reducing the time it takes for resistant strains to develop and multidrug-resistant strains of bacteria may cause life-threatening infections (Jena et al., 2017; Baran, et al., 2020; Tóth et al., 2020; Ansharieta et al., 2021). *Escherichia coli* (*E. coli*) and *Klebsiella spp.* are major pollutants in the environment that are often associated with ESBL-encoding genes (Jena et al., 2017). Milk is a food source of animal origin that can deed as a reservoir for infectious bacterial diseases. The presence of *E. coli* and *Klebsiella* in raw milk is generally reported as sources of foodborne illness (Badri et al., 2017; Athanasakopoulou et al., 2021). The prevalence of ESBL-producing *E. coli* and *Klebsiella* are very high in animal-derived food products (Odenthal et al., 2016). In the light of this information, we aim to determine the status of blaCTX-M-1, blaCTX-M-2, blaTEM and blaSHV genes, which are responsible for extended-spectrum beta-lactamase enzyme production in *E. coli* and *Klebsiella* strains detected in raw cow milk, by real-time PCR method and to present molecular epidemiological data.

MATERIALS and METHODS

Sample collection

In our study, 50 raw cow milk offered for sale in markets in Şanlıurfa province and surrounding districts were included. All samples were taken in sterile glass bottles and quickly transferred to the laboratory in the cold chain.

Isolation and identification of E. coli and Klebsiella spp

In the laboratory, 50 milk samples were examined for *Enterobacteriaceae* using the ISO 21528-1 method. 10 ml milk samples were pre-enriched using a liquid medium containing 90 ml of Tryptic Soy Broth, the samples diluted at a 1/10 ratio were inoculated on MacConkey agar medium after enrichment. After 24 hours of incubation at 37°C, the proliferated colonies were evaluated by Gram staining (Diassa et al., 2017). From suspected colonies, for the identification of the *E. coli* and *Klebsiella* strains, inoculation was done on a TSI medium, lactose and sugar fermentation and biochemical properties were utilized. Antimicrobial susceptibility tests were performed by Kirby Bauer disc diffusion method by using ampicillin, trimethoprim-sulfamethoxazole, ceftazidime, cefotaxime, meropenem, ceftriaxone, chloramphenicol, gentamicin and tetracycline antibiotic discs in Mueller-Hinton medium (Plassard et al., 2021).

Phenotypic ESBL detection

The double disc synergy method was used to detect the phenotypic ESBL in strains. For this, ceftazidime, cefotaxime, ceftriaxone and aztreonam antibiotic discs were placed in Mueller hinton medium around the amoxicillin-clavulanic acid antibiotic disc and incubated at 37°C overnight. Enlargement of the inhibition zone mediated by amoxicillin-clavulanate around the antibiotic discs of ceftazidime, cefotaxime, ceftriaxone and aztreonam was accepted as phenotypic confirmation of the presence of ESBL (EUCAST, 2016).

DNA Extraction

For the detection of the ESBL Genes which are *blaCTX-M-1, blaCTX-M-2, blaSHV* and *blaTEM*, the manufacturer's instructions for High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany) were followed in order to isolate DNA. DNAs were stored at -20°C.

Detection of blaCTX-M1, blaCTX-M2, blaSHV and blaTEM Genes with real-time PCR

Primers used for the detection of *blaCTX-M-1*, *blaCTX-M-2*, *blaSHV* and *blaTEM*, are shown in Table 1 (Casti et al., 2016; Demirci et al., 2020). qPCR protocols to detect *blaCTX-M-1, blaCTX-M-2, blaSHV* and *blaTEM* genes were described by Demirci et al. (2020) on LightCycler 480 real-time PCR system according to manufacturer's instructions.

Table 1. Primers used in study to amplify the ESBL genes

Gene	Oligo
CTX-M1	F: GCGTGATACCACTTCACCTC
	R: TGAAGTAAGTGACCAGAATC
CTX-M2	F: TGATACCACCACGCCGCTC
	R: TATTGCATCAGAAACCGTGGG
blaTEM	F: AGTATTCAACATTTYCGTGT
	R: TAATCAGTGAGGCACCTATCTC
SHV	F: ATGCGTTATATTCGCCTGTG
	R: TTAGCGTTGCCAGTGCTC

RESULTS

Enterobacteriaceae species were detected in 22 (44%) of 50 raw cow milk samples included in our study. Out of these strains, 18 (36%) were identified as *E. coli*, while 4 (8%) were found to be *Klebsiella* spp. were detected. When the antibiotic susceptibility profiles of the reproducing strains were examined, it was found that there was no resistance to the meropenem which belongs to the carbapenem group in the isolates. After meropenem, it was determined that the highest sensitivity was to quinolone group antibiotics such as ciprofloxacin. Total sensitivity to ampicillin was 5%. While all *Klebsiella* spp. strains showed ampicillin resistance, only one *E. coli* strain was found susceptible. Table 2 shows the susceptibility profiles of all strains against all antibiotics. In our study, we detected phenotypical ESBL production in 12 (54.5%) of 22 *Enterobacteriaceae* strains detected in raw cow milk. 10 of them were E. coli and 2 of them were Klebsiella spp. all of these strains were found to contain at least one ESBL gene. Table 3 shows the ESBL gene distribution in ESBL positive strains.

Table 2. *E. coli* and *Klebsiella* spp. detected in raw milk. distribution of antibiotic susceptibility status of the strains.

Antibiotic	*E. coli* (n=18)		*Klebsiella* spp. (n=4)		Total (n=22)	
	n	%	n	%	n	%
Ampicillin	1	6	0	0	1	5
Trimethoprim-sulfamethoxazole	4	22	1	25	5	23
Ceftazidime	8	44	1	25	10	45
Cefotaxime	10	56	2	50	13	59
Meropenem	18	100	4	100	22	100
Ceftriaxone	11	61	2	50	14	64
Chloramphenicol	16	89	3	75	19	86
Gentamicin	16	89	3	75	19	86
Tetracycline	14	78	2	50	16	73
Ciprofloxacin	17	94	3	75	20	91

Table 3. Distribution of ESBL genes in ESBL positive strains.

ESBL production genes	*E. coli*	*Klebsiella* spp.	Total
blaCTX-M-1	1	0	1
blaTEM	1	0	1
blaCTX-M-1 & *blaTEM*	3	0	3
blaCTX-M-2 & *blaTEM*	2	1	3
blaCTX-M-1 & *blaSHV*	1	0	1
blaCTX-M-1 & *blaTEM* & *blaSHV*	2	1	3
Total	10	2	12

DISCUSSION

Contaminated food consumption is the most effective reason for the emergence and spread of antimicrobial resistance genes and resistant bacteria. In addition to animal husbandry, the occurrence of multidrug-resistant bacteria in the community and hospitals has increased rapidly in the last decade (Waade et al., 2021). The increase of *Enterobacteriaceae* strains, especially multidrug-resistant Gram-negative bacteria, such as *Klebsiella pneumoniae* and *Escherichia coli*, is a growing concern all over the world (Yao et al., 2007; Odenthal et al., 2016; Paghdar et al., 2020; Widodo et al., 2020; Waade et al., 2021; Salinas et al., 2021). Resistance in ESBL-producing *Enterobacteriaceae* strains is predominantly formed by the plasmid-mediated *blaSHV*, *blaTEM* and *blaCTX-M* beta-lactamase genes. While TEM and SHV genes were dominant in the 1990s and early 2000s, it is known that the balance has shifted towards the newly discovered family of CTX-M enzymes in recent years (Yao et al., 2007). ESBL-producing *Enterobacteriaceae* strains have been reported in raw milk (Uraz and Aslan, 1998; Vendramin et al., 2014; Odenthal et al., 2016; Tekiner and Özpınar, 2016; Tóth et al., 2020; Athanasakopoulou et al., 2021; Ramos et al., 2021; Waade et al., 2021). When the prevalence studies in raw cow milk were examined, Diassa et al. (2017) reported *E. coli* at a rate of 39% in Ethiopia in 2017. Vendramin et al. (2014) reported *E. coli* at a rate of 53.5% in Brazil. Altun et al. (2002) detected *E. coli* at a rate of 72.6% and *Klebsiella* spp. at a rate of 41.3% in the milk they examined in Ankara. We detected *Enterobacteriaceae* species in 22 (44%) of 50 raw cow milk samples included in our study. Eighteen (36%) of these strains were found to be *E. coli*, while 4 (8%) were found to be *Klebsiella* spp. It has been concluded that there are differences in the results of the study and that the rates may be affected by the hygiene conditions, and therefore there may be similarities or differences with our study results.

When the studies examining the phenotypic ESBL productions were controlled, Uraz and Arslan (1998) found a rate of 13.99% in *E. coli* and 12.59% in *Klebsiella* spp. Tekiner and Özpınar (2016) detected 80% in *E. coli* and 3.6% in *Klebsiella* spp. In our study, we detected ESBL production in 12 (54.5%) of 22 *Enterobacteriaceae* strains detected in raw cow milk that we included in our study. It is thought that the results may contain regional differences.

When the studies examining the genes causing ESBL production were controlled, Tekiner and Özpınar (2016) found *blaTEM*, *blaCTX-M* and *blaSHV* to be 96.4%, 53.7% and 34.5% respectively. In this study, the highest rate of togetherness of *blaTEM* and *blaCTX-M* was reported at 52% (14). Jouini et al. (2007) detected 10 (26%) of 38 *E. coli* strains in Tunisia. They detected *blaCTX-M-1* in 5 of these strains. When we analyzed the genes of 12 *E. coli* strains that we detected in our study, we found *blaCTX-M-1* in 7 isolates (58.3%), *blaCTX-M-2* in 2 strains (16.7%), *blaTEM* in 8 strains (66.7%), and *blaSHV* in 3 strains (25%). Table 3 shows the genes of 12 *E. coli* strains that we detected in our study. Similar to the studies we examined in our country, it is seen that the CTX-M-based ESBL production genes are predominant.

CONCLUSION

In conclusion, our data show that there are flora originated strains such as *E. coli* and *Klebsiella* spp. and it was determined that these strains can produce ESBL. It was also determined that CTX-M-based ESBL genes predominated in ESBL productions and there is a coexistence of different ESBL production genes in the isolates. We believe that in our world where antibiotic resistance is a problem, molecular surveillance of ESBL genes in frequently used foods such as raw milk should be done routinely to determine epidemiological data.

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Evaluation of oxidative stress in dogs with demodicosis

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ABSTRACT

Objective: To investigate the effects of oxidative stress in dogs with demodicosis.

Materials and Methods: The material of the study was based on a total of 32 owned dogs, of which 21 were diagnosed with demodicosis and 11 were healthy, with different ages, genders, and breeds. Demodex examination for diagnostic evaluation was performed by examining samples under the microscope that were taken using the trichogram and deep skin scraping methods. In order to evaluate the effects of oxidative stress in dogs with demodicosis in the pre- and post-treatment groups and the control group without demodex diagnosis, the superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione (GSH) values, as antioxidants and malondialdehyde (MDA) as an oxidant, were investigated.

Results: In the clinical examinations, manifestations such as alopecia, erythema, generalized pruritus, hyperpigmentation, lichenification, pododermatitis, interdigital pruritus, and lymphadenopathy were observed in the dogs with demodicosis both pre- and post-treatment. In the analyses performed in order to evaluate the oxidative stress, MDA: 20.30 nmol/mL, GSH: 4.9 nmol/mL, GPx: 0.42 U/L, and SOD: 4.1 U/L were measured in the dogs with clinical demodicosis. Post-treatment, the average values in the same dogs were measured as MDA: 6.08 nmol/mL, GSH: 8.11 nmol/mL, GPx: 0.83 U/L, and SOD: 6.67 U/L, while in the control group, they were measured as MDA: 4.94 nmol/mL, GSH: 9.73 nmol/mL, GPx: 0.97 U/L, and SOD: 7.20 U/L. It was determined that the GSH, GPx, and SOD values in the control and post-treatment groups were significantly higher ($P < 0.001$) and the MDA values were lower ($P < 0.001$) than in the clinical demodicosis group.

Conclusion: In dogs with clinical demodicosis, when compared to the control and post-treatment groups, higher levels of MDA, which is an oxidant, and lower levels of GSA, GPx, and SOD, which are antioxidants, showed that demodex caused oxidative stress in the dogs..

Keywords: *Dermatitis, demodex, scabies, antioxidant, oxidative stress, oxidant*

INTRODUCTION

Demodex is a member of the Acari subclass of the Arachnida class of arthropods. These mites are considered a part of the normal skin microbiome of most mammals, including dogs (Gökalp and Kırbaş, 2020). Demodicosis in dogs, when the immune system is suppressed, allows the mites to overbreed and leads to the development of clinical signs. This disease is often caused by Demodex canis. However, there are other species, such as Demodex injai and Demodex cornei (Ural et al., 2019). The cause of the disease spends its whole life commensally in the skin, in the hair follicles placed on the head area, and in the follicles of the

sebaceous and embedded in the Meibomian glands. The agent cannot be separated from its host. All life stages of the agent can be found simultaneously in a follicle. The completion duration of the life cycle varies between 18 and 24 days (Hnilica and Petterson, 2017).

On the host, it completes its effective life cycle within the hair follicles and related glands. Demodex agent serum feeds on cells and debris (Aytuğ, 2012). In some generalized demodicosis patients, pyoderma occurs secondary to the overbreeding of pathogens such as Malassezia and Staphylococcus species, possibly due to the immunosuppressive effect on the skin microenvironment of the dog affected by the disease. In such cases, it is necessary to treat it with antibiotics suitable for the structure of the skin (Pekmezci et al., 2014).

Under natural circumstances, demodicosis symptoms are rarely seen. When occlusion and/or enlargement of the ostia of hair follicles and hyperpigmentation are observed, these clinical findings should be a clue to the disease (Mueller et al., 2020). During a dermatological examination, along with diffuse alopecia, seborrhea, epidermal choleretic also mediocre papulopustular dermatitis, comedones, and hyperpigmented macules can be seen (Hillier and Desch, 2002). In this disease, the proliferation of Demodex canis in the hair follicles can cause hair loss, inflammation of the hair follicle and sebaceous gland, and in severe forms, bleeding crusts and furunculosis. (Hnilica and Petterson, 2017). Pododemodicosis is characterized by interdigital pruritus, pain, erythema, alopecia, hyperpigmentation, lichenification, scaling, crusting, pustules, bullae, and drainage channels. Peripheral lymphadenopathy is common. If secondary bacterial sepsis develops, systemic findings (e.g., fever, depression, anorexia) may occur (Hnilica and Petterson, 2017). Among the clinical symptoms of the disease, there are dyskeratosis, malodor, alopecia, erythema, papules and pustules, hyperpigmentation, comedones, and secondary bacterial infection (Sgarbossa et al., 2017).

If it is not treated, hyperpigmentation and lichenification, with increased body odor due to excessive sebum production from the sebaceous glands related to hair follicles, can also arise in these patients (Mueller, 2004).

Oxidative stress can be defined as the phenomenon of cell and tissue damage as a result of the imbalance between oxidant/antioxidant substances in the body (Puppel et al., 2015). Oxidant/antioxidant balance causes piling of oxidant substances in cellular structure and molecules and failure of various physiological events by creating oxidative stress due to increased production of free radicals and deterioration of antioxidants in consequence of them being inactive or insufficient (Tabakoğlu and Durgut, 2013). Therefore, in the evaluation of oxidative stress in the body, the determination of antioxidant consumption can be made by determining the decrease in antioxidant grades or the increase in their metabolites (Puppel et al., 2015).

The leading mechanism of free radical toxicity is the peroxidation of membrane phospholipids pioneered by the creation of lipid peroxides or hydroperoxides, and peroxide radicals are formed to initiate a chain reaction (propagation) in the presence of oxygen (Abd Ellah, 2011). Lipid peroxides (lipid peroxide, cyclic peroxide, and cyclic endoperoxide), created as a result of lipid peroxidation reactions, eventually transform into aldehydes called malondialdehyde (MDA), 4-Hydroxinonenal (HNE), and hexanal, which are secondary or end products (Özcan et al., 2015). MDA is the final product that is formed as a result of the enzymatic or non-enzymatic disintegration of arachidonic acid and larger polyunsaturated fatty acids (PUFA) (Aslankoç et al., 2019).

The defense systems serving in the body to prevent the creation of reactive oxygen species, prevent the damage caused by these substances and provide detoxification are called antioxidant defense systems (Aslankoç et al., 2019). Antioxidants can be examined under two classes, as endogenous and exogenous. Endogenous antioxidants are divided into two categories, as enzymatic and nonenzymatic. Whereas the enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR); glutathione, melatonin, uric acid, albumin, and selenium, and they can be numbered among nonenzymatic antioxidants (Küçük, 2021).

Demodicosis in dogs comprises an important part of dermatological events in veterinary clinics in Turkey and around the World. The disease has a strong relationship with the immune system and other diseases. Oxidative damage formed as a result of oxidative stress is seen as the main cause of diseases characterized by tissue dysfunctions, such as aging, cardiovascular diseases, immune system diseases, degenerative diseases, and cancer. This

study, performed in light of all of this information, aimed to investigate whether oxidative stress is affected in demodicosis events observed in dogs and whether it plays a role in the pathogenesis of the disease or not.

MATERIALS and METHODS

This study was granted the approval of Kırıkkale University Animal Experiments Local Ethics Committee (decision numbered 44, dated 24.11.2021).

The animal material

The animal material of the study was based on a total of 32 dogs, of which 21 were diagnosed with demodicosis and 11 were healthy, with different ages, genders, and breeds, which were brought to Kırıkkale University Veterinary Faculty Training and Research Hospital.

Diagnosing demodicosis

In the presence of clinical symptoms on the skin when widely developed pruritic papulopustular lesions, crusting, and locally alopecia foci were observed, skin scrapings were taken from suspected animals to determine the causative agent and to diagnose them. To take the skin scraping samples from the clinically suspected dogs, the skin on the area with the lesion to be scraped was folded as much as possible, to soften the area, paraffin liquid was dripped on the area and the skin with the lesion was scraped using a scalpel until capillary bleeding occurred. Simultaneously, the hair on the lesioned area and around that area were pulled off together with their roots.

The scraped material sample and the hair pulled off together with their roots were placed on a slide. Mineral oil and 10% KOH solution were dripped on it then the preparate and the slide were covered after it was crushed thoroughly. The prepared preparate was examined under a light microscope at 10x and 40x magnifications. In the microscopic examination, the adult and developmental forms of Demodex spp. were detected on the samples taken from the dogs. In the treatment of demodicosis, until clinical improvement and a negative scraping result were obtained, Sarolaner (Simparica, Zoetis) at a dose of 3 mg/kg was orally given to the dogs, once a month.

Creating the workgroups

A total of 21 dogs diagnosed with demodicosis constituted the treatment group and 11 healthy dogs constituted the control group. For the laboratory analyses, two samples of blood were drawn, before and after treatment, from the treatment group, and the blood was drawn from the control group once. Within the scope of the study, the obtained results were examined in 3 groups, as before the Demodicosis treatment (Group 1), after the Demodex treatment of the same dogs (Group 2), and the control group.

Laboratory analyses

To evaluate oxidative stress from all of the animals, 4 mL-blood samples taken from the vena cephalica antebrachi into coagulation-activating straight tubes were centrifuged at 3500 rpm for 10 min and serum samples were extracted. The serum samples were stored at –18 °C until the analysis was performed. Each serum sample removed from the freezer and allowed to thaw until reaching 4 °C before analysis, and then they were allowed to reach room temperature. To evaluate the oxidative stress in the serum samples, the malondialdehyde (MDA) (Yoshioka et al., 1979), glutathione (GSH) (Beutler et al., 1963), glutathione peroxidase (GPx) (Paglia and Valentine, 1967), and superoxide dismutase (SOD) (Sun et al., 1989) activities were measured in accordance with their methods.

Statistical Analyses

The required sample size for the study, for all 3 groups, was calculated as 53 samples, whereas the effect size between the best and worst groups in the received responses was f = 0.50, with a power of 0.95 on error levels Type I $\propto\circledcirc$= 0.05 and Type II β = 0.05. Power Ver 3.00.10 (G*Power, Franz Foul, Universität Kiel, Germany) was used for the sample size and power analysis. The values obtained in the study were transferred to a computer environment and thereby descriptive statistical information (average, standard deviation, etc.) was obtained. The suitability of the measurement and scoring values to the normal distribution were examined graphically and using the Shapiro-Wilk test. It was observed that normal distribution occurred in all of the groups. Although the study seemed to be for three groups, pairwise comparisons were also made according to whether the groups were dependent or independent. The paired samples T-test was used for the statistical evaluation of the data in the pre-treatment group (n=21) and the post-treatment group (n=21). The independent sample T-test was used for the evaluation of the data in the treatment group (pre and post-treatment) and the data in the control group (n=11). As a result of the performed

statistical tests, p<0.05 was accepted as statistically significant.

RESULTS

Clinical findings: In the study, it was determined that the clinical findings of the study groups considerably varied based on the severity and type of disease. While there were no clinical dermatological lesions in the control group dogs, clinical pictures such as alopecia, erythema, generalized pruritus, hyperpigmentation, lichenification, pododermatitis, interdigital pruritus, and lymphadenopathy were observed in the pre- and post-treatment groups (Table 1).

Table 1. Clinical findings of treatment groups.

Clinical findings	Pre-treatment Group (n=21)	Post-treatment Group (n=21)	Control Group (n=11)
Alopecia	80%	19%	-
Erythema	71.4%	4.7%	-
Generalized Pruritus	57.1%	9.5%	-
Hyperpigmentation	38%	9.5%	-
Lichenification	23.8	9.5%	-
Pododermatitis	14.2%	9.5%	-
Interdigital Pruritus	9.5%	4.7%	-
Lymphadenopathy	4.7%	0%	-

Laboratory findings: The clinical findings of the treatment group, control group, and pre- and post-treatment groups, and the average of the groups constituted based on the diagnostic classification result along with their minimum and maximum values and their statistical significance are given in Tables 1 to 5, respectively.

The MDA levels were statistically significantly different (p<0.001) in the 21 dogs in the pre- and post-treatment groups. The arithmetic average and standard deviations of the MDA levels for all of the groups are given in Table 2. The difference between the MDA levels of the pre-treatment group compared to the control group was statistically significant (p<0.001). The difference between the MDA levels of the post-treatment group compared to the control group was not statistically significant (p>0.05).

The GSH levels were statistically significantly different (p<0.001) in the 21 dogs in the pre- and post-treatment groups, and the arithmetic average and standard deviations for all of the groups are given in Table 3. The difference between the GSH levels of the pre-treatment group compared to the control group was extremely statistically significant (p<0.001). The difference between the GSH levels of the post-treatment group compared to the control group was not statistically significant (p>0.05).

Table 2. Levels of MDA (nmol/mL) in the treatment and control groups.

MDA (nmol/mL)	n	$\bar{x} \pm S\bar{x}$	p-value
Pre-treatment (PRT)	21	$20.30^{a} \pm 4.18$	PRT-PST = <0.001
Post-treatment (PST)	21	$6.08^{b,c} \pm 1.86$	PRT-C = <0.001
Control (C)	11	$4.94^{b,c} \pm 1.58$	PST-C = >0.05

Table 3. Levels of GSH (nmol/mL) in the treatment and control groups.

GSH (nmol/mL)	n	$\bar{x} \pm S\bar{x}$	p-value
Pre-treatment (PRT)	21	$4.90^{a} \pm 1.25$	PRT-PST = <0.001
Post-treatment (PST)	21	$8.11^{b,c} \pm 1.47$	PRT-C = <0.001
Control (C)	11	$9.73^{b,c} \pm 2.04$	PST-C = >0.05

Table 4. Levels of GPx (U/L) in the treatment and control groups.

GPx (U/L)	n		P-value
Pre-treatment (PRT)	21	$0.42^{a} \pm 0.18$	PRT-PST = <0.001
Post-treatment (PST)	21	$0.83^{b,c} \pm 0.21$	PRT-C = <0.001
Control (C)	11	$0.97^{b,c} \pm 0.15$	PST-C = >0.05

Table 5. Levels of SOD (U/L) in the treatment and control groups.

SOD (U/L)	n	$\bar{x} \pm S\bar{x}$	p-value
Pre-treatment (PRT)	21	$4.10^{a} \pm 1.04$	PRT-PST = <0.001
Post-treatment (PST)	21	$6.67^{b,c} \pm 1.02$	PRT-C = <0.001
Control (C)	11	$7.20^{b,c} \pm 0.99$	PST-C = >0.05

The arithmetic average and standard deviations of the GPx levels for all of the groups are given in Table 4. The GPx levels were statistically significantly different (p<0.001) in the 21 dogs in the pre- and post-treatment groups. The difference between the GPx levels of the pre-treatment group

compared to the control group was extremely statistically significant ($p<0.001$). The difference between the GPx levels of the post-treatment group compared to the control group was not statistically significant ($P > 0.05$).

The SOD levels were statistically significantly different ($p<0.001$) in the 21 dogs in the pre- and post-treatment groups. The arithmetic average and standard deviations of the SOD levels for all of the groups are given in Table 5. The difference between the SOD levels of the pre-treatment group compared to the control group was extremely statistically significant ($p<0.001$). The difference between the SOD levels of the post-treatment group compared to the control group was not statistically significant ($p<0.05$).

DISCUSSION

Demodicosis is a skin disease caused by the ectoparasite Demodex (Demodecidae), which prognoses typically include hair loss, inflammation of the hair follicles, and sebaceous glands. Demodicosis is one of the most common skin diseases in veterinary medicine (Beyazıt et al., 2010).

Even though the diagnosis of dog demodicosis is easy to make, it can be difficult due to the healing duration, defining the underlying causes, the need for healing, the owner's expectations (time and financial commitments), and the requisite for frequent follow-up. Therefore, it is important to analyze the disease mechanism and the treatment process in more detail. Although the general complaint of the patients is alopecia, pruritus may not be observed in cases without secondary skin infection or allergy. If it is not treated, hyperpigmentation and lichenification with increased body odor due to excessive sebum production from the sebaceous glands related to hair follicles can also arise in these dogs.

While constituting the control group within the scope of the study, routine clinical cases which are been brought to the clinic for control and vaccination purposes were preferred. During the performed physical examinations, care was taken that all vital functions complied with the healthy animal profile. The clinical findings detected in the dogs with demodicosis (n=21) with the missing predisposition of gender and breed included in the study, were determined as alopecia, erythema, generalized pruritus, hyperpigmentation, lichenification, pododermatitis, interdigital pruritus, and lymphadenopathy. In addition to that, these clinical findings varied in each animal, which showed similarity to the study of Ural et al. (2019). The correlation between the number of mites detected in the microscope field and the prognosis of the disease was not related to the hypothesis and was not included in the study.

Abdulaziz et al. (2019) explained the mechanism of the hypersensitivity reaction as hyperkeratinization of the tissue in the affected area, and along with the free radical production oxidative stress was increased. They observed an increase in erythema, alopecia, severe inflammation of the skin, and allergic reactions when the free radicals took effect. Biological indicators of oxidative stress, even the measurement of antioxidant substances in serum or tissues, may lead to new findings from studies showing the relationship between free radicals and diseases as a cause or an effect of pathological conditions (Russo and Bracarense, 2016). Dündar and Aslan (2000) stated that routinizing the measuring the antioxidant values, which are biomarkers, making them more specific markers, and creating the relevant reference values were an important step in resistance to pathogens, geriatric process, condition, physiological activity, exercise life, efficiency, diagnosis, prognosis, therapy, determining and directing the protective treatment.

Sahin et al. (2004), in their submitted study, determined that oxidative stress and lipid peroxidation activities were increased in patients with dermatological problems, and plasma MDA, which is an indicator of oxidative stress, was higher in the pre-treatment group than in the post-treatment group. They also determined that contrary to the plasma MDA level, the level of GSH-Px, which is an antioxidant enzyme, was increased in the post-treatment group when compared to the pre-treatment group. When free radicals are increased in the organism, antioxidants take place and reduce their maleficence. This also indicates that the applied treatment is effective by lowering the plasma MDA level, which is the cause of cell damage and death. Abdulaziz et al. (2019) showed in their study that, regarding oxidative damage, the total antioxidant capacity (TAC) ($p<0.01$) was more significant than SOD ($p<0.05$) and MDA ($p<0.01$). In the same study, a negative correlation was observed between the MDA and SOD levels. In the current study, it was inferred that the higher levels of MDA in the control group held in the pre-treatment group, and this parameter contributed to the severity of the lesions by causing negative

consequences, such as changes in ion permeability and enzyme activity in demodex-related skin lesions. It was also inferred that the levels of MDA in the control group held in the pre-treatment group were a greater contribution to the severity of the lesions by causing negative consequences, such as changes in both ion permeability on demodex-related skin lesions and enzyme activity. Thus, it was thought that the significantly lower amount of these enzymes in the dogs with generalized demodicosis compared to the control group may have occurred due to a deficiency in the antioxidant mechanism in the dogs with severe skin lesions. In addition, it was determined that, contrary to the results of the study of Şahin et al. (2004), this enzyme level, which was determined to be lower in the pre-treatment group, increased after treatment.

In dogs in which the Demodex agent was detected and treatment was started, a decrease in the MDA levels, and increase in the SOD, GSH, and GPx levels were observed in the blood samples examined after signs of recovery were observed. Statistically significant differences were found between the p-values of the dogs with demodicosis and the healthy dogs ($p<0.001$). Dimri et al. (2008) reported that endogenous antioxidant levels were decreased in dogs with localized and generalized demodicosis, and the disease was related to the occurrence of oxidative stress. Salem et al. (2020) asserted that in dogs, there is a relationship between generalized demodicosis and oxidant-antioxidant imbalance. Proof of this relationship demonstrates itself as an increase in the MDA and TAC levels and a decrease in the GPx and CAT levels as a result of reactive oxygen species released because of Demodex infection. Moreover, herein, it was determined that the Demodex agent reduced the antioxidant capacity of the dogs, regardless of generalized or localized demodicosis.

CONCLUSION

In demodicosis in dogs, it was observed that oxidative stress was affected, and by extension, serious changes in the oxidant/antioxidant parameters occurred. It was observed that the oxidant/antioxidant parameters regressed to their normal values in the dogs that were cured with the applied treatment. It inferred that all of the obtained data herein can be used as a guide in future studies.

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Author Contribution Statement: The creation of the study design and the control of the process are carried out by BBY, while the collection of samples and the follow-up of the analysis processes are performed by GNS. Literature research, writing the article and critical reviews are done by BBY and GNS. Both of the authors have read and approved the final version of the article. BBY: Buğrahan Bekir Yağcı; GNS: Gözde Nur Sivel. All authors have read and agreed to the published version of the manuscript.

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Identification of some yeast species in traditional Turkish fermented sausage with Vitek 2 compact system

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ABSTRACT

Objective: Fermented sausage, produced by traditional methods, matures by fermentation of microbial flora originating from the raw materials and the place of production. The source of contamination of the meat industry with yeasts, which are widespread in the environment, are the surfaces of the tools and equipment used in processing. Although the presence of yeasts in meat products contributes to the formation of flavor and aroma, some yeast species can cause undesirable flavors, discoloration, and the formation of a soft texture.

Material and Method: Therefore, our study aimed to determine the yeast profile of fermented sausages using the Vitek2 Compact System, in which various biochemical tests were performed.

Results: In the sausage samples was detected *Candida zeylanoides* in 56.25%, *Candida sake* in 52.10%, *Pichia farinosa* in 25%, *Cryptococcus laurentii* in 10.42%, *Candida glabrata* in 4.17%, and *Rhodotorula glutinis* in 10.42% yeast species.

Conclusion: The difference in yeast species in fermented sausages varied depending on the microbial load of the raw material and compliance with hygiene regulations during processing and fermentation conditions.

Keywords: *Fermented sausage, Vitek2 Compact System, Yeast*

INTRODUCTION

Dry-cured meat products form a wide group of products, ranging from hams to sausages, and have been consumed since ancient times. Among them, dried fermented sausages are widely used worldwide due to their characteristic taste (Flores et al., 2015). Fermented dry sausages are products made by grinding raw meat, adding spices, and stuffing into casings, drying, and fermenting with natural microflora or commercial starter cultures (Mikami et al. 2020). During the maturation of dry-cured meat products, enzymatic and chemical reactions contribute to the development of flavor. Turkish sausage, known as "Soudjouck", a traditional Turkish sausage, is a fermented meat product. Traditionally produced sausage matures by fermentation of raw materials and microorganisms from the flora of the place of production. During production, the product has a flora that includes various lactic acid bacteria, coagulase-negative staphylococci and yeasts. (Siriken et al. 2006; Lorenzo et al., 2007; Loranjo, 2017; Loranjo, 2019). Dry-fermented sausages are considered safe meat products due to their low water activity and low pH, which inhibits the growth of pathogenic microflora during processing and storage (Meftah et al. 2018). Yeast can reach up to 106 cfu/g in dry-cured meat products such as

fermented sausages (Encinas et al., 2000; Cocolin et al. 2006; Simoncini et al., 2007). The most commonly isolated yeast species are Candida, Cryptococus, Debaryomyces, Kluyveromyces, Pichia, Rhodotorula, Saccharomyces, Trichosporon, and Yarrowia spp. (Cano-Garcíaz et al. 2014, Murgia, Marongiu et al. 2019). Yeasts with aerobic or facultative anaerobic properties as starter cultures, Debaryomyces spp. and Candida spp. are commonly used in meat products (Laranjo et al., 2017; Loranjo, 2019). Recent studies have shown that yeasts contribute to the formation of the characteristic flavors of dry-cured meat products through their proteolytic and lipolytic activities (Sørensen, 1997; Martín et al., 2004; Simoncini et al., 2007; Bosse et al., 2018). Yeast growth plays an important role in controlling pathogenic microorganisms in meat products (Metaxopoulos et al., 1996; Purriños et al., 2013). Some of the yeast strains C. catenulata, C. guilliermondii, C. edax, and Cryptococcus and Wingea isolated from Italian ham show lipolytic properties (Simoncini et al., 2007). Yeasts develop and colonize the surface of dry-cured meat products during maturation and have an antagonistic effect on mold growth. In addition, yeasts have always been recognized as safe starter cultures in foods, including meat products and beverages (Jakobsen and Narvhus, 1996; Adesulu-Dahunsi et al., 2020). For this reason, it is important to determine the natural fermentation flora in terms of safety and sensory quality of the product. The aim of this study was to determine the yeast flora of fermented sausages with the Vitek2 Compact System, using YST ID cards with different sugars, enzymes and substrates in their wells.

MATERIALS and METHODS

Material: Forty-eight fermented sausage samples sold in different markets in Erzurum in Turkey were taken in 2013, and these samples were brought to the laboratory under cold chain.

Yeast Isolation: For yeast isolation, appropriate dilutions were cultured on Rose bengal chloramphenicol agar (Merck 106867) using the smear method. Typical yeast colonies formed after 5 days of incubation at 25 °C under aerobic conditions were classified based on colony morphology and examined microscopically (Erdem büyükkiraz et al., 2020).

Yeast Identification: To identify the yeast isolates, biochemical tests were performed on the fresh cultures obtained after incubation at 25 °C for 5 days under aerobic conditions by seeding on RBC agar in a draw method using the YST ID Card in the Vitek2 Compact System. It contains various sugars, enzymes and substrates such as L-lysine arylamidase, L-malate, leucine arylamidase, arginine- GP, erythritol, glycerol, tyrosine arylamidase, beta-N-acetyl glucosaminidase, Arbutin, amygdalin, D-gentibialactose, D-glucose, lactose, methyl-A-D-glucopyranoside, D-cellobiose, gamma-glutamyl transferase, D-maltose, D-refinose, PNP-N-acetyl- BD -galactosaminidase-1, D-mannose, D-melibiose, D-melizitose, L-sorbose, L-rhamnose, xylitol, D-sorbitol, sucrose/sucrose, urease, alpha-glucosidase, D-turanose, D-trehalose, nitrate, L-arabinose, D-galacturonate, esculin, L-glutamate, D-xylose, DL -lactate, acetate, citrate (sodium), glucuronate, L-proline, 2-keto-D-gluconate, N-acetyl-glucosamine, and D-gluconate for biochemical tests (Pincus, 2006).

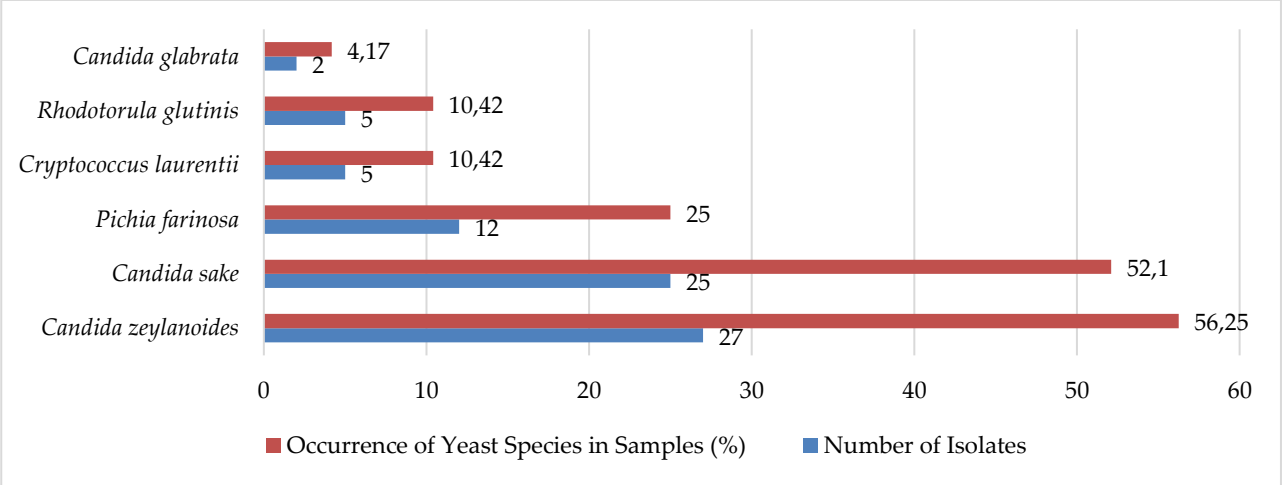

Figure 1. The number of isolates obtained from fermented sausage samples and the rate of presence in the samples

RESULTS

It was detected that *Candida zeylanoides* in 56.25%, *Candida sake* in 52.10%, *Cryptococcus laurentii* in 10.42%, *Candida glabrata* in 4.17%, *Rhodotorula glutinis* in 10.42% and 25% *Pichia farinosa* from the analyzed 48 fermented sausages (Fig 1).

DISCUSSION

The source of contamination in the meat industry by yeasts, which are widely distributed in the environment, are the surfaces of machines that come into contact with meat during processing (Hernández et al., 2018). Although yeast growth in meat products contributes to the formation of flavor and aroma, some yeast strains can cause off-flavors, discoloration, and slime formation (Nielsen et al., 2008). Yeasts can grow and multiply on complex substrates containing large amounts of sugar, salt, low pH, low temperature, and low water activity (Jakobsen and Narvhus, 1996; Lopandic et al., 2006). *Candida sake* type yeasts were also one of the yeast strains isolated from fermented sausages (Nielsen et al., 2008; Franciosa et al., 2021). *Candida zeylanoides*, which is commonly isolated in fermented meat products, is believed to be a pathogenic yeast and may pose a public health problem (Asefa et al. 2009). *Candida zeylanoides*, which is commonly isolated in fermented meat products, is thought to be a pathogenic yeast and could be a public health concern (Asefa et al. 2009). *Pichia farinosa*, a halotolerant yeast, is an important yeast strain in the fermentation industry due to its ability to produce high levels of glycerol and xylitol (Wang et al. 2006). *Cryptococcus laurentii*, a psychrotrophic yeast, may contribute to flavor formation due to its lipolytic property, although it has only been isolated in small numbers (Saldanha-da-Gama, 1997). *Rhodotorula glutinis*, which is widely distributed in the environment, is a unicellular yeast that generally produces nontoxic metabolites such as endogenous proteins, biological pigments (β-carotene, astaxanthin, lycopene), unsaturated fatty acids, and cell walls (Kot et al., 2016). *Rhodotorula glutinis*, isolated in small numbers in fermented sausages, was detected in only 10.42% of samples (Asefa et al.2009).

The difference in yeast species in fermented sausages varies depending on the microbial load of the raw material, the hygiene rules during processing, and the conditions of fermentation. In addition, the decrease in pH and water activity during maturation causes changing the yeast flora. For this reason, it is important to adjust the choice of quality raw materials and ripening conditions to obtain the standard product in fermented sausage production. The importance of using food safety systems such as HACCP and ISO 22000 in food production areas is becoming increasingly clear.

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Histopathological evaluation of uterine structure and immunohistochemical examination of MMP-1 and TIMP-1 in cows recovered from metritis

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ABSTRACT

Objective: The aim of this study is to compare the results of histopathological examination and immunohistochemical examination including matrix metalloproteinases 1 (MMP-1) and tissue inhibitor of matrix metalloproteinases (TIMP-1) of the uterus of healthy cows and the uterus of cows that have recovered from metritis.

Materials and Methods: The study materials obtained from the slaughterhouse were divided into two groups: the uterus of healthy cows (no metritis; Group N; n=10) and uterus of cows that had recovered from metritis about 45 days ago (metritis; Group M; n=10). Sections were taken from the uterus of both groups and were stained with hematoxylin and eosin (H&E) for pathological comparison. In addition, the sections were stained immunohistochemically for the examination of MMP-1 and TIMP-1 levels.

Results: The uterus showed pathological condition in Group M than in Group N. MMP1 immunopositivity was higher in the luminal epithelium ($p<0.01$), endometrial stroma, uterine gland ($p<0.05$) and myometrium sections ($p<0.01$) of the uterus in Group M compared to Group N. TIMP-1 immunopositivity of endometrial stroma and uterine gland sections decreased in Group M compared to Group N ($p<0.01$), However, TIMP-1 immunopositivity of myometrium sections was higher in Group M than Group N ($p<0.01$).

Conclusion: In conclusion, it was observed that the negative effects of metritis on the uterus persisted histologically even approximately 45 days after clinical recovery. However, this needs to be investigated extensively because time elapsed between the clinical recovery of the cows and the study may have been insufficient for the uterus to complete histological healing.

Keywords: *Cow, Metritis, MMP-1, TIMP-1, Uterus*

INTRODUCTION

It is known that one of the most important factors affecting the profitability of dairy animals is reproductive performance (LeBlanc, 2008; Almughlliq et al., 2017), which is expected to be reduced through uterine diseases (Baez et al., 2016). Uterine infections have an incidence of up to 50% in the postpartum period in dairy cows (Manimaran et al., 2016). It has also been reported that the most common uterine disease is metritis in dairy cows (Kurt et al., 2019), with an approximately 20% incidence (Pérez-Báez et al., 2021). Moreover, uterine diseases can occur in the form of endometritis or metritis. Endometritis is known as inflammation of the endometrium in the uterus and rarely causes systemic symptoms. Metritis is defined as inflammation of the uterine wall caused

by various pathogens and is a disease characterized by systemic findings such as fever, watery red-brown and foul-smelling uterine discharge, loss of appetite, and high heart rate (Genís et al., 2018). Metritis result in negative outcomes such as prolongation of the calving to conception interval, delayed resumption of ovarian cyclicity, decrease in conception rates, and milk yield and increase in culling rate in farm animals (Manimaran et al., 2016; Kurt et al., 2019; Pérez-Báez et al., 2021). Because uterine infections directly disrupt the uterine environment (Sheldon et al., 2006) within connective tissues and endometrial cells, histological recovery of the uterus can take time. Unless histological improvement of uterine occurs, the uterus cannot be considered to be ready for a new pregnancy. So, it is critical for uterine health that connective tissues and endometrial cells can regain their previous functions. As consequence, we think that the histological recovery of the uterus in cows suffering from metritis should be investigated comprehensively. In animals suffering from metritis, the histological status of the uterus can be monitored with Matrix metalloproteinases 1 (MMP-1) and the tissue inhibitor of matrix metalloproteinases (TIMP-1). Nikolov et al. (2020) reported that MMPs are known as zinc-dependent endopeptidases and have the ability to bind to components of the extracellular matrix. MMPs have different groups including collagenases (MMP-1 and MMP-13), gelatinases (such as MMP-2 and MMP-9), stromelysins (such as MMP-3), and membrane MMPs. Moreover, MMPs are involved in the remodeling of various tissues and organs, in many physiological and pathological processes (Chen et al., 2017; Li et al., 2017). It has also been stated that some of the MMP family can play a role in the remodeling of endometrial tissue (Chen et al., 2017). Activated macrophages in the wall of arterial vessels secrete MMPs. On the other hand, MMPs have the ability to change tissues, which is important for normal and pathological physiology (Nikolov et al., 2020). Therefore, it is understood that MMP-1 secretion increases in pathological conditions such as uterine infection. In this case, level of TIMP secretion, which is an inhibitor of MMPs, can be expected to change. Considering the above information, it is thought both MMP-1 and TIMP-1 levels can be related to the damage caused by the infection in the tissue.

The present study hypothesized that the negative effects of metritis on the uterus could persist even after clinical recovery. Therefore, the aim of this study is to compare the results of histopathological examination and immunohistochemical examination including MMP-1 and TIMP-1 of the uterus of healthy cows and the uterus of cows that have recovered from metritis.

MATERIALS and METHODS

Ethical approval

Ethical approval for this study was obtained from the Local Ethics Committee of Ceyhan Veterinary Faculty, Cukurova University, Adana, Turkey (Approval number: 08/01, date: 16.06.2021).

Animals and Groups

The present study was carried out on the uterus of a total 20 Holstein dairy cows obtained from the slaughterhouse. Study materials were divided into two groups: the uterus of healthy cows (no metritis; Group N; n=10) and uterus of cows that had recovered from metritis approximately 45 days ago (Group M; n=10). In addition, the cows in both groups were between about 3.5-5 years old, and did not have any clinical problems at the time of study. The previous health and disease information of the cows was obtained from the records, and metritis was characterized as previously described, taking into account findings such as fever, and fetid watery red-brown uterine discharge (Genís et al., 2018; Kurt et al., 2019). In addition, animals in two groups were included in the study according to the results of microbiological examination.

Microbiological examination

Swab samples were taken from the uterus under aseptic conditions for microbiological examinations immediately after slaughter in both groups. Gram-negative and Gram-positive bacteria isolation was performed in all swab samples taken. All processes were done according to standard procedures previously described (Markey et al., 2013). In Group N and Group M, animals that any bacteria could not be isolated in their intrauterine environment were included in the study.

Tissue collection, preparation and staining

Uteri of both groups were harvested immediately after slaughter. Then, the uteri were randomly divided into small pieces from their middle regions and immediately fixed in 10% neutral buffered formalin solution. These uteri were dehydrated with increasing series of alcohols and clarified using xylene. After these procedures, all fixed uteri were embedded in paraffin blocks, and serial sections of 5 μm thick were taken using a Rotary microtome.

Sections were stained with hematoxylin and eosin (H&E) for histopathological comparison (Feldman and Wolfe, 2014). In addition, MMP-1 and TIMP-1 immunohistochemistry were applied to these sections.

Immunohistochemistry and obtaining quantitative data

The prepared sections were stained for immunohistochemical examination of MMP-1 and TIMP-1 levels (Nagel et al., 2004; Naruse et al., 2009). The samples were washed in PBS (Phosphate-buffered saline without calcium and magnesium) and antigen retrieval was applied in citrate buffer. Then the samples were incubated in 3% H_2O_2. MMP-1 (Bioss Antibodies, MA, USA) and TIMP-1 (Biorbyt, Cambridge, UK) antibodies diluted 1:300 were dripped onto the samples, followed by overnight incubation. In order to prevent non-specific binding, the blocking solution was used before the antibody application, and the subsequent steps were performed with a ready-to-use kit (Thermo Scientific, Waltham, MA, USA). The chromogen reaction was performed with DAB (3,3 Diaminobenzidine) using a commercial kit (Thermo Scientific, Waltham, MA, USA). Samples that developed a reaction were counterstained with hematoxylin and covered with entellan and examined under a light microscope at 10x and 20x magnification (Zeiss Axio Imager 2).

Quantification of Immunohistochemistry Analyses

In order to examine MMP-1 and TIMP-1 immunoreactivities in stained sections, a scoring was done in three different areas determined in each section. The immunopositivity of MMP-1 and TIMP-1 was determined using H scoring. H-score classification was done with a scoring grade of 0 to 3 as previously described (Nakopoulou et al., 2003; Nagel et al., 2004) and detailed below:

- Grade 0: Absence of immunoreactivity
- Grade 1: Low level of immunopositive
- Grade 2: Increased level of immunopositive
- Grade 3: Very densely immunopositive

Finally, the obtained scores were analyzed statistically.

Statistical analysis

Obtained MMP-1 and TIMP-1 immunopositivity levels were analyzed statistically (IBM SPSS Statistics 24). For this purpose, independent samples t-test was used. Results were shown as mean ± standard deviation (mean ± SD) and $p<0.05$ was considered statistically significant.

RESULTS

Histopathology

In Group N, the presence of normal histological structures was observed in H&E sections. It was found that the luminal epithelium had a characteristic structure, the stroma was wide and regular, and the uterine glands were normally distributed. In addition, it was observed that the myometrium muscle tissue was thick and the vascular structures were in normal histological view between the fiber bundles.

In Group M, it was determined that epithelial vacuolization was intensely increased in the luminal epithelium. There was a deformative appearance in the uterine glands in the stroma, and an increase in mononuclear cell infiltration was observed in the stromal area. It was determined that the hyalinization image was intensified in the endometrium and myometrial transition region (Figure 1).

Table 1. Results of MMP1 and TIMP1 immunopositivity scoring in Group N and Group M.

		Group N	Group M	p value
MMP-1	Luminal Epithelium	1.20±0.61	2.27±0.69	<0.01
	Endometrial Stroma	0.90±0.48	1.23±0.57	<0.05
	Uterine Gland	0.93±0.58	1.30±0.65	<0.05
	Myometrium	0.57±0.50	2.07±0.87	<0.01
TIMP-1	Luminal Epithelium	0.57±0.63	0.40±0.56	>0.05
	Endometrial Stroma	1.27±0.58	0.57±0.63	<0.01
	Uterine Gland	1.40±0.56	0.50±0.63	<0.01
	Myometrium	0.27±0.45	0.70±0.70	<0.01

Immunohistochemistry

In the uterine sections of the two groups, luminal epithelial stroma and endometrial gland epithelium showed varying levels of TIMP and MMP positivity (Figure 2). On the other hand, although TIMP-1 immunopositivity was generally low in the myometrium of Group M, it was highly concentrated in the transition regions (Figure 3). MMP-1 immunopositivity was found to be significantly higher in the luminal epithelium ($p<0.01$), endometrial stroma, uterine gland ($p<0.05$) and myometrium sections ($p<0.01$) of the uterus in Group M compared to Group N. No significant difference was observed in the Luminal Epithelium of Group N and Group M in terms of TIMP-1 immunopositivity ($p>0.05$). TIMP-1 immunopositivity of endometrial stroma and uterine gland sections decreased in Group M compared to Group N ($p<0.01$), However, TIMP-1 immunopositivity of myometrium sections was higher in Group M than Group N ($p<0.01$). MMP-1 and TIMP-1 immunopositivity results are presented in detail in Table 1.

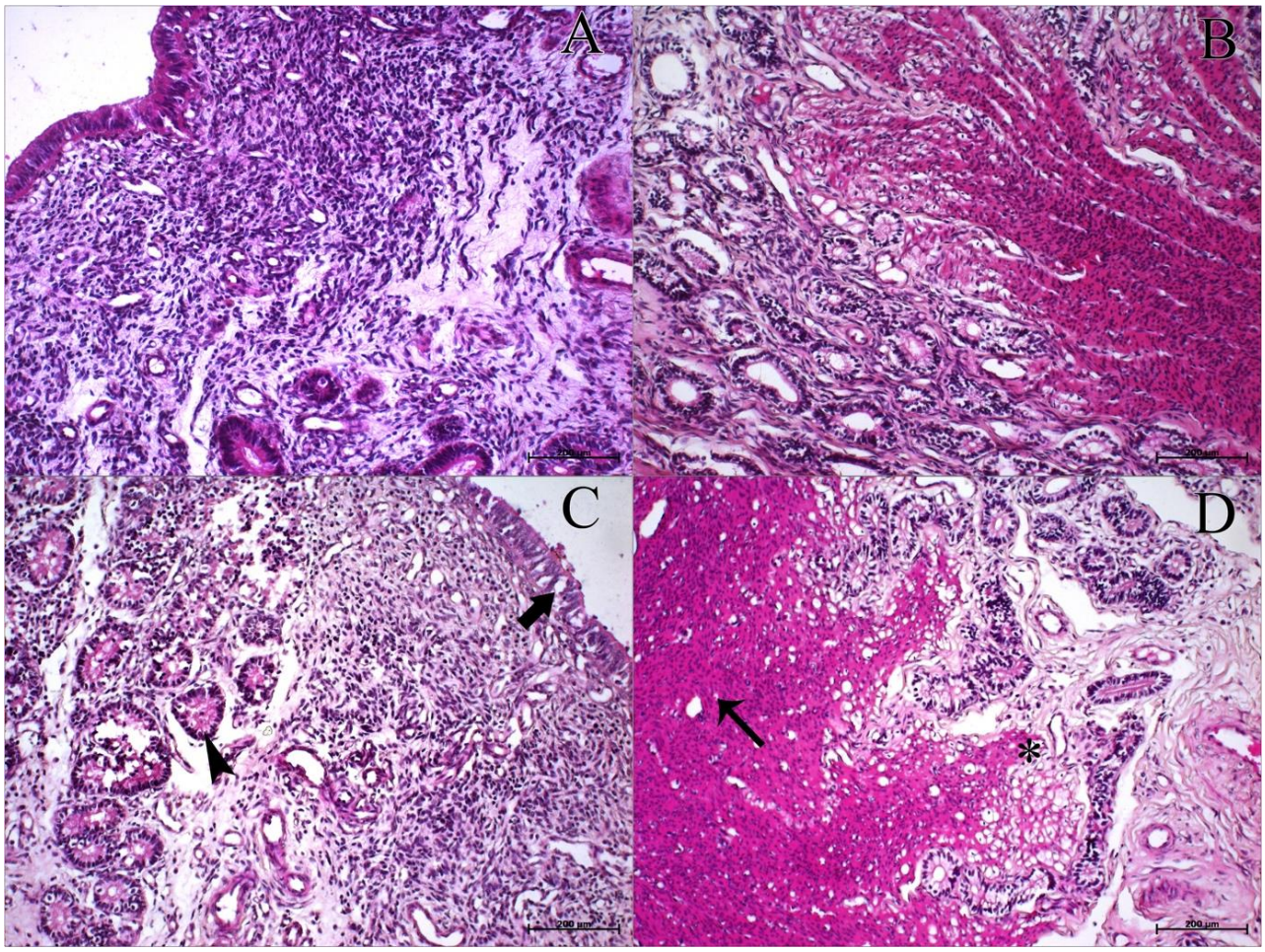

Figure 1. Light microscope examination of uterus sections in Group N (A, B) and Group M (C, D). Vacuolized epithelium image in uterine luminal epithelium (thick arrow), irregular image of the uterine glands (arrowhead), an increase in the appearance of hyalinization in the transition zone between the endometrial stroma and the myometrium (*) and vacuole and edema in the muscular layer of the myometrium (arrow). Staining: H&E, Bar: 200 µm.

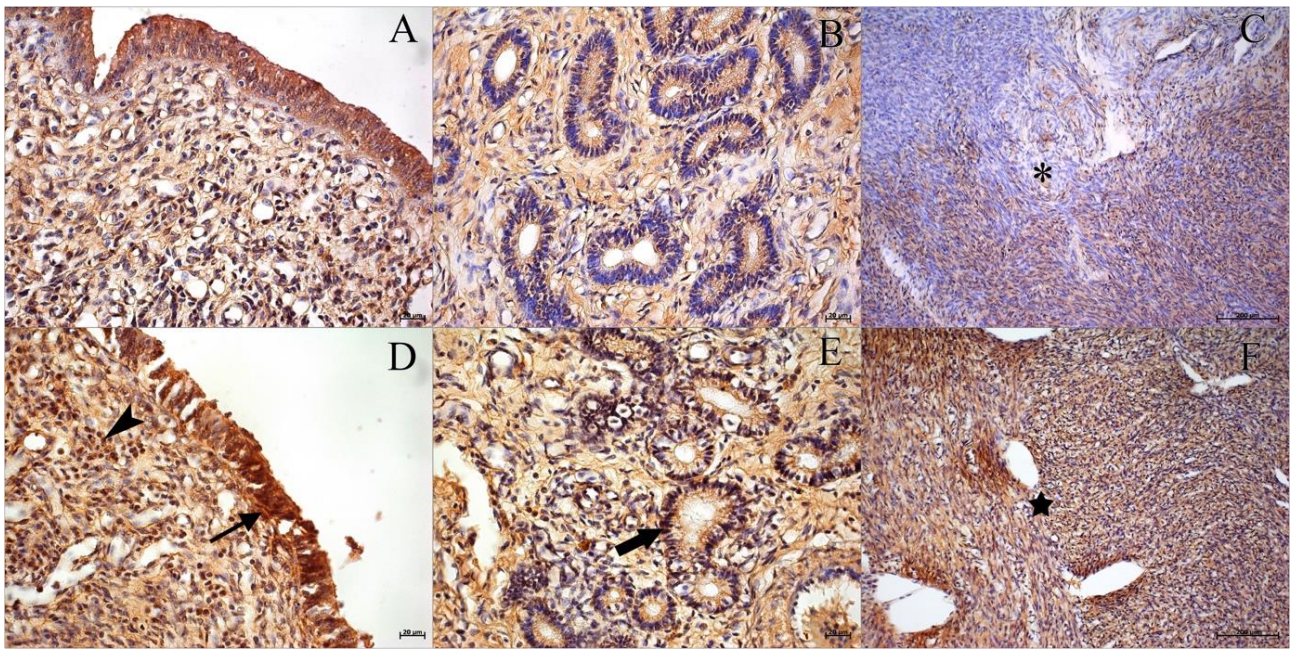

Figure 2. MMP-1 immunohistochemistry in Group N (A, B, C) and Group M (D, E, F). Presence of condensation of MMP-1 immunopositivity in the lumial epithelium (arrow) and stroma (arrowhead). Similar immunopositivity in uterine gland epithelium in Group N and Group M. Density difference in terms of MMP-1 between inner and outer muscle fiber bundles of myometrium (star). Concentration of MMP-1 immunopositivity in the myometrium (star). Staining: MMP-1 immunohistochemistry, Bar: 20 μm (A, B, D, E) 200 μm (C, F).

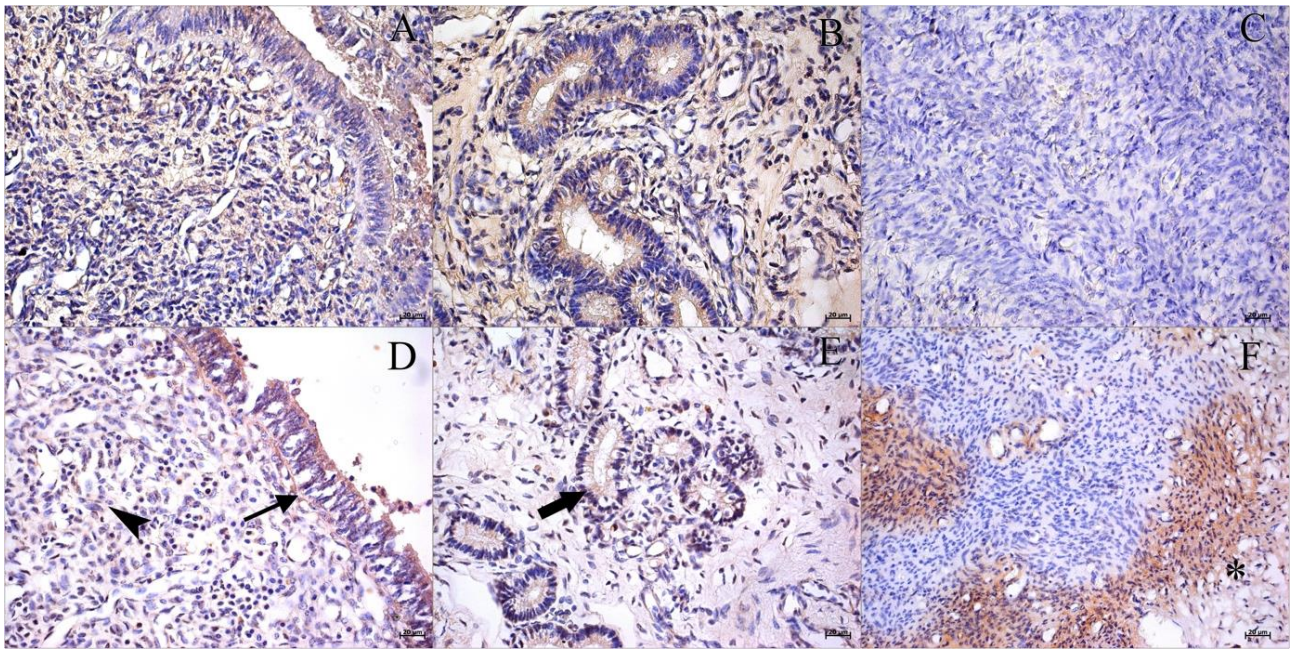

Figure 3. TIMP-1 immunohistochemistry in Group N (A, B, C) and Group M (D, E). In Group M, TIMP-1 condensation in the basal lamina of the luminal epithelium (arrow) and decreased TIMP-1 immunopositivity in the stroma (arrowhead). Low level of immunopositivity at the apical pole of the uterine gland epithelium (thick arrow). Although negative TIMP-1 areas are observed in the myometrium of Group N and Group M, areas of immunopositivity at different densities in the myometrium, especially in the edometrio-myometrial transition region, in Group M (star). Staining: TIMP-1 immunohistochemistry, Bar: 20 μm.

DISCUSSION

The present study investigated the histopathological effect of metritis on the uterus in cows recovered from metritis. For this purpose, the uteri of healthy cows (no metritis) and uteri of cows that have previously recovered from metritis were compared in terms of MMP-1 and TIMP-1 immunopositivity.

It is well known that metritis causes inflammatory symptoms such as edema in all layers of the uterus, leukocyte infiltration, and myometrial degeneration (Sheldon et al., 2006). On the other hand, it is stated that ovarian function is impaired in cows with uterine infection (Sheldon and Owens, 2018). As a result, fertility decreases. The only way to reverse this situation is to treat the disease allowing the return of the physiological uterine function. It is known that the uterus of cows that have recovered from metritis is restored histologically (Sheldon et al., 2006; Hansen, 2013). However, it cannot be estimated exactly how long this period lasts. Sheldon et al. (2008) reported that although epithelial regeneration is completed approximately 25 days after parturition, a period of 6-8 weeks after calving can be required to restore deeper tissue layers. However, some cases of uterine infections are known to prolong this period (Koh et al., 2018). On the other hand, it is a matter of interest whether the uterus returns to its histologically healthy function in cows with metritis. We suppose that one way to assess this was to investigate the level of regeneration in uterine cells and connective tissue. For this purpose, we investigated the positivity of immunohistochemistry of MMP-1, which is involved in the remodeling of various tissues including endometrial tissue (Chen et al., 2017; Li et al., 2017), and tissue inhibitor of MMP (TIMP-1) in the uterine tissue. We found that the uterus was in a pathological state since the expression levels of MMP-1 and TIMP-1 differed in Group M compared to Group N. However, no significant difference was found between the groups in terms of TIMP-1 in the luminal epithelium of the uterus. In fact, a previous report stated that the luminal epithelium may be the most sensitive site in the rat uterus (Seker et al., 2020). Thus, with this sensitivity, we think that the uterine lumen epithelium may be the first site to provide histological recovery in cow uterus with metritis. We were focused on two assumptions as the reason for this situation. The first is that metritis causes damage to the uterus that is histologically difficult to reverse. The second assumption is that the uterus in Group M has not yet recovered histologically and more time is needed for complete histological recovering. The second assumption is more likely to be true because cows in Group M are known to have clinically recovered from metritis approximately 45 days ago. Therefore, we think that this period cannot be sufficient to complete the histological healing of the uterus. We think that the histopathological results of the study also support this. However, in order to clarify this assumption, the histological recovery period should be determined by taking repeated biopsy samples from the uterus of live cows using biopsy forceps. For this purpose, we think that a similar study should be conducted on both live cows and slaughtered cows at different time periods of clinical recovery. We also think that factors such as immune system function, metabolic conditions, care, feeding, management, individual differences or the type of bacteria that cause metritis can affect the histological recovery period in the uterus. Furthermore, it is noted that a normal endometrium is required for a successful breading (Sheldon and Owens, 2018). Similarly, Koh et al. (2018) reported that an abnormal uterine environment can affect normal embryo development and reproductive performance in a variety of ways. In the present study, results of histopathological MMP-1 and TIMP-1 show that the histological restoration of the uterus is not complete and they do not yet have a normal endometrium in Group M compared to Group N. Considering above information, it is estimated that cows with metritis may experience fertility problems even approximately 45 days after the disease has clinically resolved.

CONCLUSION

In conclusion, it was observed that MMP-1 immunopositivity increased in various parts of the uterus of cows recovered from metritis. However, TIMP-1 immunopositivity decreased in endometrial stroma and uterine gland sections and increased in myometrial sections in the uterus of cows recovered from metritis. This demonstrated that the negative effect of metritis on the uterus persisted histologically even approximately 45 days after clinical recovery. However, this needs to be investigated extensively because time elapsed between the clinical recovery of the cows and the study may have been insufficient for the uterus to complete histological healing. Therefore, whether the negative effect of metritis on the uterus is

permanent or temporary should be clarified with further studies.

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Incidence of gastrointestinal parasites by stool examination methods in horses in Van

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ABSTRACT

Objective: This study was carried out to determine the distribution of digestive system parasites in horses in İpekyolu, Tusba, Özalp, Saray, and Gürpınar districts in Van Province, Turkey between June 2019 and May 2020.

Materials and Methods: Materials for this study consist of a total of 95 horse stool samples, 58 male and 37 female, from different races, ranging in age from 1 to 12 years.

Results: Various parasitic infections were detected in 65 (68.42%) of the 95 horses examined by stool examination. While 55 of the 65 infected horse stool samples were infected with a single species, the remaining 10 stool samples were infected with two species. In infected horses, the most common parasites were *Strongylidae* spp. (44.21%) followed by *Parascaris equorum* (11.57%), *Eimeria leuckarti* (6.31%), *Anoplocephala* spp. (5.26%), *Oxyuris equi* (4.21%), *D. dendriticum* (3.16%), and *Fasciola* spp. (4.21%). When the infected horses were evaluated according to age groups, parasitic infections were detected in 34 (35.79%) horses aged 8 years and above, 29 (30.53%) between the ages of 5-7, and 12 (12.63%) between the ages of 1-4. As for gender groups, parasitic infections were found in 38 (65.51%) of the 58 male horses and 27 (72.92%) of the 37 female horses. When the parasite species were examined, it was determined that the highest infection rate was in the Strongylid type egg. There was a statistically significant relationship between parasite species and infection rate ($p<0.05$). A statistically significant relationship between parasite species and age was determined only in the Strongylid type egg parasite ($p<0.05$).

Conclusion: This study concluded that the horses in Van Province were infected with various parasite species. Helminth infections were found to be the most common type of parasites, and we think that more frequent examination and treatment should be conducted to fight parasites.

Keywords: *Gastrointestinal parasite, Horse, Van, Turkey*

INTRODUCTION

Horses are animals that people cannot live without not only for sporting activities but also for agriculture and transportation purposes in rural areas in our country. In the Van region, horses are used for sporting purposes as well as in agriculture and transportation. The fact that horses are not healthy in terms of bacteriological, virological, parasitological, and mycotic diseases leads to serious performance degradation and even death. Parasitic diseases, which are among the important infectious diseases in horses, can significantly affect the performance of these animals. Therefore, effective parasitic treatment and control are important for both animal health and animal welfare. In the digestive system of horses, nematodes, cestodes, trematodes, and protozoa are found (Jacobs, 1989; Öge, 2002).

In studies based on stool examination and necropsy, the prevalence of equine helminths was determined at a rate of (27.6-100%) and (16.2-100%) in the world and in Turkey respectively and the most common helminths are strongyloid infections (62.7-100%), followed by *Parascaris equorum, Oxyuris equi, Dictyocaulus arnfieldi, Strongyloides westeri, Trichostrongylus axei, Draschia megastoma, Habronema spp., Fasciola hepatica, Dicrocoelium dendriticum, Anoplocephala magna, Anoplocephala perfoliata* and *Paranoplocephala mamillana* (Dunsmore and Jue, 1985; Öge, 1991; Özer and Küçükerden, 1992; Tınar et al., 1994; Arslan and Umur, 1998; Pişkin et al., 1999; Gül et al., 2003; Bakırcı et al., 2004; Pereira and Vianna, 2006; Ulutaş and Efil, 2012; Kozan and Güzel, 2015). In addition, protozoal infections caused by *Eimeria leuckarti, E. solipedium* and *E. uniungulati* have also been reported in horses (Oğuz, 1971; Özer and Küçükerden, 1992; Tınar et al., 1994; Öge, 2002; Bakırcı et al., 2004). This study was carried out to determine the prevalence of digestive system parasites in horses in Van Province.

MATERIALS and METHODS

This study was carried out in İpekyolu, Tusba, Özalp, Saray, and Gürpınar districts in Van Province between June 2019 and May 2020. The materials for this study consist of a total of 95 horse stool samples, 58 male and 37 female, from different races, ranging in age from 1 to 12 years. Fecal samples were gathered from the horses which were not given any parasitic drugs before. Collected fresh stool samples were brought to the laboratory on the same day after being placed in separate stool containers. Stool samples were examined for nematodes, cestode eggs, and protozoan oocysts by Fülleborn's flotation method prepared with saturated salt water, by sedimentation method for trematode eggs and *E. leuckarti* oocysts, and by Baermann-Wetzel methods for *D. arnfieldi* larvae (Stoye, 1984; Thienpont et al., 1986). This study was approved by Van Yuzuncu Yil University Animal Researches Local Ethic Committee with the number of 2022/04-01.

Evaluation of research data was done with SPSS (Ver. 13.0) package program. In the evaluation of the data, descriptive statistics are given as numbers and percentages. The relationship between categorical variables was evaluated with the chi-square test. Statistical significance was accepted as $p<0.05$ (SPSS. IBM SPSS statistics version 13.0 for Windows. New York: IBM Corp.)

RESULTS

Various parasitic infections were detected in 65 (68.42%) out of the 95 horses. Infection with one species was found in 55 of the 65 infected stool samples, and with two species in 10 stool samples (Table 1, Table 2).

Table 1. The rate of infections (%) with a single species in horses according to their stool examination (n: number of infected animals).

Parasitic Species	n	Infection rate* (%)
Strongylid type egg	34	35.79
Parascaris equorum	6	6.31
Eimeria leuckarti	3	3.16
Anoplocephala spp.	4	4.21
Oxyuris equi	3	3.16
Dicrocoelium dendriticum	2	2.10
Fasciola spp.	3	2.16

*: There was a statistically significant relationship between parasite species and infection rate ($p<0.05$). When the parasite species were examined, it was determined that the highest infection rate was in the *Strongylid* type egg.

Table 2. The rate of infections (%) with two species in horses according to their stool examination (n: number of infected animals).

Parasitic Species	n	Infection rate (%)	p*
Strongylid type egg + *Parascaris equorum*	3	3.16	
Parascaris equorum + *Eimeria leuckarti*	1	1.05	
Eimeria leuckarti. + *Strongylid* type egg	2	2.10	
Anoplocephala spp.+ Strongylid type egg	1	1.05	0.853
Oxyuris equi + *Parascaris equorum*	1	1.05	
Dicrocoelium dendriticum + *Strongylid* type egg	1	1.05	
Fasciola spp. + *Strongylid* type egg	1	1.05	

*: There was no statistically significant relationship between parasite species and infection rate ($p>0.05$).

In infected horses, the most common parasites were Strongylid-type eggs (44.21%) followed by *Parascaris equorum* (11.57%), *Eimeria leuckarti* (6.31%), *Anoplocephala* spp. (5.26%), *Oxyuris equi* (4.21%), *D. dendriticum* (3.16%), and *Fasciola* spp. (4.21%) (Table 3).

Table 3. Parasites detected in horses according to stool examination results (n: number of infected animals).

Parasitic Species	n	Infection rate* (%)
Strongylid type egg	42	44.21
Parascaris equorum	11	11.57
Eimeria leuckarti	6	6.31
Anoplocephala spp.	5	5.26
Oxyuris equi	4	4.21
Dicrocoelium dendriticum	3	3.16
Fasciola spp.	4	4.21

*: There was a statistically significant relationship between parasite species and infection rate ($p<0.05$). When the parasite species were examined, it was determined that the highest infection rate was in the *Strongylid* type egg.

When the infected horses are evaluated according to their age groups; parasitic infections were detected in the feces of 34 (35.79%) horses aged 8 and older, 29 (30.53%) in the 5-7 age group, and 12 (12.63%) horse feces in the 1-4 age group (Table 4). As a result of the examination, Strongylid-type eggs were found to be the most common parasites in all three age groups, followed by *P. equorum* eggs and *Eimeria leuckarti* oocysts. As for gender groups, parasitic infections were found in 38 (65.51%) of the 58 male horses and 27 (72.92%) of the 37 female horses (Table 5).

When the parasite species were examined, it was determined that the highest infection rate was in the Strongylid type egg. There was a statistically significant relationship between parasite species and infection rate ($p<0.05$). A statistically significant relationship between parasite species and age was determined only in the Strongylid type egg parasite ($p<0.05$).

Table 4. Distribution of parasites by age.

Parasitic Species	The age range of infected animals			p*
	1-4 ages	5-7 ages	8 ages and older	
	n (%)	n (%)	n (%)	
Strongylid type egg	6 (6.31)	16 (16.84)	20 (21.05)	0.013
Parascaris equorum	2 (2.10)	4 (4.21)	5 (5.26)	0.516
Eimeria leuckarti	3 (3.26)	1 (1.05)	2 (2.10)	0.600
Anoplocephala spp.	1 (1.05)	2 (2.10)	2 (2.10)	0.816
Oxyuris equi	-	3 (3.26)	1(1.05)	0.312
Dicrocoelium dendriticum	-	2 (2.10)	1 (1.05)	0.561
Fasciola spp.	-	1 (1.05)	3 (3.26)	0.312
Total	12 (12.63)	29 (30.53)	34 (35.79)	

n: number of infected horses; *: A statistically significant relationship between parasite species and age was determined only in *Strongylid* type egg parasite ($p<0.05$). Infection rate of *Strongylid* type egg parasite was determined as 21.05% in horses aged 8 years and older. There is a statistically significant relationship between other parasite species and age ($p<0.05$).

Table 5. Parasite infection distribution by gender.

Gender	Male (%)	Female (%)	p*
Infected	38 (65.51)	27 (72.92)	0.446
Non-Infected	20 (34.48)	10 (27.02)	-
Total	58	37	-

*: There was no statistically significant relationship between gender and infection rate ($p>0.05$).

DISCUSSION

A number of studies conducted in Turkey to determine helminth infections of equidae according to stool examination. It has been reported that the majority of helminth infections seen in equidae originate from pastures. In studies conducted in various provinces, it has been reported that parasitic infection rates of equids range from 16.2% to 100% (Aydenizöz, 2004; Karaca et al., 2005; Altaş et al., 2005; Uslu and Güçlü, 2007; Umur and Açıcı,

2009; Ulutaş and Efil, 2012; Kozan and Güzel, 2015). Studies conducted in Turkey and worldwide have reported *Strongylidae*, *Anoplocephalidae*, *P. equorum*, and *O. equi* species as the most frequently detected helminths in Equidae (Karaca et al., 2005; Pereira and Vianna, 2006; Uslu and Güçlü, 2007; Umur and Açıcı, 2009). In this study, 65 (68.42%) of the 95 horses examined were infected with various parasites with strongyloid (44.21%) being the most common form of In infection, followed by *P. equorum* (11.57%), *E. leuckarti* (6.31%), *Anoplocephala* spp. (%) 5.26), *O. equi* (4.21%), *Fasciola* spp. (4.21%), and *D. dendriticum* (3.16%).

In studies conducted in Turkey, *Strongylidae* are reported to be the most common helminths in equidae (Öge, 1991; Özer and Küçükerden, 1992; Tınar et al., 1994; Arslan and Umur, 1998; Pişkin et al., 1999; Gül et al., 2003; Bakırcı et al., 2004; Ulutaş and Efil, 2012; Kozan and Güzel, 2015). *Strongylidae* spp. has been reported in 88.86% of the horses in agricultural holdings, 100% of equids in the Kars region, 71.76% of Gemlik Military Stud farm Horses, 63.04% of purebred Arabian horses in the Şanlıurfa region, 100% of horses and donkeys in Konya, and 100% of horses in Afyon region (Arslan and Umur, 1998; Bakırcı et al., 2004; Altaş et al., 2005; Uslu and Güçlü, 2007; Kozan and Güzel, 2015). In Van Province, Karaca et al. (2005), and Erdoğan (2019) reported *Strongylidae* spp. in 96.4% and 60% of the horses respectively. In this study, Strongylid type eggs were detected in 44.21% of the horses and this rate was found to be significantly lower than the rate reported by other studies conducted in Turkey (60-100%) and Van (60-96.4%). We believe that the reason for this may be horse breeds, pasture or intensive nutritional status, etc.

The prevalence of *Parascaris equorum* has been reported to be between 0.5% and 42.9% in different studies conducted in Turkey according to stool examinations (Gülbahçe, 1990; Demir et al., 1995; Pişkin et al., 1999; Aydenizöz, 2003; Gül and ark., 2003; Bakırcı et al., 2004; Altaş et al., 2005; Karaca et al., 2005; Uslu and Güçlü, 2007; Umur and Açıcı, 2009; Toktamis and Yaman, 2012; Ulutaş and Efil, 2012; Kozan and Güzel, 2015). Karaca et al. (2005) reported the prevalence of *Parascaris equorum* to be 35.8% in their study in Van while *P. equorum* was not found in another study also conducted in Van (Erdoğan, 2019). In the current study, *P. equorum* was found in 11.57% of the horses in the Van region. We think that the reason for the different findings in these two studies is that the samples were collected from different regions of Van. However, the rate determined in this study is compatible with other studies reported in Turkey (Gülbahçe, 1990; Demir et al., 1995; Bakırcı et al., 2004; Çırak et al., 2005; Uslu and Güçlü, 2007; Umur and Açıcı, 2009).

Studies have reported that the most common types of cestode infections in horses are *Anaplocephala perfolitica* and *A. manga*. The spread rate of these parasites has been recorded as 0.2-15.8% (Özer and Küçükerdan, 1992; Tınar et al., 1994; Burgu et al., 1995; Arslan and Umur, 1998; Pişkin et al., 1999; Karaca et al., 2005; Erdoğan, 2019). While *Anoplocephalidae* spp. was reported as 2.9% in a study conducted in Van Province, it was reported as 8% in another study (Karaca et al., 2005; Erdoğan, 2019). In the present study, *Anoplocephala* spp. was detected at a rate of 2.9%, and the results are compatible with the above-mentioned studies conducted in Turkey according to stool examinations.

In autopsy studies reported in different parts of the world, *Oxyuris equi* has been recorded at a rate of 7-90% (Bucknell et al., 1995; Barbosa et al., 2001; Pereira and Vianna, 2006), and 4.1-17% in stool examinations (Sotiraki et al., 1997; Eslami et al., 2005). In Turkey, Burgu et al. (1995) found *Oxyuris equi* as 30% in their autopsy study, while it was reported to be between 0.6-7.6% in some studies based on stool examinations (Demir et al., 1995; Pişkin et al., 1999; Gül et al., 2003; Bakırcı et al., 2004; Altaş et al., 2005; Uslu and Güçlü, 2007; Umur and Açıcı, 2009). Erdoğan (2019) detected 4% of *Oxyuris* spp in his research in Van. In this study, *Oxyuris equi* was found to be 4.21% and this rate was found to be consistent with studies conducted in Turkey through stool examination.

In studies conducted in different regions of Turkey on the presence of *F. hepatica* in horses (Arslan and Umur, 1998; Gül et al., 2003) and *D. dendriticum* (Demir et al., 1995), *D. dendriticum* was found to be 0.9-1%, (Demir et al., 1995; Aydenizöz, 2004; Uslu and Güçlü, 2007; Umur and Acıcı, 2009), and *Fasciola* spp. as 0.9-5.8% (Demir et al., 1995; Arslan and Umur, 1998; Gül et al., 2003; Karaca et al., 2005; Uslu and Güçlü, 2007; Umur and Açıcı, 2009). However, Bakırcı et al. (2004), reported that they did not find these trematodes in their study on Gemlik military stud farm horses. In Van Province, Karaca et al. (2005) reported that they detected Fasciola hepatica at a rate of 5.8% in horses, and Erdoğan (2019), detected *Fasciola* spp. at 2% and *Dicrocoelium* spp. at 2% in horses. In this study, *D. dendriticum* was detected at a rate of 3.16%, and

Fasciola spp. at a rate of 4.21%, and these rates are consistent with reported studies in Turkey.

In this study, *Eimeria leuckarti* was found to be 6.31% in the stool examination of horses. *E. leuckarti* has been reported in Germany in 80% of foals (Beelitz et al., 1994), and in 7.6% of equidae in Iran (Karimi ghahfarrokhi et al., 2014). In Turkey, *Eimeria leuckarti* in horses was first reported by Oğuz (1971). It was reported that this parasite was encountered at rates between 0.4-5.88% (Tınar et al., 1994; Arslan and Umur, 1998; Bakırcı et al., 2004). *Eimeria leuckarti* was reported in 0.4% of horses in Bursa Province (Tınar et al., 1994), 3.8% of equidae in Kars Province (Arslan and Umur, 1998), 4.5% of horses in Konya Province (Uslu and Güçlü, 2007) and 1% of horses in Van Province (Erdoğan, 2019). A study conducted in Erzurum Province reported no *E. leuckarti* in the horses examined (Avcıoğlu et al., 2016).

CONCLUSION

This study found that the horses in Van Province were infected with various parasite species. Strongylid-type nematode eggs were found to be the most common type of parasite in the examined horse stools. The current study also concluded that helminth infections were more widespread, indicating the development of resistance against these parasites. Intense helminth infections in horses cause loss of productivity and poor performance. Therefore, it is necessary to inform the breeders about these parasites and to warn them to take the necessary precautions. Effective parasite control is recommended and antiparasitic drugs should be used in antiparasitic control programs carried out in horses with focus on fight against this group of helminths.

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Author's Contributions: VD and AK designed the study. VD and AK performed surgeries. VD performed statistical analysis. AK participated in drafting and revising the manuscript. VD: Vural Denizhan, AK: Ayşe Karakuş. All authors have read and agreed to the published version of the manuscript.

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Evaluation of hemogram parameters in neonatal diarrhoeic calves with and without gastrointestinal protozoa infections

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ABSTRACT

Objective: The aim of this study was to compare the hemogram analysis results of neonatal diarrheal calves with and without gastrointestinal protozoa infection.

Materials and Methods: A total of 21 neonatal calves with diarrhoea were examined within the scope of the study. Eleven of the cases were calves with gastrointestinal protozoa infection and 10 were calves without gastrointestinal protozoa infection. Demographic, clinical, and laboratory data of calves were evaluated.

Results: When demographic data and vital signs were evaluated between the two groups, no statistically significant difference was found between the two groups ($p>0.05$). However, when the hemogram values between the two groups were compared, it was determined that there was a significant difference in white blood cell ($p=0.003$) and neutrophil ($p=0.01$) numbers.

Conclusions: Evaluating hemogram parameters should be taken into account as it is an inexpensive and easy-to-apply analysis and offers important outputs in the control and follow-up of neonatal calf health especially in neonatal calf diarrhoea cases which is one of the common diseases.

Keywords: *Neonatal, Calf diarrhoea, Hemogram, Protozoa*

INTRODUCTION

Calf mortality is one of the major problems of animal husbandry worldwide. Factors such as low calf performance, genetic losses due to reduced stocks in the herd during the breeding process and therefore reduced selection probability, and the budget spent on the diagnosis and treatment of the disease negatively affects the breeder and the country's economy (Smith, 2012; Yimer et al., 2015).

Veterinarians are often requested to investigate animal death or illness, including those related to neonatal calf diarrhoea, and to prevent calf death as soon as possible. The most common agents causing neonatal calf diarrhoea have been reported as bacterial (*Escherichia coli, Salmonella* spp., etc.), viral (Rota virus, Corona virus, etc.), and protozoan (*Cryptosporidium* spp. and, *Eimeria spp*.) infections (Altug et al., 2013; Cho and Yoon, 2014). Studies conducted in different countries have shown that neonatal calf diarrhoea due to protozoan agents is very common. Studies conducted in our country show that *Cryptosporidium* spp. and, *Eimeria* spp. are found to be positive in newborn calves up to 63% and 90%, respectively (Çitil et al., 2004).

The etiology of neonatal calf diarrhoea is quite complex and multifactorial and also environmental factors and practice differences in farm management play an important role, as well as the vulnerability of calves to infectious agents due to their predisposition. Therefore, whether preventive medicine practices are applied in the herd, feeding of pregnant and post-partum animals and related

diseases and the seasonal variations are effective in calf mortality rates (Singh et al., 2009).

Many enteric pathogens can play a role in calf diarrhoea, having fecal-oral route for transmission (Cho and Yoon, 2014). This increases the possibility of the rapid spread of pathogens, especially in farms where hygiene rules are not followed. For this reason, it is important to isolate the sick animal in cases where signs such as watery diarrhoea and/or blood in the stool, dehydration, anorexia, and lethargy are detected (Cho and Yoon, 2014; Elitok, 2020). In these acute neonatal diarrhoea cases, information obtained from the clinical examination findings of the patient as well as from easy-to-apply and low-cost analysis methods such as evaluation of hemogram parameters gains even more importance (Smith, 2012; Song et al., 2020).

This study aims to compare the results of hemogram analysis in diarrheal calves with and without gastrointestinal protozoa infections, which are the most determined etiologic agents in neonatal calf diarrhoea, and to contribute to the evaluation of the patient according to these parameters in field applications.

MATERIALS and METHODS

Animal material

Cases of neonatal calf diarrhoea brought to Ankara University Faculty of Veterinary Medicine Animal Teaching Hospital between February 2018 and June 2022 were determined retrospectively from the hospital software database. A written owner consent form was obtained from all animal owners. This study was reviewed by the Local Animal Ethics Committee of Ankara University it was decided that the study was not subject to Ethics Committee approval (Decision number: 2022-18-163)

The keywords 'calf' for animal species and 'diarrhoea' for diagnosis were used to search for cases. Records of cases were reviewed, and eligible calves meeting the inclusion criteria for neonatal calf diarrhoea were selected. Neonatal calf diarrhoea was defined as diarrhoea in calves aged 1-month-old or younger that was triggered by protozoans or other causes. Only the animals have the clinical examinations details as well as the results of both the hemogram and faecal examination findings were included in the study.

The animal material was consisted of 21 calves. Among the cases 11 calves were included in the first group infected with gastrointestinal protozoa infections while 10 calves were in the second group without gastrointestinal protozoa infections.

The breeds, ages and genders of the calves were noted for the analysis in both groups. The dates when patients were first brought to our clinic were also analysed to understand whether there was a distribution difference in the seasons in which the disease occurred between the groups.

Clinical examination and laboratory analysis

Clinical examination records including temperature, pulse and respiratory rate (TPR), mucous membrane color and dehydration status were noted. The hydration status was classified as normal <5%, mild 6-8%, moderate 8-10%, and severe >10 (Smith, 2009).

The fecal examination was performed within half an hour after fresh sample collection. The protozoan infections were diagnosed by fecal flotation and Ziehl–Neelsen staining under a light microscope (Cho and Yoon, 2014).

The hemogram (complete blood cell count) analysis measured in the Central Diagnostic Laboratory (by Exigo H400 Veterinary Hematology Analyzer, Boule Medical AB, Sweden) of Ankara University Animal Hospital including white blood cell (WBC), lymphocyte (LYM), monocyte (MONO), lymphocyte % (LYM%), monocyte % (MONO%), neutrophil % (NEUT%), red blood cell (RBC), hemoglobin (HGB), packed-cell volume (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MHC), red cell distribution width (RDW) and platelet (PLT) were also studied within the scope of this paper.

Statistical analysis

To analyze any differences in the parameters between the 1st and 2nd groups, we applied the student's t-Test for parametric values while Mann-Whitney U-Test was performed for non-parametric values. The gender differences between the groups were analyzed with Chi-Square test. The analyses were performed in SPSS 22.0 for Windows, SPSS Inc, Chicago, IL, USA. Significance was defined as $p<0.05$.

RESULTS

There were 9 Simental (81.8%) and 2 Holstein (18.2%) calves in the first group while the second group consisted of 7 (70%) Simental and 3 Holstein (30%) calves. While male calves were in the majority in the first group (54.54%), the number of female calves was higher (80%) in the second group. The

mean age at the presentation was 10.9 and 10.12 days in the first and second groups, respectively. When the demographic data of the calves were examined, no statistical difference was found between the two groups in terms of breed, age, and gender (p>0.05).

The dates of the first admission of the patients to our clinic were examined and the seasonal distribution was evaluated. While 7 out of 11 calves in the first group (63.63%) were first presented in the spring months (March, April, or May), the remaining 4 were (36.36%) presented during winter (December, January, or February). In the second group, while the majority of calves were presented in the winter season (n=6, 60%), 3 were presented in the spring and 1 was in the summer (August).

When the temperature, pulse, and respiratory rate data were examined to evaluate clinical examination findings, no statistically significant difference was found between the two groups (p>0.05). However, it was observed that the body temperature of the neonatal calves with protozoan infection (37.83°C±0.89) was slightly lower than the calves in the 2nd group (38.58°C±1.01). Moreover, the pulse and respiratory rates were also found to be higher in the second group compared to those in the first group. The pulse rate was 95±11.36 per/min in the first and 100.5±9.06 per/min in the second group while the respiratory rate was 40.1±6.42/min and 41.6±5.31/min in the first and second groups, respectively.

When the hydration status of the calves in both groups was evaluated, the average dehydration level of 8-10% was determined in both groups (p>0.05). Although the mucous membranes were noted as "pale" in 73.7% of the calves in the first group, this rate was found to be %60 in the second group.

Fecal examinations revealed 7 calves diagnosed with *Cryptosporidium* spp. (63.6%) while 4 with *Eimeria* spp. (36.3%) in the first group. We did not determine any gastrointestinal parasites from the samples of the second group.

The hemogram data of the calves in both groups were compared. While WBC (p=0.003) and NEUT (p=0.016) counts significantly differed between the two groups, we did not find any significant differences in the other parameters. The mean±std value of each parameter in the groups has shown in Table 1.

Table 1. The findings of hematological parameters in the first and second groups.

Parameters	First group (Mean±std)	Second group (Mean±std)	p value
White Blood Cell (×10⁹/l)	10.60±5.17	17.63±5.45	0.003
Lymphocyte (×10⁹/l)	6.45±3.61	5.21±3.71	0.30
Monocyte (×10⁹/l)	1±0.6	1.63±1.1	0.07
Neutrophil (×10⁹/l)	4.56±3.65	9.54±5.23	0.016
Lymphocyte %	48.59±21.48	36.81±21.89	0.36
Monocyte %	8.96±3.83	8.09±4.50	0.89
Neutrophil %	41.58±24.13	49.16±23.46	0.88
Red Blood Cell (x10¹²/l)	6.81±2.16	7.81±1.34	0.34
Hemoglobin (g/dl)	9.45±1.92	10.63±1.74	0.17
Packed-cell volume (%)	26.45±6.45	26.90±6.22	0.26
Mean Corpuscular Volume (fl)	39.18±3.51	38±2.57	0.48
Mean Corpuscular Hemoglobin (pg)	14.09±1.67	13.90±0.83	0.83
Red Cell Distribution Width(fl)	21.36±2.26	20.54±2.74	0.58
Platelet (10⁹/l)	402.27±213.31	527.45±226.40	0.16

DISCUSSION

Calf diarrhoea is a disease that highly affects the livestock economy worldwide. Although the overall liveborn calf mortality due to gastrointestinal system diseases including neonatal calf diarrhoea should be around 1% on a farm, previous studies conducted in different European countries reported the mortality rates varying between 1.5% to 17 while it is around 15% in Turkey (Norberg, 2008; Naylor, 2009; Raboisson et al., 2013; Elitok and Elitok, 2016; Motus et al., 2017). In the United States, these rates are between 6 to 8%, and the annual economic damage resulting from the

loss of calves is calculated to be about $125 million (Meyer et al., 2001; Jorgensen et al., 2017). The survival rate of calves due to the presence of protozoan-induced diarrhoea is reported to be significantly different compared to those without diarrhoea (Glombowski et al., 2017).

Due to the multifactorial and multi-etiological nature of the disease, the change in the severity of the disease also affects the chance of success in field practice (Cho and Yoon, 2014). In the presented study, demographic factors that may be effective in the development of the disease were evaluated among the calf groups with and without diarrhoea associated with gastrointestinal protozoa. According to our analysis results, no significant difference was determined between the groups included in the study in terms of breed, presentation age, and gender parallel to the findings of Lanz Uhde et al. (2008). Although the number of females in the second group was higher in percentage compared to the first group in our study, there was no statistical difference between the two groups, which may be due to the low number of animals in the groups, which is a limitation of our study.

In the present study, seasonal distribution was found to be associated with diarrheal pathogens. This finding is consistent with previous studies conducted in our country as well as in the world (Brenner et al., 1993; Raboisson et al., 2013; Chao et al., 2019; Berber et al., 2021). In our study, it was determined that neonatal calf diarrhoea cases intensified in both groups in the winter and spring months. While the majority of the cases are seen in the spring months of protozoan-induced diarrhoea, it is noteworthy that the first incidence of the disease in the calves in the second group increases in the winter months. This increase in cases in winter and spring may be due to the fact that cold weather is more optimal for the infectious effects of pathogens, as well as the fact that the herds are mostly kept indoors during these months and thus the spread of infections becomes more difficult to control (Berber et al., 2021).

Calf mortality during the first month of life is one of the biggest problems of farms. The morbidity and mortality rates in neonatal calf diarrhoea and sepsis lead to failure of colostral transfer and absorption of antibodies. Bacterial, viral, and parasitic infections can cause neonatal diarrhoea and sepsis, and in this case, morbidity and mortality rates increase considerably due to the failure of colostral transfer and absorption of antibodies (Naylor, 2009; Abuelo, 2017). Abnormal changes in temperature, heart and respiratory rate, and increased total leucocyte count are considered to be compatible with systemic inflammatory response syndrome (SIRS) findings (Singer et al., 2016) and any changes in these parameters should be a warning alarm for veterinarians in the field.

In this study, we have found a significant increase in the WBC and NEUT counts in the second group compared to those in the first group. The increase in these parameters is thought to be related to bacterial or viral aetiology of the diarrhoea in this group leading to more noticeable inflammatory response signs. Although there is no significant difference between etiological factors in terms of disease development, identification of pathogens is important in the treatment of patients (Cho and Yoon, 2014). Therefore, laboratory findings are also of great importance in terms of rational antibiotic use, since it is easier and cheaper to detect the presence of protozoa in stool samples from calves with diarrhoea than viral or bacterial agents, and since it is possible to evaluate the variability in the inflammatory response based on blood parameters.

Eimeria spp. is known to cause to malabsorption and enteritis with epithelial cell destruction in the intestines and can lead to enteric bleeding and eventually anemia in patients with an excessive protozoan load and severe disease (Elitok, 2020). However, this agent was diagnosed in one-third of the first group in this study and probably that is why there was not a significant difference in the RBC or the other RBC indexes. However, when the overall results of the groups are considered, the absence of a statistical difference in erythrocyte levels between the groups is in line with the previous study of Atcalı and Yıldız (2020).

CONCLUSION

In conclusion, since hematological parameters including WBC and Neutrophils show significant increase in non-protozoa induced diarrhoea cases, performing complete blood count analysis, which is an inexpensive and easy-to-apply analysis may help for preventing the losses due to neonatal calf mortality.

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The therapeutic effectiveness of thyme extract in naturally infected puppies with ascariasis

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ABSTRACT

Objective: This study aimed to investigate the therapeutic efficacy of thyme extract in puppies naturally infected with ascarids.

Material-Method: The study consisted of 20 puppies of different sexes, 2-4 months old, naturally infected with ascarid. There were given an oral 20% concentration of thyme extract for 3 days to puppies, and faecal egg counts were conducted on the 0th, 1st, 2nd, 3rd, and 7th days after the treatment was started (day 0). Also, serum urea, creatinine, AST, ALT levels were monitored on the 0th and 3rd days, together with daily clinical examination, to monitor possible toxic effects.

Result: In 2 puppies (10%), the fecal egg count was highly variable at post-treatment examinations, but no reduction in egg count was observed. Egg shedding in 7 (35%) of the treated puppies was zero. It was observed that egg shedding was not completely zero in 11 of the puppies (55%). However, the egg counts decreased by 25% to 98.3%. It was also observed that the values of the measured blood biochemical values were within reference range except serum urea levels and the puppies did not show any clinical sign of toxicity during the treatment.

Conclusion: It was concluded that the thyme extract did not have any toxic effect in the puppies at the concentration studied, and it could be effective in the treatment of ascariasis.

Keywords: *Toxocara spp., Puppy, Ascariasis, Treatment, Thyme extract*

INTRODUCTION

Ascarids are among the most common gastrointestinal parasites encountered in dogs (Overgaauw and Van Knapen, 2013; Becskei et al., 2020a). Globally, *Toxocara canis* and *Toxascaris leonina* in particular, are the most common canine ascarids (Becskei et al., 2020b). These parasites, which settle in the small intestines of their main hosts, carnivores can also infest paratenic hosts such as; humans, mice, earthworms, ticks, chickens, sheep, pigs and birds (Glickman and Schantz, 1981). Ascarids are important zoonoses because they can be easily transmitted to humans from cats and dogs with whom we are in constant contact in our daily lives (Despommier, 2003).

The development and course of the infection in dogs; the age, sex of the animal, the number of infected eggs exposed, hormonal status, immune system and the migration route of the larvae

directly affect it (Lloyd et al., 1998). It can cause growth retardation, diarrhoea, dehydration, abdominal bloating, intestinal obstruction, ascarid toxicity and death, especially in young dogs (Burrows et al., 1995).

In the fight against ascariasis in dogs, it is recommended to first deworm puppies from parasites, and then apply ascarid treatment to adult dogs four times a year, even if there is no effective and regular examination (Becskei et al., 2020b). Due to its zoonotic importance, it has been reported that dogs that come into contact with individuals with compromised immune systems should be treated monthly (Companion Animal Parasite Council, 2020). In the treatment of ascariasis in dogs, medications such as; pyrantel pamoate, ivermectin, selamectin, eprinomectin, moxidectin, nitroscanate, mebendazole, milbemycin, sarolaner, spinosad, lotilaner, praziquantel are used either alone or in combinations (Genchi et al., 1990; Clark et al., 1991; Bowman et al., 1998; McTier et al., 2000; Kozan et al., 2008; Cardenas et al., 2017; Young et al., 2021). However, there is no 100% effective treatment protocol for *T. canis* in dogs, since its biology is very complex and there are still aspects of it that cannot be clarified (Doğanay et al. 2018).

In its simplest form, phytotherapy can be defined as the treatment made with plants. Phytotherapy, together with chemical treatments or as an alternative treatment, has attracted the attention of researchers in recent years (Sarışen and Çalışkan, 2005). When the phyto-therapeutic potentials of thyme were examined; it was reported to have antibacterial, antiviral, antiparasitic, antifungal and antioxidant potentials (Burt, 2004). The essential oils of thyme contain compounds such as; carvacrol, borneon, tanen, pimen, cymol, and flavones in addition to their intense thymol quantity. Their thymol ratio is around 50% (Benli and Yiğit, 2005).

In this study, it was aimed to investigate the treatment efficacy of thyme extract as an alternative to chemical treatments in naturally infested puppies with ascarid.

MATERIALS and METHODS

The study was carried out with the permission of Kırıkkale University Animal Experiments Local Ethics Committee, dated 19/12/2019 and numbered 63. The study consisted of 20 puppies of different sexes, 2-4 months old, which were brought to Kırıkkale University, Veterinary Faculty, Animal Hospital for medical examination and vaccination. Puppies that were mono-infected with ascaris upon stool examinations were included in the study.

Before the administration of thyme to the animals included in the study, blood and stool samples were taken, it was administered to orally 3 times in total on the 0^{th}, 1^{st} and 2^{nd} days, by diluting the commercial thyme extract (Thy717, BioArt, Turkey) to a concentration of 20%, at a dose of 1 ml/kg.

Stool samples were taken from the puppies for a total of 5 times, on the first 4 days and on the 7^{th} day of the beginning of the treatment, to count eggs in each gram faeces. In order to monitor for possible toxicity; serum urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were measured by taking blood samples twice, before thyme extract administrations (day 0) and after thyme extract administrations (3th day).

To determine the presence of *Toxocara* spp. eggs in stool samples, the Fülleborn flotation technique was used. The Mc Master technique was adopted to determine the number of eggs in 1 g stool before, during, and after the treatment. For this, the average of 40 glass beads were thrown into 100 ml jars. Three g of faeces was weighed and put into the jar and 42 ml of saturated salt water was added. Stool was homogenized by closing the lid of the jar. The resulting solution was transferred into centrifuge tubes and centrifuged at 1500 rpm for 3 minutes. The upper liquid was removed without moving the sediment at the bottom, and the same amount of saturated salt water was added to the faecal residue at the bottom of the tube, and the centrifuge tube was turned upside down 5-6 times to homogenize the sediment. The chambers of the Mc Master slide were filled with the help of a Pasteur pipette. Eggs were counted at x10 magnification under the light microscope and the number of eggs detected in both chambers was multiplied by 100 to determine the total number of eggs in 1 gram of stool (Şenlik, 2006). Faecal egg reduction test (FECRT) was also adopted to determine the efficacy of the thyme extract against *T. canis* (Doğanay et al., 2018). Egg reduction was calculated according to the formula below.

$$\text{FECRT} = \frac{\text{EPG value before treatment - EPG value after treatment}}{\text{EPG value before treatment}} X100$$

RESULTS

The daily change in *Toxocara* spp. egg counts in the puppies given the thyme extract is presented in Table 1. According to the data, it was determined

that the number of eggs was zeroed after the administration of the thyme extract in 7 out of 11 puppies which had faecal egg counts between 200 and 1650 on day 0, and in 1 out of 9 puppies which had faecal egg counts more than 1650. In total, faecal egg counts zeroed in 7 dogs (35%).

There was no decrease in the number of eggs laid in 2 of the 20 puppies. In 11 puppies, the egg laying rate showed a wide distribution between 25% and 98.3% (Table 1). Considering the general distribution, the percentage of egg reduction was found to be higher in puppies with relatively low egg counts prior to treatment.

Table 1. Faecal egg counts results of the puppies

No.	Day 0	Day 1	Day 2	Day 3	Day 7	% of Reduction
1	200	0	0	0	0	100
2	200	700	400	0	0	100
3	500	0	0	200	0	100
4	500	Not Sampled	50	0	250	50
5	500	150	0	0	50	90
6	750	Not Sampled	0	0	50	93.3
7	1000	900	1350	5200	3700	No Reduction
8	1100	7450	0	0	0	100
9	1500	1650	1200	1250	0	100
10	1500	Not Sampled	0	100	900	40
11	1650	Not Sampled	700	50	0	100
12	2100	1950	4950	9800	4600	No Reduction
13	2350	3900	5000	6000	1150	51.1
14	2500	4100	7100	6000	1050	58
15	3000	3000	2350	500	50	98.3
16	4250	12000	11650	0	0	100
17	8400	5800	1200	4000	6300	25
18	8400	5100	6250	12150	6050	28
19	15000	18200	2700	6250	1450	90.3
20	28150	5050	9600	16250	17900	36

Table 2. Mean changes in blood biochemical parameters before and after treatment.

Parameters	Before Treatment (n=20)			After Treatment (n=20)			Reference Values
	Min	Max	$\bar{x}$	Min	Max	$\bar{x}$	
Urea (mg/dl)	10	28	15.38	13	57	24.52	10-26
Creatinine (mg/dl)	0.16	0.48	0.26	0.12	0.57	0.32	0.5-1.3
AST (U/L)	15	88	28.84	7	58	21.15	10-88
ALT (U/L)	9	21	15.15	3	26	15.63	10-90

An adult worm was detected on the 7th day after the treatment in puppy number two and on the 3rd day in puppy number 10. On the 3rd day after the treatment, a large number of adult parasite excretions were observed in dogs with 17 and 19 numbers. It was observed that the general condition of dog number 15 was deteriorated prior to the treatment, however, the general condition improved after 3 days of treatment.

The mean values obtained from the measurements made from the blood samples taken before and after the treatment are given in Table 2. It was observed that serum urea, creatinine, AST and ALT values in the blood samples taken 24 hours after the last treatment dose were within the normal reference ranges. Although the mean value of urea after treatment approached the upper limit, it was still within the normal reference limit.

All the animals included in the study were clinically completely healthy during the study and on the 7th day examinations. During this period, there were no findings such as diarrhoea, vomiting, depression and anorexia that could indicate clinical toxication.

DISCUSSION

Canine ascariasis can cause fatal diseases, especially in susceptible dogs, and draws attention as it is a zoonotic condition (Macpherson, 2013; Hassanain et al., 2015). Especially in areas where people and dogs are in close contact, such as parks and gardens, the parasite can easily be transmitted to both susceptible dogs and humans by faecal contamination (Fankhauser et al., 2016). For this reason, in the fight against ascariasis in dogs, it is recommended to break the parasitic cycle by spraying dogs at least 4 times a year, even if no parasite is found (Becskei et al., 2020b). Due to widespread resistance to anti-helminthics (chemical drugs) used in the treatment of canine ascariasis, there is the need to constantly seek for new remedies (Coles et al., 1992; Hoekstra et al., 1997; Silvestre and Humbert, 2002). Among the strategies developed against helminths is phytotherapy (Jahangir et al., 2001).

Considering studies investigating the antiparasitic activities of thyme: Hafez et al. (2019) and Amin et al. (2016) *T. canis* in experimentally infected rats; Amin and El-Kabany (2013), *T. vitulorum*; Luis et al. (2016) anti-helminthic against *Haemonchus contortus* in experimentally infected sheep; Malatyalı et al. (2009) antileishmanial in vitro; Behnia et al. (2008) *Entamoeba histoytica* trophozoites in vitro; Gaur et al. (2018) in cell culture, Kara et al. (2022) anti-protozoan on *Cryptosporidium parvum* in experimentally infected rats; Attia et al. (2015), intestinal and cystic stages of *Trichinella spiralis*; Morsy et al. (1998) *Lucilia sericata* larvae; Remmal et al. (2011), Abbas et al. (2012), Arczewska-Wlosek and Swiatkiewicz (2012) studied the anticoccidial activity of thyme.

Studies have shown that the ant-parasitic activity of thyme extract is mediated by thymol, which constitutes 50% of the active ingredients (Ferreira et al., 2016). It has been reported that the anti-helmintic effect of tymol is based on its paralysing of the parasite, similar to the mechanism of action of macrocyclic lactones, and the inhibition of movement and feeding functions (Kotze et al., 2012; Lynagh et al., 2014). It has been reported that when macrocyclic lactones such as ivermectin, moxidectin, and eprinomectin are used for treatment in canine ascariasis, it reduces the number of faecal eggs by 100%, while this rate is 99.7% with topical use of selamectin, a member of the same group (Pal et al., 1995, Gargılı et al., 1999; Payne- Johnson et al., 2000; Kozan et al., 2008). Despite its success in treatment, it should not be forgotten that ivermectins are neurotoxic, especially in sensitive breed dogs such as Collies (Pronk and Schefferlie, 1998).

In canine ascariasis, both the toxic/side effects of chemical agents and the development of antiparasitic resistance, and the costs of continuous new drug development have led researchers to seek for treatment options (Pronk and Schefferlie, 2022; Jackson and Miller, 2006). Hassanain et al. (2015) compared the efficacy of mebendazole and sugar lemon (*Citrus aurantifolia Swingle*) seed extract in dogs naturally infected with *Ancylostoma caninum* and *T. canis*. According to the study, the number of faecal eggs was taken as a reference in the evaluation of treatment efficacy, faecal egg numbers; decreased by 74.10% in the group given only mebendazole, by 91.08% in the group given only sugar lemon extract, and by 98.20% in the group given mebendazole and sugar lemon extract. In our study, egg laying decreased by 25-100% in 18 dogs given thyme extract, while egg laying did not decrease in 2 dogs.

Amin et al. (2016) evaluated the therapeutic efficacy of thyme oil in experimentally infected rats with *T. canis*; they took the number of larvae detected in the brain tissue and brain damage as success criteria and reported that the treatment with thyme oil was found to be effective compared to the control groups. Hafez et al. (2019) examined the therapeutic efficacy of thyme oil in rats experimentally infected with *T. canis* larvae in a similar study. In the evaluation of the efficacy of the treatment, the histological changes observed in the testis tissue due to larval migration were taken as reference; while moderate and reversible changes were observed in the testicular tissue of the group treated with the thyme oil, serious histopathological changes were detected in the control group. In both studies, thyme oil was found to be effective in the treatment of experimental *T. canis* infection in rats. This result obtained in the experimental animals inspired this clinical study in dogs, the definitive host of *T. canis*.

In our study, it was determined that the number of eggs in the faecal samples taken 24 hours after the first treatment application increased significantly. This is thought to be due to increase intestinal peristalsis of thyme extract, induced fragmentation of adult parasites and increase in the number of eggs excreted in faeces due to fragmentation of adult female parasites.

Yıldız et al. (2011) in a study in which they examined the efficacy of *Artemisia absinthium* extract in cats naturally infected with *Toxocara cati*, they reported that egg laying stopped in only two of the 8 cats given the extract, and in the remaining cats, although the number of eggs decreased compared to the first day, the laying did not stop. In our study, egg laying stopped in 8 dogs (40%) after the administration of the thyme extract against *T. canis*, which is in the same family as *T. cati*. Similar to the related study, in 3 dogs egg shedding was reduced by 90.3-98.3%, while there was no decrease in 2 dogs. In 7 dogs, egg laying decreased by 25-58%. When the results of these studies were compared, it was found that the zeroing rate of thyme extract (35%) was higher compared to the zeroing rate of *A. absinthium* extract against ascarides (25%).

Hassanain et al. (2015) applied *Citrus aurantifolia* (lemon blossom) extract against *T. canis* in Egypt. In that study, they reported that the number of eggs decreased by 91.8% in the group in which only *C. aurantifolia* was administered. In our study, when the egg reduction in the stools examined before and after the treatment was calculated, it was determined that the egg reduction varied individually in the animals and the egg reduction was between 25-100%. In addition, it was recorded in 2 dogs that thyme extract had no effect on the number of eggs excreted in the faeces.

Although some of the cat and dog owners believe that natural products are safer than synthetic chemicals in parasitic control, it should not be forgotten that essential oils and plant extracts also have toxic effects on animals (Villar et al., 1994; Woolf, 1999; Genovese et al., 2012). Genovese et al. (2012), in their retrospective study covering a 2-year period, in which they compiled the cases of poisoning reported as a result of the use of topical products containing essential oil in cats and dogs; they evaluated different products made of oils such as peppermint, cinnamon, lemongrass, clove, thyme, cedarwood, rosemary, wheat germ oil. In the evaluation, clinical findings such as lethargy, weakness, desire to lie down, hyperactivity, tachycardia, hypothermia, hyperthermia, seizures, skin erythema, vomiting, diarrhea, edema, ataxia, agitation, anorexia, fasciculation, hiding, hypersalivation, panting, tremor were observed in animals. They mentioned that kidney failure may occur. Bischoff and Guale (1998) reported that liver enzymes such as ALT and AST increased in cats exposed to tea tree (*Melalleuca alternifolia*) oil poisoning. In this study, in which ascariasis was treated with the oral use of thyme extract, no clinical symptoms of poisoning were observed during or after the study. In order to monitor whether the treatment with thyme extract caused organ failure or not, urea and creatinine were measured for kidney failure, and AST and ALT values were measured for liver failure. Although all values before and after treatment were within their normal physiological reference limits, it was noted that the urea value approached the upper reference values after treatment. In subsequent studies, if the treatment period of 3 days is extended, it would be beneficial especially, in monitoring values for kidney functionality closely. Although there are reports of cats and dogs with a history of poisoning after topical applications with products such as thyme oil, no signs of poisoning were observed in any of the 20 dogs in this study. The products used in the summarized literature consisted of a combination of many oils and carrier substances but, in this study, no other substance was used aside from the thyme extract (as revealed by its content analysis) thus, it is thought that the non-toxicity of the thyme extract may be due to the fact that no other active substance was used.

CONCLUSION

In this study, it was concluded that oral use of thyme extract once a day for 3 days is safe for dogs at the concentration and dose used in this study. Considering that the rate of zeroing egg excretion is high in dogs with lower egg numbers in our study, it is predicted that our study will make a significant contribution to research works to be done on thyme extract in different doses or application days in patients with high egg excretion.

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Right lateral paracosto-abdominal hernia in a cat

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ABSTRACT

A 2-years-old, 2.4 kg – male crossbred cat was brought to Siirt University, Faculty of Veterinary Medicine, Clinic of Surgery Department, due to a traffic accident. A diffuse, palpable, painless swelling was detected under the skin starting from the right cranio-lateral abdomen to the right cranio-lateral thorax. In the orthopedic examination, there was a pain in the pelvic region and asymmetry at the right coxa-femoral joint. In the radiological examination of the thorax and abdomen, there was damage to the right lateral thoracoabdominal muscle in the abdominal region. There were also fractures in the sternum and pelvis, a diffuse interstitial lung pattern in the lungs and right coxa-femoral luxation. In the ultrasonographic examination, bloated bowel segments at the line of the right lateral thorax were monitored. Ventral median laparotomy was performed under general anesthesia. Herniated intestines at the right paracostal region were placed back into the abdominal cavity, and herniorrhaphy was performed. The abdominal cavity was closed according to the routine technique. As a result, paracostal hernia is a pathology that is rarely seen in cats after being hit by a car. Many operative techniques have been defined in hernia treatment. Median laparotomy was preferred for our patient and the hernia was treated successfully. This case report was prepared in order to contribute to our colleagues.

Keywords: *Cat, Paracostal, Hernia*

INTRODUCTION

Hernia can be defined as the protrusion of an internal organ through a tear in the wall surrounding it (Pavletic, 2005). The abdominal wall, diaphragm, and perineal hernias are the most encountered in small animals (Pratschke, 2002). The most common causes of traumatic abdominal wall hernias are traffic accidents and animal fights. The regions affected the most due to these traumas are the caudo-ventral abdominal wall and paracostal region (Langley-Hobbs et al. 2013). Injuries to the thoracoabdominal region, such as paracostal hernia, are often accompanied by diaphragm rupture. Traumatic paracostal hernia is a type of abdominal hernia and formed by the protrusion of organs into a space that is not anatomically present between the outer surface of the ribs and the abdominal muscles. In this case, the trauma affecting the patient causes a tear on the inner surface of the thoracoabdominal muscles, indirectly causing a hernia (Trindade et al., 2013). In some cases, the clinical findings are unclear and the desired results cannot be obtained from radiographic examinations. As a result, the diagnosis cannot be made, or the diagnosis is significantly delayed in many cases. Small hernia lesions may not be diagnosed even after months or even years. In such cases, a hernia is suspected

when significant dyspnea occurs or herniated intra-abdominal organs are strangulated (Lenot et al., 1990). The classical clinical findings of hernias manifest as painless swellings that suddenly occur in the region. Hernias are diagnosed with detailed clinical examination (inspection, palpation, etc.) and imaging techniques (CT, X-ray, Ultrasonography, etc.) (Hassen et al., 2017). Sometimes, intraoperative hernias are encountered during laparotomy performed for organ damage in animals with multiple traumas (e.g., liver rupture) and the diagnosis is made in the meantime (Soldá, 2002). Many interventions such as herniorrhaphy or hernioplasty have been defined in the operative treatment of hernias. However, an excellent surgical intervention in hernia cases is directly associated with the success shown in the postoperative period. This report describes the diagnosis and treatment of a traumatic paracostal hernia in a cat.

CASE HISTORY

A 2-years-old, 2.4 kg - male crossbred cat was brought to Siirt University, Faculty of Veterinary Medicine, Clinic of Surgery Department, due to a traffic accident. In routine clinical examinations, the patient's mucous membranes were anemic; the submandibular lymph nodes were hypertrophic; the body temperature was low (34.4°C). No abnormality was found in other routine clinical examination findings (respiration rate, pulse rate, heart rate). A diffuse, palpable, painless swelling was detected under the skin, starting from the right cranio-lateral abdomen to the right cranio-lateral thorax. On palpation, this swelling was soft and had limited movement in different directions under the skin with the pressure. In the orthopedic examination, a mild bilateral lameness was observed in the hindlimbs, and the patient was reluctant to move. Palpation revealed pain in the pelvic region and asymmetry at the right coxofemoral joint. In ventro-dorsal (V/D) and latero-lateral (L/L) radiographs of the thorax and abdomen, there was damage to the right lateral thoracoabdominal muscle in the abdominal region. Although no pathology was observed in the ribs, there was a fracture in the sternum and a diffuse interstitial lung pattern in the lungs. In the positive contrast radiographs of the gastrointestinal tract, a large number of intestinal segments were detected starting from the cranial of the right lateral abdominal wall to the right lateral cranial of the thorax (Figure 1). In ventro-dorsal (V/D) and latero-lateral (L/L) radiographs of the pelvis, there were fractures in tuber ischii at many different points and luxation in the right coxa-femoral joint. In the ultrasonographic examination, bloated intestines were monitored at the right lateral thorax.

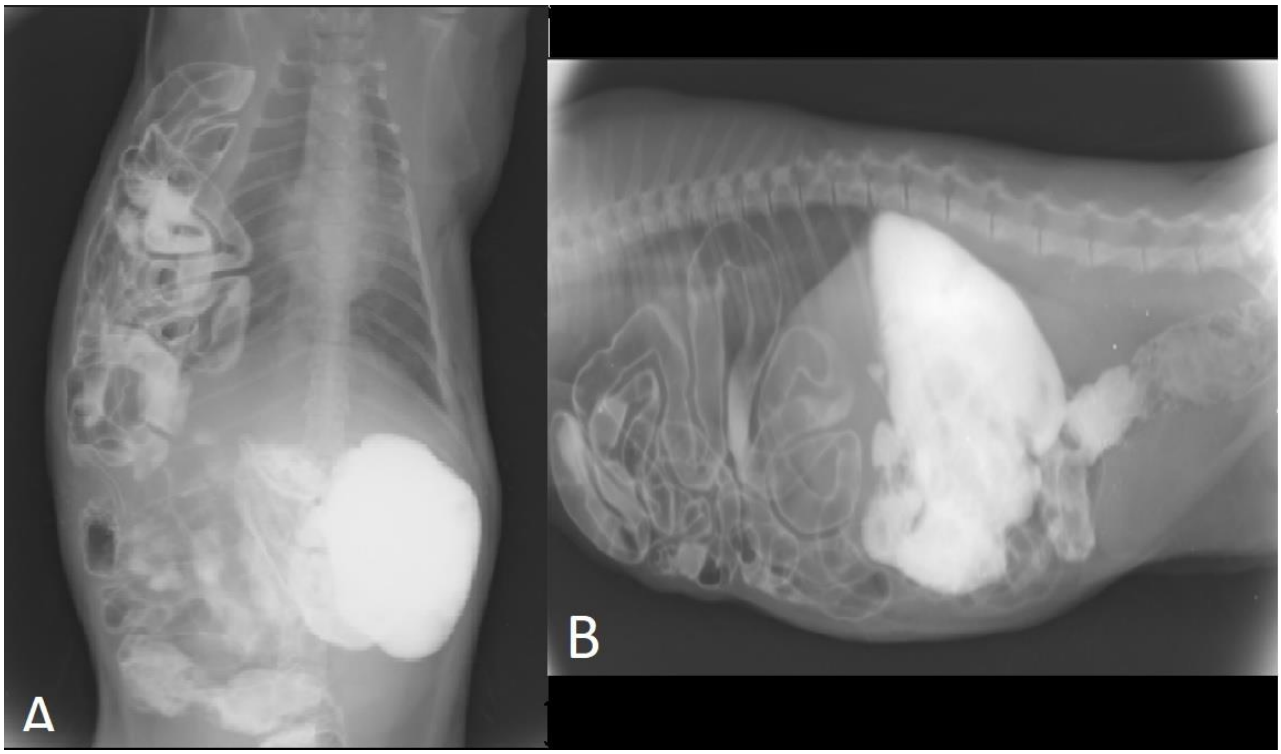

Figure 1. The ventro-dorsal (V/D) (A) and latero-lateral (L/L) (B) positive contrast radiographs of the patient. Herniated intestinal segments are observed on the right lateral thorax.

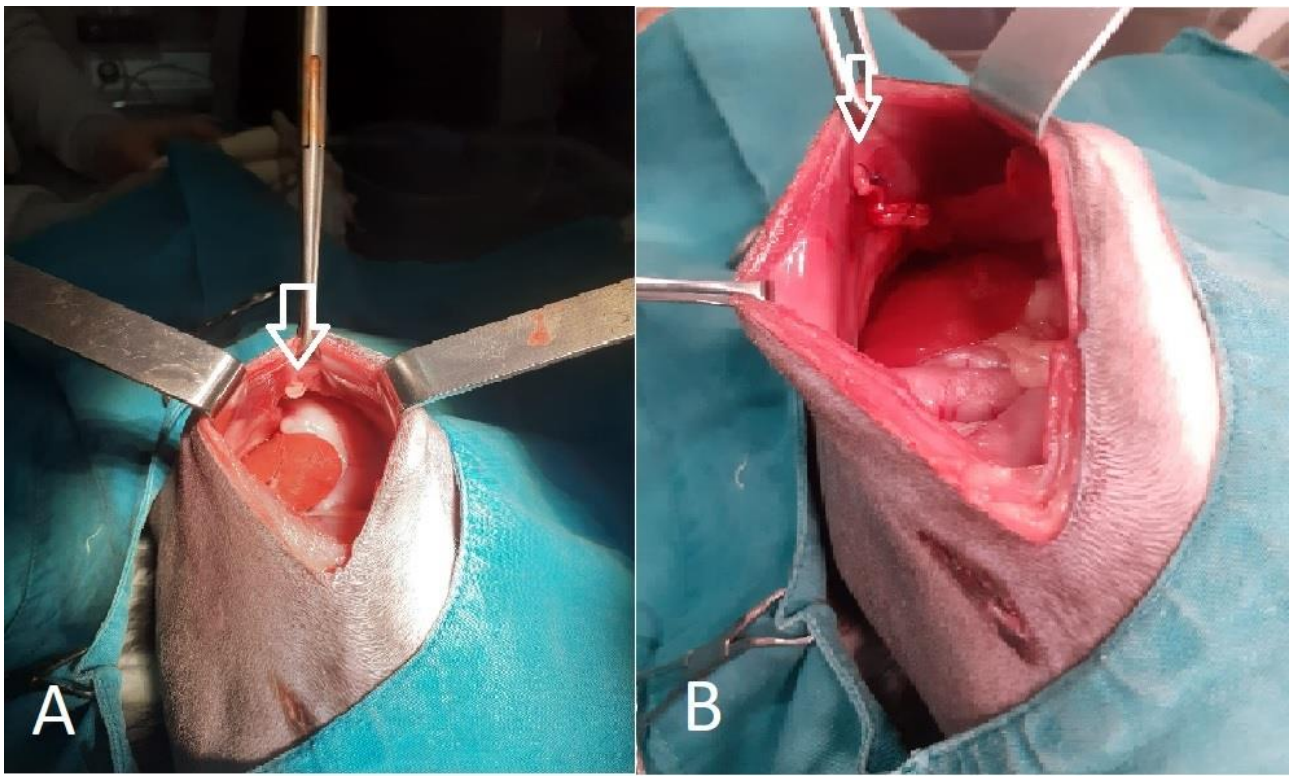

Figure 2. Intraoperative intestinal segments herniated from the paracostal region (A, White arrow). Hernia passage repaired with simple continuous suturing (B, White arrow)

The hematological examination revealed that leukocytosis (20.7 x 109/L) suggesting the onset of an infection. Although the hematocrit value was 27%, anemia onset was detected because dehydration was 8% in the patient in the clinical examination and it was thought that the cat might have suppressed anemia. A thrombocytopenia (174 x 109/L) was also detected.

The case was evaluated as a traumatic paracostal hernia and the operation was decided. The patient was sedated with 2 mg/kg, intramuscular xylazine HCL (Xylazınbio 2%, Intermed, Ankara), and 8 mg/kg intramuscular Ketamine HCL (Ketasol 10%, Interhas, Ankara) were administered. Maintenance of the anesthesia of the intubated patient was carried out with 2% sevoflurane (Sevorane Liquid Abbvie, Istanbul). Intravenous fluid therapy (0.9% isotonic saline; 10 ml/kg/hour) was provided to patient during operation. Preoperative 20 mg/kg intravenous ceftriaxone disodium (Unacefin® 1000 mg, Yavuz Drug, Istanbul) was applied.

The animal was placed in dorsal recumbency and the ventral abdominal area was prepared for aseptic surgery. We performed a ventral midline abdominal incision to allow the entire abdomen to be explored. In the examination of the right abdominal wall through the abdomen, it was observed that the muscle tissue was torn approximately 4 cm in the right paracostal line. A large segment of the small intestine was herniated into this tear. No other pathology was found in the abdominal exploration. Herniated small intestine segments were easily placed into the abdominal cavity with gentle manipulations (Figure 2). There was no incarceration or strangulation. The hernia hole was closed using the simple continuous suture technique with polydioxanone (USP: 2/0). Subcuticular suturing was done with polyglycolic acid (USP: 2/0), and the skin was closed in a simple interrupted suture pattern using polyglycolic acid (USP: 2/0).

After the hernia operation, a closed reduction procedure was performed to the luxation of the right coxa-femoral joint under general anesthesia. However, the joint was luxated again when abduction and adduction movements were performed on the extremity. Therefore, it was decided to delay the open reduction to another date. Considering the patient's general condition we preferred conservative treatment with cage confinement for hip fractures. Postoperative medical treatment with 20 mg/kg ceftriaxone disodium, i.v, twice daily (Unacefin® 1000 mg, Yavuz Drug, Istanbul) was applied for 5 days. 16 mg/kg Vitamin-C, i.v, once daily (Tekno-C® 250 mg, Teknovet, Istanbul) was applied for 5 days. 0,2

mg/kg, Meloxicam, s.c (Maxicam, Sanovel, Istanbul) was administered to reduce postoperative inflammation and pain for 3 days. Medical treatment and oxygen therapy were administered for lung contusion. The patient's anemia progressed and his general condition worsened. The patient died on the postoperative 6th day.

DISCUSSION

A hernia is the protrusion of an organ or an organ's part through a tear that is not anatomically normal. Hernias can be congenital or may occur later. They are named as true or false hernias according to the presence or absence of a hernia sac (Smeak, 2007). In small animals, abdominal, diaphragmatic, and perineal hernias are generally seen. Hernias are named lateral, ventral, prepubic, umbilical, intercostal, paracostal, diaphragmatic, and perineal according to their regions (Soldá, 2002). Paracostal and intercostal hernias are less common than other hernias (Pavletic, 2005). This case report in a cat, describes a traumatic paracostal hernia which is etiologically and clinically rare incident.

Traumatic body wall hernias can be defined as the protrusion of visceral organs from a traumatic defect in the thoracic or abdominal wall. Traumatic hernias can be classified as false hernias since they have no hernia sac (Kraus, 1990). Hernias in the lateral abdominal wall are the most common among traumatic hernias considering the region (Smeak, 2007). The size and location of a traumatic hernia may vary according to the severity of the trauma as well as to the anatomical characteristics of the patient (Pavletic, 2005). Considering the patient's body structure and weight (2.4 kg), his condition was within normal limits. From this point of view, the car hit, which can be considered a high-energy traumatic factor, created an approximate 4 cm hernia hole in the patient's right paracostal region. Traumatic hernias can occur as a result of blunt traumas to the abdomen such as traffic accidents, falls, kicks, bites, stab wounds, and gunshot wounds. When exposed to these forces, a large surface area of the patient is affected. Since the skin tissue is relatively more elastic, it may not be injured due to these forces, but defects may occur in the muscles and fascias since they are not as elastic as the skin tissue (Perez et al., 1998). In the examination of the patient's right lateral thoracic and abdominal region, no injury was observed on the skin. Despite this, there was a rupture in the right lateral thoracoabdominal muscles and a hernia hole was formed. An important point that should not be forgotten in traumatic hernias is that skin injury may not always occur. In such cases, the most prominent clinical finding is the varying degrees of swelling under the skin. Since anamnesis information is lacking, especially in street animals, the patient should be carefully examined and it should be considered that a traumatic hernia may occur even if there is no injury on the skin. Even in small-scale hernia cases in which the diagnosis is overlooked, organs may be herniated or strangulated in the advancing processes (Lenot et al., 1990). A sizeable swelling spread over a wide area was detected in the patient's right lateral thorax region and soft tissue structures were palpated on palpation. No strangulation was observed in the intestines during the operative intervention. This was interpreted as proof that the hernia was newly formed. Although the clinical findings vary according to the region of the hernia in hernia cases, weight loss and weakness are generally seen and, in some cases, abdominal breathing, restlessness, and swelling in the hernia region are observed. The most frequently herniated structures include the omentum, mesenterium, and intestines whereas stomach, spleen, and liver herniation rarely occur (Kumar, 2020). Since the patient had more than one pathology, the patient's general state of health was evaluated as moderate. In general, it was observed that the patient's interest in the environment was low and fatigue.

In some cases, the hernia sac may continuously expand as a result of the protrusion of more organs such as the omentum or intestine from the hernia hole. In addition, the herniated organ may adhere to the surrounding soft tissues in undiagnosed or untreated hernias. When the differential diagnosis is examined, traumatic hernias can also be confused with other diseases such as abscess, phlegmon, cyst, hematoma, and soft tissue tumors (Pavletic, 2005). During the operation, it was determined that a large part of the intestinal segment was herniated. The fact that the herniated intestine was larger than the size of the hole explains the excessive swelling observed in the preoperative examinations. The easy placement of herniated contents into the abdominal cavity during the operation was interpreted as an indicator of the lack of adhesion.

In paracostal hernias, abdominal organs may herniate along the thorax wall. In rare cases, abdominal organs may protrude from a defect between the intercostal muscles into the chest cavity. Studies have shown that many different pathologies such as orthopedic problems (eg,

pelvis) and soft tissue injuries may occur in half of the animals with traumatic abdominal wall hernia. For this reason, it is crucial to perform a detailed clinical examination of the affected animals (Fossum, 2013). The right coxa-femoral luxation, the paracostal hernia, and the fractures in tuber ischii detected in the patient support the literature. Paracostal hernia has been associated with diaphragmatic rupture in people and it has been suggested that it is more common in cats than in dogs. Traumatic abdominal wall hernia (TAWH) associated with pelvic fracture gaps has occasionally been reported in dogs but not cats (Dubois et al., 1981; Dorn et al., 1976; Mann et al., 1989). In one report of traumatic abdominal hernias only 18% of feline hernias were due to dog-bite wounds; however, a subsequent report identified a 40% incidence (Shaw et al., 2003; Waldron et al., 1986). This difference may merely be a reflection of the different patient populations seen by the two centers in question. TAWH is thought to have a relatively low incidence overall, with one report of 600 vehicular accidents documenting only two TAWH, and another of 123 cats with high rise syndrome injuries again identifying only two (Kolata et al., 1975; Whitney et al., 1987). Due to the genus of the patient (cat) and the fact that the case was etiologically (car crash) rare, we thought that this case report should be shared with veterinarians.

Muscle defect reconstruction is recommended in the operative treatment of paracostal hernias (Smeak, 2007). Depending on the size and tension of the defect, muscle flaps or synthetic meshes can be used (Pavletic, 2005). In some cases, herniated organs have been tried to be approached over the swelling; however, in our case, there was a need for an abdominal exploration since the patient had multiple traumas. For this reason, laparotomy was performed through the median line and muscle defects could easily be repaired without any mesh application.

CONCLUSION

In conclusion, paracostal hernia is a rare pathology in cats after being hit by a car. Even if there is no injury on the skin, it is important to clinically examine the patient in detail in terms of traumatic abdominal wall hernias and, if necessary, confirm the diagnosis using auxiliary examination methods such as radiography and ultrasonography. Many operative methods have been defined in the treatment of hernias. Median laparotomy was preferred in our patient and the hernia was repaired successfully. This case report was prepared in order to contribute to our colleagues.

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Author's Contributions: MBA designed the study. MBA, OY, SK applied the operation technique. KK and MBA evaluated the results. AG and MBA wrote the manuscript. GA provided hematological, technical and supervisory support MBA: M. Barış Akgül, OY: Onur Yıldırım, SK: Sevdet Kılıç, KK: Kezban Kaçak, GA: Gülşah Akgül, AG: Ali Gülaydın. All authors have read and agreed to the published version of the manuscript.

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Evaluation of feline infectious peritonitis in a Persian Cat using different diagnostic methods in pet hospital, Dhaka

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ABSTRACT

Feline infectious peritonitis (FIP) is a viral contagious disease of all domestic and wildcats. A 2.5 years old male Persian breed cat was brought to the Teaching and Training Pet Hospital and Research Center, Purbachal, Dhaka, with a history of the lateral recumbent, swollen abdomen, breathing difficulty, and without urination. A clinical examination revealed a collection of fluid in the abdomen and chest, and the cat was suspected of FIP. The blood sample was collected for the estimation of biochemical parameters of total protein (TP), albumin, bilirubin, SGPT, and SGOT. An X-ray and ultrasonography were performed to check the chest and kidney morphology. Collection of fluid in the abdomen and chest, and decreased levels of total protein, albumin, and albumin-globulin ratio confirmed that the cat was infected by FIP. A Rivalta test was performed to observe the changes in effusion fluid and also performed an FIPV antibody test for the qualitative detection of FIPV antibody in feline serum. As the prevalence of FIP is increasing nowadays, a proper diagnosis of this disease is required.

Keywords: *Feline infectious peritonitis, Diagnosis, Blood test, Rivalta test*

INTRODUCTION

Feline infectious peritonitis is a viral disease caused by feline coronavirus (FCoV) that mainly affects wild and domestic cats. FCoV is an RNA virus with enclosed positive strands and is frequently seen in cats (Hartmann et al., 2003).Together with canine coronavirus, they are members of the family Coronaviridae and the order Nidovirales (Kipar and Meli, 2014). FCoV has been divided into two forms based on serological and genetic characteristics, with type 1 being the most common worldwide. Feline coronavirus can persist for seven weeks in a dry environment and can spread indirectly, notably during cat exhibits, via litter trays, shoes, hands, and clothing (Addie et al., 2009).

Epidemiological studies of FIP have identified the highest prevalence in young cats (3 months to 3 years of age) with the majority of cases (75%) in multi-cat environments (Pesteanu-Somogyi, et al., 2006). Domestic cats are frequently infected with the feline coronavirus, and wild felids may also be seropositive. This infection is more prevalent in several pets than it is in single ones. A fatal disease called FIP, which is most prevalent in environments with many cats, develops in about one in nine FCoV-infected cats. Breeds like the Bengal and particular lineages within breeds have a higher mortality rate from FIP. Age is a significant risk factor, and 70% of cases are kittens under 1-year-old. FCoV-infected cats who are under stress over time are more likely to develop FIP (Addie et al., 2009). Susceptible cats are most likely to contract

FCoV after coming into touch with it in asymptomatic cats' excrement. Within one week of a natural infection, cats start spreading the virus in their feces, and they continue to do during for weeks, months, and in some cases, their entire lives (Pedersen et al., 2008).

The early symptoms of disease cats with FIP disease may be very asymptomatic. Clinical symptoms that are frequently described include listlessness, lethargy, diminished appetite, weight loss, and a fluctuating fever (Riemer et al., 2016). Other symptoms often start to appear after a few days to a few weeks. Most cats at this stage will develop the "wet" or effusive type of FIP, characterized by the buildup of fluid in body cavities. Fluid may build up in the abdomen, causing an enlarged abdomen, or in the thoracic cavity, causing breathing difficulties. In some felines experience "dry" or non-effusive FIP develops, in which little or no fluid builds up. The eyes, brain, liver, intestine, or other organs of the body are frequently severely affected in dry form, which can result in various clinical symptoms. The only clinical symptom in many cats with non-effusive FIP will be ocular problems. Once its infected, most affected cats decline quickly, however, some cats continue to function normally for a few weeks. Unfortunately, practically every occurrence of the illness will end in death (Riemer et al., 2016).

Infection with FCoV is widespread in cats. The FCoV infection rate in domestic cats is approximately 40%, and it reaches 90% in multi-cat households (Tasker, 2018). An increase in FCoV-related disease is most likely a result of changing trends in feline management (Addie, 2012). However, FCoV infection causes feline infectious peritonitis (FIP) in a limited percentage of cases, a deadly condition that is a common cause of mortality in young cats (Pedersen, 2009). FIP outbreaks in multi cat homes or shelters are occasionally documented, possibly more frequently recently (Wang et al., 2013). It might be challenging to accurately diagnose FIP ante mortem in many clinical situations. Therefore, the objective of this case report is to introduce the common and available diagnostic method to identify Feline Infectious Peritonitis in Bangladesh.

CASE PRESENTATION

A Persian male cat who was 2.5 years old and complaining of not being able to urinate and having an enlarged abdomen was taken to the Teaching and Training Pet Hospital and Research Center, Purbachal, Dhaka, Bangladesh. The cat was not vaccinated and also one of the cats the of the owner died with the same symptoms previously. On clinical examination, the cat was found dehydrated with lateral recumbence. The cat showed 39.4°C body temperatures on clinical examination. The cat was initially suspected of having FIP based on clinical symptoms.

Sample collection

Blood samples were taken into vacutainers without anticoagulants for the measurement of total protein (TP), albumin, and globulin to confirm the presence of FIP. Thoracentesis was done through Ultrasonography guidance using a 22 Gauge butterfly needle connected with a 5 mL syringe to collect fluid (Figure 1) and the color of the fluid was yellow (Figure 2).

Figure 1. Collection of fluid from the chest

Laboratory investigation of blood

For a biochemical test, anticoagulant-free blood was allowed to coagulate for 30 minutes in a slant position before the serum and supernatant were properly separated. Then, the HumaLyzer 3000® carried out the biochemical test following the prescribed procedure and the manufacturer's guidelines.

The biochemical analysis of blood revealed that TP (12.1 gm/dL) is higher than the reference value and albumin (2.3 gm/dL) is less than the reference value as shown in Table 1. The value of globulin was also found through TP and albumin values. The value of

globulin was 9.8 gm/dl. The ratio between albumin and globulin was 0.23. The value of bilirubin, SGPT, and SGOT was 2.1 mg/dL, 185 u/L, and 172 u/L respectively (Table 1).

Figure 2. Yellow color thoracic fluid.

Table 1. Biochemical parameters of blood serum.

Blood analysis	Result (gm/dL)	Reference value
Total protein	12.1	5.2-8.8 (gm/dL)
Albumin	2.3	2.5-3.9 (gm/dL)
Bilirubin	2.1	0.1-0.4 (mg/dL)
AIT/SGPT	185	10-100 (u/l)
AST/SGOT	172	10-100 (u/l)

Ultrasonography and X-ray

To determine the morphology of the abdomen and both kidneys, a USG (ultrasonography) of the ventral lower abdomen was performed. The animal was prepared for this by using a disposable razor to shave the ventral lower abdomen. After the animal had been properly restrained, a USG probe was positioned on the ventral lower abdomen to locate the cortex of both kidneys and to assess the internal condition of the abdomen. USG was carried out at a 15A and 4.0MHz frequency.

An X-ray was also performed to know the condition of the abdomen and chest. After the animal had been restrained, the animal was placed under the light in lateral recumbency and also ventral recumbency to take the pictures of abdomen and chest.

The most common Ultrasonic and X-ray findings of FIP are the presence of free fluid in the abdomen and chest (Lewis and O'Brien, 2010). In this case, the presence of free fluid is observed in the abdomen which appeared as black in USG (Figure 3) and was also observed in the abdomen and chest through X-ray (Figure 4).

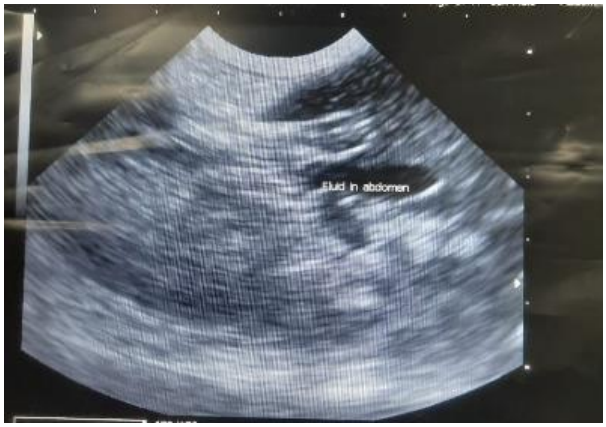

Figure 3. Fluid in abdomen.

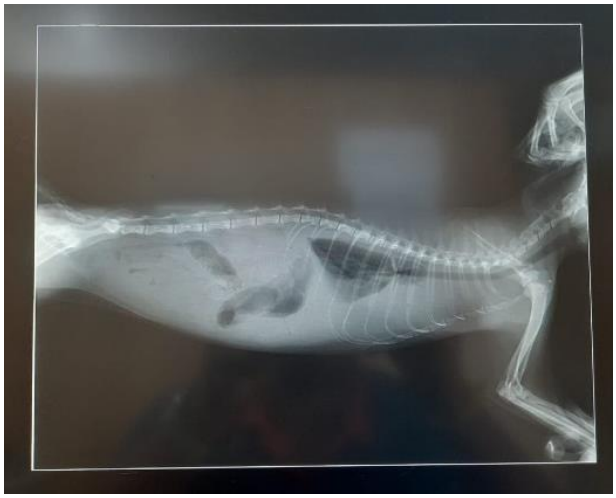

Figure 4. Fluid in chest and abdomen.

Rivalta test

Rivalta's test can distinguish between transudates and exudates (Fischer et al., 2012). Rivalta test is performed on cat effusions suspected of having FIP. The test is based on the idea that when an effusion fluid sample is put into an acetic acid solution, a precipitate will form.

A reagent tube was filled with 5 mL of distilled water, and 1 drop of acetic acid (98 percent), and the tube's contents were vigorously mixed (Figure 5). One drop of the effusion fluid was carefully put on top of this solution (Fischer et al., 2012).

The Rivalta test was considered effective if a precipitate formed and remained stuck to the surface, maintained its shape, or gradually drifted to the bottom of the fluid. The Rivalta test was considered negative if the effusion fluid drop

disappeared and the solution remained clear. The Rivalta test was regarded as doubtful when slightly opaque swirls occurred that neither formed a clear precipitate nor entirely disappeared (Fischer et al., 2012). In this case, a precipitate was formed and remained stuck to the surface (Figure 5).

Figure 5. Rivalta test positive.

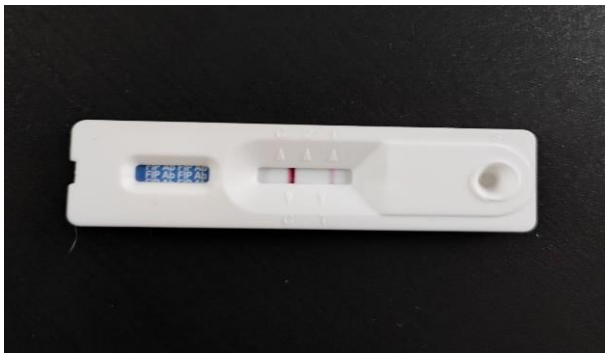

Figure 6. FIP antibody rapid test positive.

Feline Infectious Peritonitis Antibody Rapid test

The Feline infectious peritonitis antibody rapid test device uses lateral flow immuno-chromatographic assay to identify FIPV antibodies in feline serum or ascites in a qualitative manner and the origin of it is Hangzhou Zhejiang, China. The test device is for in vitro diagnostic use only for felines (www.teastsealabs.com). All kit components (Testsealabs®) and samples (fresh serum) were initially allowed to adapt to room temperature before testing. After waiting for 30 to 60 seconds, 1 drop of serum was added to the sample well. Within 8 to 10 minutes, the result was read after adding 3 drops of buffer to the sample well. In this case, the test kit result was positive (Figure 6).

DISCUSSION

Ante mortem diagnosis of FIP is challenging that's why the combination of clinical signs and symptoms with diagnostic aid is needed to do the diagnosis of FIP.

Biochemical parameters of blood play an important role in the diagnosis of FIP. In this case, an increased value of TP, bilirubin, SGPT, and SGOT and a decreased value of albumin was found in the biochemical test of blood (Table1). An increase in the concentration of total serum proteins, primarily due to an increase in gamma globulins, is a common laboratory finding of FIP (Paltrinieri et al., 2001). Depending on the extent and location of organ damage, liver enzymes and bilirubin can also be increased, although they are typically not helpful in making a diagnosis. The presence of elevated liver enzyme activity and high bilirubin levels without hemolysis should raise the possibility of FIP (Hartmann et al., 2003). Another important finding of this case was the albumin-globulin ratio which was 0.23. Compared to total serum protein or gamma globulin concentrations, the albumin / globulin ratio has a higher diagnostic value since, if the liver is affected, both albumin and globulin levels will fall (Jeffery et al., 2012). It is hypothesized that Low albumin is frequently accompanied by protein loss carried on by immune complex-induced glomerulopathy or by the extravasation of protein-rich fluid during vasculitis (Fujii et al., 2015). If the serum albumin to globulin ratio is less than 0.8, there is a high probability that the cat has FIP (Julie and Staci, 2016).

The Rivalta test is a common and easy test to suspect FIP. It is used to determine the differentiation of FIP effusions. According to a study of cats who presented with effusion, the test has a strong negative predictive value for FIP of 96% and a positive predictive value of 86% (prevalence of FIP, 51%) (Hartmann et al., 2003). The findings of the Rivalta test, in this case, were a precipitin formed which remains attached to a surface (Figure 5). Compared to blood tests, effusion tests have a better diagnostic value(Giori et al., 2011). Clear yellow effusions with a sticky substance are sometimes referred to as typical findings in the case of FIP but their lone presence in body cavities does not confirm a diagnosis. Pure chylous effusion cases have also been documented in the case of FIP (Addie et al., 2009).

An important diagnostic tool for FIP is FCoV antibody detection. Most likely, healthy cats with negative antibody results are neither FCoV execratory nor carriers. Rapid FIP is the screening test for the accurate detection of FCoV antibodies in whole blood, plasma, serum, and effusion of the cat since it is based on highly specific and recombinant FCoV antigens (Vetlab®). According to the manufacturer's declaration there are two lines on the test cassette. One is C (control) line, and another is T (test) line. The presence of both lines within 10 minutes indicates the presence of FIP, no matter T line is clear or vague. If only a C line has appeared, then the test result is negative (Testsealabs®).

There is a limitation of this case being unable to do CBC (complete blood count). Normocytic, normochromic, non-regenerative anemia, neutrophilic leukocytosis with lymphopenia, eosinopenia, and monocytosis are CBC abnormalities found in FIP-infected cats (Diaz and Poma, 2009).

CONCLUSION

The clinician must incorporate diagnostic tests to help guide a more definitive diagnosis as the index of FIP suspicion rises. The sensitivity and specificity of the chosen diagnostic test, as well as the limitations of each diagnostic test, must be evaluated.

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Full Text

For Original Research articles, the full text of the manuscript should be divided into the following sections: *Introduction, Materials and Methods, Results, Discussion, Conclusion*. For other articles, the text may be divided into sections if desired. Microsoft Word is preferred. All text should be double spaced, and all text elements, including tables, should be combined in a single document. Manuscripts should include continuous line numbering.

Acknowledgments

On a separate page preceding the references, contributions made by colleagues who are not authors of the paper should be acknowledged. This page should also include acknowledgments of published material reproduced with permission, previous presentations of the work at meetings, related grant support received, and disclosures of relevant commercial interests.

Footnotes

Footnotes are to be used when referencing each of the following types of information:

- Abstracts
- Conference presentations
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References

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Abstract of a Paper Presented at a Meeting

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Whole Book

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Edited Book

Underwood LE, Van Wyk JJ. Normal and aberrant growth. In: Wilson JD, Foster DW, eds. Wiliams' Textbook of Endocrinology. 1st ed. Philadelphia: WB Saunders; 1992. p.1079-1138.

Website

Animal and Plant Health Inspection Service. Bovine spongiform encephalopathy (BSE). Available at:

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